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Contents

1. INVITED SPEAKERS

Climate Change and Good Practices to protect Oceans in Hospital 4.0

Rosa M. Orriols[(1)*], Marina Rovira[(2)], Júlia Seco[(3)]

Board International Commission on Occupational Health (ICOH)
Board on the Board at Prevint[(1)]
International Commission on Occupational Health (ICOH) [(2)(3)]
Institut Català de la Salut (ICS) [(1)]
Hospital Universitari de Bellvitge (HUB) [(1)]
Hospital del Mar[(3)]
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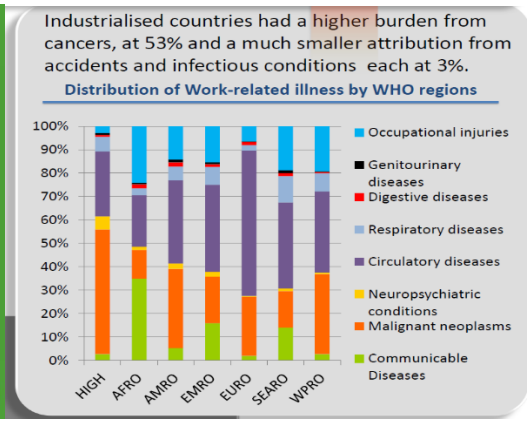
The 70th session of General Assembly has oponed with a towering achievement: the adoption of the 2030 Agenda, including 17 inspiring Sustanaible Development Goals, the SDGs. The COVID-19 acclerate the needs of hospitals to invest in Tech and Sustainability.
At Hospital de Bellvitge, we are committed to caring for the environment and people. Our goal is to provide the best health care to citizens while maintaining the sustainability of our processes, which places plastic recycling as one of the most beneficial activities in this regard, since we can reincorporate part of it to the products we use producing less. waste and lowered the cost of the products we need for our daily activity; to fight against Climate Change and to protect the Oceans.
The authors will expose the new technology in the sirurgical rooms to implement Circular Economy and how to monitoring and prevent the COVID-19 for the Healthworkers with Tech.

Figures:



***Biography (Prof. Dr. Rosa M. Orriols)**
Master in Environmental Management Engineering received her Graduate of Science in Chemistry Engineering from Barcelona University, Post degree Occupational Health Safety from Suffolk University. Currently, she is Executive Assitant General Management in Hospital Bellvitge (ICS) the main health institution in Spain. Her research and work focus on preventing harmful exposures and creating healthy environments at work.
Previous, Dra. Orriols was Technical Officer Occupational Health & Safety in National Occupational Safety and Health (INSHT) at the Spanish Government. She is Auditor. Advisor EU-Commission. Internationl Commission Occiàtopnañ Heath (ICOH) Board.
Her research interests include:1)occupational&environmental exposure assessment for studies of human health risks;2)intervention research to prevent illness and injury and promote healthy environments in healthcare;3)methods to integrate occupational&environmental health and safety into production and consumption of goods and services.
Dra. Orriols has spoken, written and testified extensively on health issues, including indoor air quality (specially surgical hume),prevention of blood borne exposures,radiations exposures, as well as about the effects of work organization.

Oxidative stress and pro-inflammatory status in patients with non-alcoholic fatty liver disease according to intrahepatic fat content

Antoni Sureda

Research Group in Community Nutrition and Oxidative Stress, University of the Balearic Islands and Health Research Institute of Balearic Islands (IdISBa), 07122 Palma de Mallorca, Spain; CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain

Abstract

Non-alcoholic fatty liver disease (NAFLD) is characterised by excessive fat accumulation, especially triglycerides, in hepatocytes. If not adequately treated, It can progress to non-alcoholic steatohepatitis (NASH) and continue to fibrosis, cirrhosis or hepatocarcinoma. Up to now, therapeutic approaches to cope with this disease are basically dietary and lifestyle interventions since there are no effective pharmacological therapies against NAFLD. The aim of the present work was to identify plasma biomarkers of liver damage, oxidative stress and inflammation that facilitate the early diagnosis of the disease and monitor its progression. Antioxidant and inflammatory biomarkers were determined in plasma of patients diagnosed with NAFLD (n=100 adults; 40-60 years old) living in the Balearic Islands, Spain. Patients were classified attending to the amount of intrahepatic fat content (IFC) measured by Magnetic Resonance Imaging (MRI). Circulating glucose, triglycerides, low-density lipoprotein-cholesterol, glycosylated haemoglobin, aspartate aminotransferase and alanine aminotransferase were higher in patients with IFC ≥2 of NAFLD with respect to patients with IFC 0 and 1. Plasma activity of catalase and superoxide dismutase and the levels of irisin, interleukin-6, malondialdehyde, and cytokeratin 18 were higher in stage ≥2 subjects, whereas resolvin D1 levels were lower. No differences were observed in xanthine oxidase, myeloperoxidase, protein carbonyl and fibroblast growth factor 21 depending on liver status. In conclusion, the quantity of intrahepatic fat in NAFLD patients is associated with an increase in oxidative stress and pro-inflammatory status. Because diagnostic tests such as MRI are not routinely carried out in clinical practices to diagnose or monitor fatty liver disease, combining various non-invasive markers could be useful for monitoring NAFLD.

Systems biology for newly bioproduction technology

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Abstract

In bioproduction and biotechnology field, improvement of the bioproduction ability of efficient microorganisms is one of the fascinating themes, and several types of empirical breeding approaches have been applied over the years. Those approaches are useful for constructing an efficient host strain, but their development is quite time-consuming and costly. To solve this costly problem, systems and synthetic approaches have been developed and applied in Japanese National Project, and we developed and applied statistical network modelling methods to reveal the mechanism of bioproduction systems. In this presentation, I'll introduce our developed computational approaches, and show you some application examples. Our developed methods are useful for improvement the productivity in microbial bioproduction and will be one of the newly biotechnology approach in Bioproduction field.

Keywords: Network Modelling, Bioproduction, Gene expression

Heavy metal role in medicinal plants related to health issue in the world

Aliakbar Maleki Rad[1&2] *(https://orcid.org/0000-0002-3436)*, Habibollah Nazem[1] *(https://orcid.org/0000-0003-4767-1904 1993)*, Mohammad Fazilati [1] *(https://orcid.org/0000-0003-0491-5497)*, Hossein Salavati[1] *(https://orcid.org/0000-0002-3407-269X)*, Ebrahim Alinia-Ahandani[1*] *(https://orcid.org/0000-0002-1633-086X)*, Mohammad Rezaei[2] *(https://orcid.org/0000-0002-8410-272X)*

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Abstract

Heavy or toxic metals are trace metals that are at least five times denser than water. Medicinal plants accumulate heavy metals from contaminated soil, and their consumption can cause poisoning. Environmental contamination by heavy metals such as mercury, cadmium and lead is a serious problem throughout the world. A survey by the World Health Organization showed that about 80% of the world's population depend on indigenous medicinal plants for the treatment of various ailments. Slightly above 35000 species of plant generally employed ethnomedicinally worldwide and a vast number of this are marketed uncontrollably. Toxic industrial wastes mixing with liquid agricultural fertilizers disperse farmlands. In this review article, an attempt has been made to collect relevant articles in recent years according to the importance and place of medicinal plants in the lives of people today. We tied to collect the information regularly in some databases source such as Web of Science, PubMed, Scopus, Islamic World Science Citation Center, and Magiran and etc., and then the relevant articles were detected. The results showed that many medicinal plants are affected by the more than usual accumulation of heavy metals in their structure and should be more careful from the moment of harvesting and cultivation of medicinal plants related to human health.

Keywords: Heavy metal, Health, Review, database, soil.

2. ORAL PRESENTATIONS

2.1. ABSTRACTS

➢ *ORAL PRESENTATION*

Lignin as an effective adsorbent of aflatoxin

Irma Mikade PhD, Rusudan Uridia Doctor, LeilaTatiashvili PhD,
Rusudan Tsiskarishvili pHD, Liparit Dolidze PhD

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Abstract

Aflatoxins represent one of the most aggressive and dangerous groups of mycotoxins with strong carcinogenic properties. Aflatoxins are isolated from two strains of fungi: *Aspergillus flavus Link* and *Aspergillus parasiticus Speare*. These fungi belong to the mesophilic microscopic order with the ability of growth at a temperature of 26-28°C.
It has led to the necessity of development of detoxication methods of raw materials, foodstuffs and feeds.
The chemical method causes breaking down of aflatoxins and useful nutrients as well. The use of various types of adsorbents in warehouses is hightly effective to prevent contamination by aflatoxins of vegetables and wheat crops, and act. Adsorbents of aflatoxins are devided into several groups.
The samples of the filings were selected: sawdust of coniferous and deciduous wood, and their mixture as well. The technical lignin was obtained by two methods: 1. Hydrolysis of the sawdust samples, then treatment by 4-6% dilute NaOH solution, then by Na_2S; 2. Extraction of lignin by water solution acidified with sulfuric acid, then treatment by 4-6% dilute NaOH solution. The matter gained by both methods was concentrated, dried at 20-30°C and crushed up, brown matter of 7-8% humidity had been obtained.
Among the preparations used for the detoxication of aflatoxins, we have selected the technical lignin that is distinguished by low cost price and high efficiency. Sulfated lignin belongs to non-toxic compounds, it is used as wet pastes, does not produce dust and is not fire-dangerous.
Therefore, considering urgency of the issue and the above mentioned factors, elaboration of a simplified scheme of obtaining technical, sulfated lignin from mixed sawdust and preparation of preparative forms for adsorption of aflatoxins is one of the ways for resolving this problem. The development of effective and selective methods using various physicochemical and microbiological methods is required.

Keywords: Aflatoxin, Lignine, Adsorbtion, Agricultural, Raw materials.

➢ *ORAL PRESENTATION*

Evaluation of the anti-oxydant activity of the two extracts of *Anvillea radiatta*

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Abstract

Natural substances from plants have multiple interests related to biotechnology in the food, cosmetic and pharmaceutical industries. Among these compounds we find a large part of secondary metabolites that have been particularly successful in therapy. For a long time, traditional herbal remedies have been used without knowing what their actions were due to. Studies of secondary metabolites are the subject of much research based on in vitro and in vivo cultures of plant tissue. This is notably the case of the phenolic compounds that are the subject of our study, compounds widely used in therapy as vasculoprotectors, anti-inflammatory drugs, enzyme inhibitors, antioxidants and antiradicals. In this context, the present work focuses on a phytochemical and antioxidant study of the n-butanolic and ethyl acetate phases from an endemic plant traditionally used to treat several diseases in Algeria and Morocco. The level of polyphenols is remarkably high in the n-butanolic phase (347.55± 7,387 µg EAG/mg EXS) and the ethyl acetate phase (319.28 ± 4,418 µg EAG/mg EXS). Additional analyses have highlighted the antioxidant and antiradical capacities of these extracts according to the DPPH- method. The results of this work allowed us to affirm that the two extracts of the studied plant present an interesting activity that gives IC 50 as follows of n-butanolics is (26.571 µg/ml), ethyl acetate (41.364 µg/ml) compared to that of quercetin (17.498 µg/ml) and ascorbic acid (16.681 µg/ml) very good antioxidant properties that could allow us to recommend them in biotechnology**.**

Keyword: antioxidant, IC50, Anvillea radiate, polyphenols.

➢ *ORAL PRESENTATION*

Sequential Ultrasonic Extraction of Pectin and Pentacyclic Triterpenoids from Apple pomace

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Abstract

Sequential extraction method is a possibility for recycling agricultural waste. Modern concept of utilization of agricultural wastes requires complete utilization, when mass of waste reduces more than 80%. Well-designed sequential extraction method make available selective and quantitative extraction of more than one valuable product. Selection of suitable techniques for sequential extraction method depends on: the desired class of compounds to be extracted; the process conditions and economic feasibility for scaling up the process.
The aim of the present study was to develop a simple, effective, eco-friendly, reproducible and high-yield two-step ultrasound-assisted extraction (UAE) procedure combined with quantitative determination high performance liquid chromatographic (HPLC) method for obtaining pectin and isomeric triterpene acids - oleanolic acid (OA) and ursolic acid (UA) from apple processing agro-industrial waste material.
The ultrasound frequency was 20 kHz; the temperature was controlled at 25±2ºC during 60 min. ultrasonication of extraction mixture of apple pomace and distilled water (solid-to-liquid ratio 1:10), acidified with citric acid for extraction of pectin.
Solid residue remained after pectin extraction was used for ultrasonic-assisted extraction of pentacyclic triterpenoids on the second step of sequential extraction. Acetone was selected as a most selective solvent for extraction of ursolic and oleanolic acids. Optimal conditions were: solid-to-liquid ratio 1:10, temperature-25^{0}C, extraction time-60 min. Such sequence of steps provides high yield and quality of pectin and easy purification and separation of UA and OA from extract.
A rapid, sensitive and specific HPLC method was developed and validated with respect to robustness, specificity, linearity-range, accuracy, precision and sensitivity for quantitative estimation of target products.

Keywords: Extraction, Pectin, Pentacyclic Triterpenoid

➢ *ORAL PRESENTATION*

Comparative study of the chemical composition of essential oils of *Tetraclinis articulata* (Vahl) Masters from Morocco

Rabib Halima[1*] (https://orcid.org/0000-0002-3641-9570), Hssain Mohamed [2], Fougrach Hassan [2], Wadii Badri [2], Koussa Tayeb [1]

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Abstract

The quality and variability of the chemical composition of the essential oil isolated from the leaves of Tetraclinis articulata was studied in three ecologically different regions of Morocco. The average yields obtained by hydrodistillation are 0.5% for the Benslimane region, 0.56% for Ras Elma Tazekka region (Mountain) and 0.36% for Debdou ragion (plain). The GC / MS analysis allowed us to identify the major components for each essential oil from the three regions show quantitative variability and are, respectively, Bornyl acetate (35.05%); 34.84%; 32.55%), Camphor (11.17%; 11.24%; 11.31%), and α-Pinene (10.84%; 11.41%; 18.83%).

Keywords: Tetraclinis articulate, Essential oil, Chemical composition, Morocco.

➢ *ORAL PRESENTATION*

Antioxidant and Antibacterial Activities of the Essential Oil of Moroccan *Tetraclinis articulata* (Vahl) Masters

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Abstract

The analysis of the main compounds of the essential oil isolated from the aerial part of *Tetraclinis articulata* (Vahl) leaves, Masters originating in Morocco (Benslimane Region, Atlantic-influenced plain) carried out by gas chromatography and mass spectrometry showed that this oil is dominated by Bornyl acetate (35.05%), Camphor (11.17%) and α-Pinene (10.84%). The antioxidant properties were evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical trap test the antimicrobial activity of the essential oil of T. articulata was tested against clinical isolates of Staphylococcus aureus, *Pseudomonas aeruginosa* and *Escherichia coli* which were inhibited from 25 µg / mL.

Keywords: Tetraclinis articulate, Essential oil, Antioxydant Activity, Antibacterial activity.

➢ *ORAL PRESENTATION*

Diphenyltin(IV) compounds as potential anticancer agents

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Abstract

There is an urgent need for substantial investigation of non-platinum drugs with higher activity and improved selectivity to address the problem associated with the use of platinum-based compounds as therapeutic agents. In light of this, diphenyltin(IV), dimethyltin(IV) and tin(IV) compounds were synthesised from the Schiff bases of dithiocarbazate and thiosemicarbazides with 2-hydroxy-3-methoxybenzaldehyde (oVa). The tin(IV) compounds formed were found to have a general formula of $[R_2Sn(ONS)]$ and $[Sn(ONS)_2]$ (where R = Me and Ph). The compounds were fully characterised by physico-chemical and spectroscopic methods. The spectroscopic results supported the coordination geometry in which the Schiff bases behaved as tridentate ONS donor ligands coordinating via azomethine nitrogen, thiolo sulphur and phenoxide oxygen atoms. Diphenyltin(IV) compounds showed the most promising cytotoxicity against a panel of twelve cancer cell lines (RT-112, EJ-28 (bladder), HT29 (colon), U87, SJ-G2, SMA (glioblastoma), MCF-7 (breast), A2780 (ovarian), H460 (lung), A431 (skin), Du145 (prostate), BE2-C (neuroblastoma) and MIA (pancreatic)). The three diphenyltin(IV) compounds were able to induce the production of Reactive Oxygen Species (ROS) and acted as a cell apoptosis inducer. Good binding interactions for all the diphenyltin(IV) compound series were observed and supported by molecular docking analysis, where hydrogen, electrostatic and hydrophobic binding interactions were observed. This highlights the important of two phenyl groups coordinated directly to the tin ion to enhance the cytotoxicity by strong π-π stacking interactions to biomacromolecules. Diphenyltin(IV) compounds could bring hope in the field of drug development against various diseases including cancers.

Keywords: Dithiocarbazate, tin(IV) compounds, cytotoxicity, molecular docking

➢ *ORAL PRESENTATION*

Formulation, characterization and studies of biodegradable nanocomposites films based on chitosan, polyvinylpyrrolidone and zero-valent copper nanoparticles stabilized by graphene nanosheets

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Abstract

The purpose of this study is to synthesize novel biocompatible and biodegradable nanocomposites films by solution casting method. Sayed nanocomposite is formed by zero-valent copper nanoparticles supported on functionalized graphene (Cu@FG) as a filler and chitosan/Polyvinylpyrrolidone (CP) polymer blend as a matrix. The filler was synthesized by in situ chemical reduction method of a blend composed of graphene and copper (II) sulfate powder using hydrazine hydrochloride as a reductant in an aqueous alkaline ethylene glycol organic solvent. It is noteworthy that the graphene was pre-functionalized (FG) for the better stability of zero-valent copper. The effect of the weight ratios on the polymer-matrix performance was tested to evaluate the compatibility of the blended materials. Furthermore, the influence of Cu@FG loading (0.1, 0.3, 0.5, 0.75 and 2 wt%) on the functional and physicochemical properties of the nanocomposites films was evaluated using FTIR, TGA, SEM, mechanical testing, biodegradation and barrier properties. The addition of Cu@FG significantly enhanced the thermal stability and mechanical properties of the nanocomposites films. FTIR measurement reveals a strong physical interaction between the filler and the polymeric matrix. SEM analysis shows the uniform dispersion of all the components of nanocomposite film. In addition, swelling degree, moisture uptake, water vapor permeability and biodegradability within 110 days in soil of nanocomposites films decreased with the increase in Cu@FG loading. These findings confirmed the efficient use of Cu@FG nanosheets in CP matrix to manufacture a green, cohesive and flexible nanocomposites films with improved thermal, mechanical and barrier properties for innovative food packaging applications.

Keywords : chitosan, polyvinylpyrrolidone, copper, graphene, films.

➢ *ORAL PRESENTATION*

Isolation and identification of *Staphylococcus aureus* and studying effect of physical and chemical mutagenesis on Hyaluronidase production from Iraqi patients

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Abstract

Isolation and identification of S.aureus bacteria from Iraqi patients usimg microbiological and molecular identification by 16s rRNA system using PCR amplification. Secondary metabolite production from wild strains is very low for economical purpose therefor certain strain improvement strategies are required to achieve hundred times greater yield of metabolites. The present study was conducted for enhanced production of hyaluronidase from Staphylococcus aureus S9 by mutagenesis using ultraviolet (UV) radiation, and Mitomycin C (MitC) as a mutagens. Results showed that a hyaluronidase over producer mutant symbol S.aureus S9-63 was obtained after mutagenesis with UV irradiation with higher enzyme specific activity (209.0 U/mg protein) in comparison to the wild type (118.02 U/mg protein). On the other hand, another overproducer mutant symbol S.aureus S9- 121) was obtained after chemical mutagenesis with MitC characterized with high hyaluronidase production. the enzyme specific activity in its crude filtrate was 188.4 U/mg protein in comparison with wild type. It could be concluded that physical mutagenesis using UV irradiation was more efficient than the chemical mutagenesis by MitC to enhance the ability of S.aureus A9 in hyaluronidase production.

Keywords: *Staphylococcus aureus*, mutant, UV, Mitomycin C

➢ *ORAL PRESENTATION*

Effects of different drying methods on the quality of dried *Serapias vomeracea*

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Abstract

Serapias vomeracea is a tuberous plant that belongs to *Orchidaceae* family. Salep is obtained by exposing the successive processes these tubers include drying. Salep is consumed as a traditional hot beverage, especially in Turkey, and it is used as a raw material of the drugs due to its therapeutic properties on the cough, headache, flu, intestine, and sexual problems. It is also an indispensable stabilizer for Maras ice-cream due to certain qualities like relative delay in melting and improved taste. These qualities depend on the chemical composition of salep, especially the glucomannan level. Other important quality indicators of salep are the moisture level and ash content.

The drying process is the main step for the production of salep and the sun-drying method is utilized in the conventionally producing of it. In this study, the effects of drying methods on quality parameters of salep were investigated. For this purpose, Salep was obtained from *Serapias vomeracea* with sun-drying, hot-air drying, and freeze-drying methods, and they were compared for the moisture level and physical appearances.

Keywords: Drying, Salep, *Serapias vomeracea*

Acknowledgements: This study was supported by The Scientific and Technical Research Council of Turkey,TUBITAK (Under 1002 Program – Project No: 119 O 893)

➢ *ORAL PRESENTATION*

The Effect of Lycopene Supplementation on Endothelial Nitric Oxide Synthase (eNOS) Levels in the Cortex and Hippocampus after Pentylenetetrazole-induced Epileptic Seizures in Rats

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Abstract

Several results have suggested that lycopene, a natural antioxidant, has positive effects on the nervous system. The aim of this study was to investigate the effect of lycopene supplementation on endothelial nitric oxide synthase (eNOS) levels in the cortex and hippocampus after pentylenetetrazole-induced epileptic seizures in rats. In this study, 30 male Wistar Albino rats were used. Animals were divided into five groups as control, saline (1 mL/kg/day serum physiologic), positive control (2 mg/kg/day diazepam), and lycopene (5 and 10 mg/kg/day) for ten days. Pentylenetetrazole (45 mg/kg) was given to induce a seizure in the tenth day except for the control. After 24 hours of PTZ injection, brain tissues of all groups animals were removed. eNOS levels in the cortex and hippocampus were measured by using the ELISA kit. Statistical evaluation of the data was performed by one-way ANOVA, and multiple comparisons were determined by the Tukey test. Statistical significance was defined at $P < 0.05$. Obtained data suggest that eNOS levels in the saline group were higher than the control group in the cortex and hippocampus ($P < 0.05$). However, there is no statistical significance in the eNOS levels between the saline group and the lycopene pretreatment group both in the cortex and hippocampus ($P > 0.05$). In conclusion, lycopene supplementation did not change eNOS levels in the brain after pentylenetetrazole-induced epileptic seizures in rats.

Keywords: Epilepsy, Pentylenetetrazole, Lycopene, Endothelial Nitric Oxide Synthase, Rats

➢ *ORAL PRESENTATION*

Aflatoxin M_1 Contamination of Buffalo Milk in Aksaray

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Abstract

Aflatoxin M_1 (AFM_1), a hepatotoxic metabolite, occurs as a result of consumption of feeds contaminated with aflatoxin B_1 (AFB_1) by lactating animals. AFM_1 has been reported to be resistant to pasteurization or other heat treatments. Thereby, it has been reported that dairy products may also contain AFM_1 depending on the contamination in the raw material. The purpose of this study is to specify the presence and levels of AFM_1 in buffalo milk which is produced widely in our region. The study can be considered as a pioneer in that uses buffalo milk as a material in Aksaray province.

In the year 2019, a total of 173 raw buffalo milk samples used as material. All samples were transported to the laboratory in cold chain (4 °C) and analyzed as soon as possible. Tests were done with ELISA (Enzyme-Linked Immunosorbent Assay) technique and AFM_1 specific test kits Ridascreen® Aflatoksin M1, r-biopharm were used for detection of AFM_1. The samples were prepared as described as kit manufacturer's instructions. Later, for calculation of AFM_1 levels, RIDASOFT WIN.NET software was also used as recommended.

173 raw buffalo milk samples were analyzed in duplicate and the results of each sample averaged in the study. While AFM_1 was not detected in 122 sample (70.5%). 51 sample (29.5%) was contaminated with AFM_1. However, it was observed that the AFM_1 levels of these 51 samples did not exceed the levels specified in the Turkish Food Codex.

In conclusion, although milk and dairy products pose a potential risk in terms of AFM_1, this risk was found low in samples belonging to our region. However, this situation may vary depending on the feeding conditions of lactating animals and sampling season. Therefore, it is recommended that similar studies could be diversified the data in the future.

Keywords: Aflatoxin M1, buffalo, milk, ELISA

➢ *ORAL PRESENTATION*

The Role of Bee Pollen's Protective Effect Against Copper Chloride Damage on Protein Expression in *Saccharomyces cerevisiae*

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Abstract

Bee pollen is used in public to alleviate colds, ulcers, anemia and allergies. Bee pollen contains the male seeds of plants, flowers or flowers, as well as flavonoids, which are products of plant metabolism that present various phenolic structures. For this reason, it is known for its antioxidant and radical scavenging properties, as well as having strong immunomodulatory and anti-inflammatory activities. In this study, the protective role of bee pollen against oxidative damage caused by copper chloride ($CuCl_2$) in *Saccharomyces cerevisiae* was investigated. Four groups were formed in the study. Groups: (i) Control group: Group in which only yeast is cultivated; (ii) Bee polen group: Bee polen given group (10%); (iii) $CuCl_2$ group: The group given $CuCl_2$ (30 mM); (iv) Bee polen + $CuCl_2$ group: The group given Bee polen (10%) + $CuCl_2$ (30 mM). *Saccharomyces cerevisiae* cultures were grown at 30 °C for 1, 3, 5 and 24 hours. Cell growth, lipid peroxidation, MDA (malondialdehyde) analyzes, GSH (glutathione) levels and catalase activity determinations were determined by spectrophotometer. Total protein changes were detected by SDS-PAGE electrophoresis and calculated by the Lowry protein method. As a result of this study, when compared to the $CuCl_2$ group, cell development (1, 3, 5 and 24 hours), total protein synthesis, GSH level (24 hours) and catalase activities increased in bee polen groups, while MDA level (24 hours) decreased. As a result of this study, it was determined that bee polen has a role to stimulate cell growth and total protein synthesis by reducing oxidative damage in *Saccharomyces cerevisiae* culture. Moreover, the high antioxidant capacity of bee polen suggests that its positive effects on *Saccharomyces cerevisiae* may also be on humans in both traditional and modern medicine.

Keywords: Bee polen, MDA, Oxidative damage, *Saccharomyces cerevisiae*, SDS-PAGE

➢ *ORAL PRESENTATION*

Investigation of protease productivity of marine bacteria isolated from *Axinella damicornis* sponge and partial characterization of produced protease

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Abstract

Proteases from the proteolytic enzyme group have an important position in the commercial enzyme market. It is crucial to bring the superior properties of the proteases, which are desired to have different characteristics according to their usage, to the industry. Therefore, isolation of new microbial species and enzyme productions from those is a critical study subject. In this study, protease productivity of the 81 bacteria were screened which were isolated from Kas, Antalya, Turkey. It was determined that the most productive strain belongs to the *Microbacterium genus*, and this strain produced 166 U ml^{-1} protease activity in shake flask productions. In addition, the optimum temperature and pH values of the produced protease were determined to be 35^{o}C and pH 8. Also, protease enzyme activity increase under the influence of 10 mM $MnCl_2$ and 4^{o}C condition is more advantageous than of -20^{o}C in long-term storage of the enzyme.

Keywords: Protease, Marine bacteria, Enzyme production, Enzyme characterization

➢ *ORAL PRESENTATION*

Experimental approach to tacrolimus measurement after radiopaque use in kidney transplant patients

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Abstract

Objective: The measurement of tacrolimus levels used for immunosuppression in kidney transplant patients is performed by immunoassay and LC-MS / MS methods. Although the LC-MS / MS method is the reference method, some molecules in the blood matrix may cause false measurements. Patients with renal transplantation may have the potential to affect tacrolimus levels due to radiopaque in blood after contrast-enhanced imaging. The aim of this study is to experimentally investigate the effects of gadobutrol, iohexol, gadopentetic acid, gadodiamide and ioversol on tacrolimus concentration.
Methods: Gadobutrol, iohexol, gadopentetic acid, gadodiamide and ioversol were added to the control material containing tacrolimus. 20µL of the radiopaque solution and 180µL of the control solution were taken and mixed. The level of tacrolimus was studied in the LC-MS / MS device from the mixture prepared by dropping each radiopaque. The same work was repeated by adding 20uL of distilled water. Deviation amounts were calculated with bias%.
Results: All radiopaques produced false negative results. Tacrolimus results affected by gadobutrol - 21.02%, iohexol - 24.46%, gadopentetic acid - 34.39%, gadodiamide - 25.80%, ioversol - 21.56%. The greatest deviation occurred with gadopentetic acid.
Conclusion: Radiopaque agents caused false results in tacrolimus concentrations measuring by LC-MS / MS. Inaccurate measurement of immunosuppressant levels reveals the risk of organ rejection in kidney transplantation. Blood samples should be taken prior to contrast agent injection to measure immunosuppressants.

Keywords: Tacrolimus, Iohexol, Gadopentetic Acid, Ioversol, False Measurement

➢ *ORAL PRESENTATION*

The Effect of Royal Jelly on Bax, Bcl-2, Caspase-3 and Caspase-6 Protein Signaling Pathways in Rats with Muscle Damage with Fluoride

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Abstract

Fluoride is among the most abundant elements on earth. It plays a role in the growth of the teeth and bones of humans and animals. However, in many studies, it is known that fluoride causes damage to the stomach, intestine, lung and nervous system, and in chronic fluoride poisoning, it causes disorders in the skeletal system, kidneys and testicles. Royal jelly, it is a viscous milky substance secreted from the mandibular and hypopharyngeal salivary glands of young worker bees. Royal jelly is stated to strengthen memory, increase physical performance and relieve fatigue, including pharmacological activities such as anti-oxidative, anti-tumor, and anti-inflammatory. In this study, it was investigated whether Royal jelly had a protective role against fluoride-induced muscle damage in rats. The animal experiments part of this study was carried out in F.U Experimental Animal Research Center (FUDAM) with the permission of F.U. Animal Experiments Ethics Committee on 02/09/2020 meeting date and number 2020/12. The rats were divided into 6 groups and each group included 7 rats. Groups: (1) Control Group: Group fed with standard diet; (2) Royal jelly Group: Royal jelly (100 mg/kg CA, gavage); (3) Fluoride-50 Group: Fluoride (50 mg/kg CA, drinking water); (4) Fluoride-100 Group: Fluoride (100 mg/kg CA, drinking water); (5) Fluoride-50 + Royal jelly Group: Fluoride (50 mg/kg CA, drinking water) + Royal jelly (100 mg/kg CA, gavage); (6) Fluoride-100 + Royal jelly Group: Fluoride (100 mg/kg CA, drinking water) + Royal jelly (100 mg/kg CA, gavage). Animals were decapitated after 8 weeks and their muscle tissues were received and investigated. Expression levels of caspase-3, caspase-6, bcl-2 and bax proteins in muscle tissue were determined by western blotting technique, lipid peroxidation MDA (malondialdehyde) analysis, catalase activities and GSH (glutathione) levels were determined by spectrophotometer. Royal jelly with application, bcl-2 protein expression levels and MDA levels decreased, caspase-3, caspase-6 and bax protein expression, GSH levels and catalase activities increased in Fluoride-50 + Royal jelly and Fluoride-100 + Royal jelly group. The results obtained from this study show that Royal jelly protects muscle tissue from the intoxication of fluoride and minimizes the damage to muscle tissue as a result of toxicity. This work was supported by Firat University Scientific Research Projects Unit (FUBAP) with FF.19.16 project number.

Key words: Caspase-3, Fluoride, Royal jelly, MDA, Muscle damage

➢ *ORAL PRESENTATION*

Biyodizel Üretim Yöntemleri: Kritik Karşılaştırma

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Özet

Artan enerji ihtiyacı ve çevre sorunları nedeniyle bilimsel çalışmalar yenilenebilir alternatif enerji kaynaklarına odaklanmıştır. Biyodizel, dünyanın pek çok ülkesinde alternatif dizel yakıt olarak kullanılan önemli yenilenebilir enerji kaynaklarından biridir. Biyodizel genellikle bitkisel ve hayvansal yağların geleneksel karıştırma altında kesikli ve sürekli proseslerle katalizörle transesterifikasyon reaksiyonu ile üretilir. Ekonomik nedenlerden dolayı, biyodizel üretimi için verimli transesterifikasyon yönteminin seçilmesi son yıllarda önem kazanmıştır. Bu bağlamda, araştırmacılar verimsiz süreçlerden kaçınmak için süper kritik, mikrodalga destekli ve ultrason destekli süreç gibi farklı yeni süreçleri araştırmaktadır. Bu yöntemlerin geleneksel yöntemlerle karşılaştırıldığında birkaç farklılığa sahip olduğu bulunmuştur. Homojen katalizörler (sülfürik asit, sodyum hidroksit, potasyum hidroksit, sodyum ve potasyum metoksit vb.), heterojen katalizörler (ZnO, SiO, MgO, BaO, SrO vb.) ve enzimatik katalizörler (lipaz) de mikrodalga ve ultrasonik uygulamalarda rahatlıkla kullanılmaktadır. Ancak bitkisel yağların süper kritik transesterifikasyon reaksiyonu katalitik olmayan bir reaksiyondur ve geleneksel yöntemlere göre daha yüksek verim elde edilebilir. Biyodizel üretimi için yeni yöntemler daha fazla avantaj sunmaktadır ancak bu yöntemlerin bazı olumsuz etkileri de vardır. Örneğin enerji tüketimi, fazla miktarda alkol kullanımı süper kritik sürecin dezavantajlarıdır.
Mikrodalga destekli biyodizel sentezi hala laboratuvar ölçekli üretim aşamasındadır ve mikrodalga radyasyonunun emici malzemelere nüfuz etme derinliği nedeniyle endüstriyel üretim için büyük ölçekte geçerli değildir. Ayrıca mikrodalga reaktörlerin güvenlik yönü, endüstri için başka bir dezavantajıdır. Ultrasonik biyodizel üretimi ise küçük üreticiler için avantajlı olabilir, ancak büyük ölçekli işletmelerde birçok ultrason probunun gerekliliği nedeniyle zor olabileceği düşünülmektedir. Biyodizel üretiminde yeni yöntemlerin bazı dezavantajları olmasına rağmen, bu yöntemler reaksiyon süresinin ve reaksiyon sıcaklığının azaltılması, istenmeyen yan ürünlerin oluşmaması, ester veriminin daha yüksel olması, geleneksel yöntemlere kıyasla daha kolay dönüşüm sağlaması gibi avantajları bulunmaktadır. Sonuç olarak, önemli avantajları ile bu yöntemler artık geleneksel yönteme göre daha çok tercih edilebilmektedir.

Anahtar Kelimeler: Biyodizel, enzimatik biyodizel üretimi, mikrodalga yöntemi, ultrasonik yöntem, süper kritik yöntem

➢ *ORAL PRESENTATION*

Foreign Body Intestinal Obstruction in a Dog

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Abstract

Material of this case report has created by a male Pitbull dog which is 18 months old, 15 kg, brought to clinic of Kyrgyzstan- Turkey Manas University of Veterinary Medicine. Patient came to clinic with the complaint of vomiting, difficulty in stool, reluctance of eating and drinking and pain. With taken history, it was learned that the patient swallowed two rubber balls, one of which came out with defecation and the other one was inside. It was decided to take the image of the abdominal region radiologically. It was obviously seen that rubber ball obstructed inside of the animal's small intestine with the examination. It was decided to carry out an operation. Abdominal was opened with the proper premedication and anesthesia. The intestine area where the ball was attached was found, and intestine taken out. We entered abdominal lumen by longitudinal incision in the cranial of the obstruction. The rubber ball was taken out from there. Antibiotic was given to animal for 10 days. It was suggested that animal fed by liquid food. After 10 days, animal started to eat normally. As a result, it is thought that in foreign body obstructions in dogs, early intervention is very important in success in and operation is final result among treatment options.

Key words: foreign body, obstruction, dog

➢ *ORAL PRESENTATION*

A Macroscopic Study on the Tendons of Manus in the Gissar Sheep

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Abstract

This study was perfomed to reveal the course and intertio of the extensor and flexor the tendons of manus in the meat type three Gissar sheep. The extensor and flexor tendons were macroscopically dissected and photographed in the distal to carpal joints. The superficial and profund digital flexor tendons distally coursed on the caudal face of third and fourth metacarpal bone. The interosseus tendon located in the depth of this region ended the proximal sesamoid bones. Prior to the intertion, the tendon give rise the two branches to the common digital extensor tendon on the dorsal face of the proximal phalanxes. It was observed that the common and lateral digital extensor tendon run distally on dorsal face of the third and fourth metacarpal bones and the common digital extensor tendon take the two branches from medail and lateral in the distal to fetlock. It was determined that the lateral digital extensor tendons inserted to dorsal face of the medial phalanxes of the third and fourth digits, while the common digital extensor tendons end to the extensor process of the distal phalanx on both third and fourth digits. It was seen that the superficial digital tendon arrived at the palmar aspect of the basis medial phalanx both third and fourth digits, while the profund digital flexor tendon reached the flexorial aspect of the distal phalanx both third and fourth digits. As a result, it was concluded that the interosseus tendo was import to stabilize the joints of the manus. In addition to, the medial and lateral braches splitted the interosseus tendon was also import.

Keywords: Gissar sheep, manus, flexor tendon, extensor tendon.

➢ *ORAL PRESENTATION*

Anaerobic Degradation of Polycyclic Aromatic Hydrocarbons (PAHs) by an Enriched Bacterial Consortium Isolated from Riverbank Sediments Contaminated with PAHs

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Abstract

The Mahoning River, in Northeast Ohio and Western Pennsylvania, USA is known to have been heavily contaminated with polycyclic aromatic hydrocarbons (PAHs), metals, and polychlorinated biphenyls that remain in riverbank sediments. The river is considered one of the most contaminated sites in northeast USA and around the world. Between 1900 and the 1970s the river received over 32,000 kg of oil and grease each day. The aim of this research was to isolate potential PAHs-degrading bacteria from river sediments in Ohio, USA and investigate the effect of anthraquinone-2,6-disulfonate (AQDS) on the anaerobic degradation of PAHs. A modified culture never tested before was used to enrich an anaerobic consortium which yield 200 clones. Only 38 clones were identified by partial 16S rRNA gene sequences. Molecular analyses showed that enriched anaerobic bacteria included members of Gammaproteobacteria, Sphingobacteria, Clostridia and Actinobacteria (96-99% similarity). The same enriched bacterial consortium was used to test anaerobic degradation of fluorene, phenanthrene and pyrene in the presence and absence of AQDS. The highest degradation was achieved after 120 days (67%, 66% and 66% respectively) in the presence of AQDS and bacteria consortia. With bacteria and without ADQS fluorene, phenanthrene and pyrene degradation was lower (59%, 58% and 58% respectively) than with AQDS yet statistically significantly different. This study warrants the use of indigenous bacteria for future in situ degradation studies of similar highly contaminated environments. This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) awarding a grant to Omer ACER and Youngstown State University Biological Science Department where degradation studies and analyses were conducted.

Key words: Anaerobic degradation, PAHs, Mahoning river, sediment, AQDS

➢ *ORAL PRESENTATION*

Investigation of the relationship between obesity and rs9939609 and rs1421085 single nucleotide polymorphisms in the *FTO* gene

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Abstract

Body mass index between 25-29.9 kg/m² reflects overweight, 30-39.9 kg/m² obese, 40 kg/m² and above represent morbid obesity. *FTO* (fat mass and obesity associated gene) is the first obesity susceptibility gene discovered during genome-wide association studies. Today, it continues to be the gene locus with the broadest impact on body mass index (BMI). It is the most variable gene region in different lineages throughout life, depending on various types of obesity.
One hundred and forty-two overweight and obese (BMI≥25 kg/m^2), and 142 healthy individuals (BMI <25 kg / m^2) as a control from the Turkish population living in Malatya were included in the study. The rs9939609 and rs1421085 genotypes were detected by the PCR-RFLP method. Obese and control groups were compared for possible differences between genotype and allele distributions. Besides, whether there is a relationship between rs9939609 and rs1421085 and BMI was investigated.
The obese-control study showed that there was no significant difference between the obese and control groups screened in terms of genotypic and allelic distributions of rs9939609 and rs1421085 single nucleotide polymorphisms. Additionally, no relationship was found between rs9939609 and rs1421085 and BMI.

Keywords: *FTO*, obesity, BMI, rs9939609, rs1421085

➢ *ORAL PRESENTATION*

The protective effects of nerolidol in thioacetamide induced oxidative damage in heart and kidney tissue

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Abstract

Thioacetamide (TAA) is an antifungal pesticide used to eliminate agricultural pests. TAA, known for its hepatotoxicity in literature studies, has toxic effects on many organs. This study aims to revert the oxidative stress created by TAA on the heart and kidney, and increased lipid peroxide peroxidation back with antioxidant-properties nerolidol. Nerolidol is a bioflavoid-shaped occipital and its antioxidant effects have been shown in the literature studies.
The rats were randomly divided into four groups (control, TAA, NRL, TAA+NRL). A total of 32 adult male Wistar Albino studies, eight rats in each group. The control group was given only gavage of corn oil. TAA was administered to the TAA group as 200 mg/kg/day ip twice a week for three weeks. NRL was administered daily to the NRL group 100 mg/kg with a by gavage. TAA+NRL group therapy was administered at a dose of 200 mg / kg/ TAA and 100mg/kg/day NRL. These applications were carried out for 21 days. As a result of these dose practices, TAA has formed free oxygen radicals in the heart and kidney tissues, creating oxidative stress. TBARS levels, SOD, CAT, GSH, GPx levels were detected. The finding of this study the nerolidol treatment significantly decreased TBARS levels and increased antioxidant enzymes. Histopathological changes in organs were significantly changed after TAA+ NRL application.
In conclusion, this study clearly demonstrated that NRL has protective effects on TAA-induced heart and kidney toxicity depending on its antioxidant properties. While increasing the nerolidol antioxidant defense system, TAA has also made significant improvements to histopathological changes in tissues. As a result, it has been shown that TAA, which does multiple organ damage, is treated with an antioxidant bioflavonoid such as a nerolidol.

Keywords: Nerolidol, TAA, oxidative stress, lipid peroxidation

➢ *ORAL PRESENTATION*

Kalp ve böbrek dokularına tiyoasetamid ile indüklenen oksidatif hasar üzerine nerolidolün koruyucu etkileri

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Özet

Tiyoasetamit (TAA) tarım zararlılarını öldürmek için kullanılan antifungal bir pestisittir. Literatür çalışmalarında hepatotoksisite oluşturması ile bilinen TAA birçok organ üzerine toksik etkiler oluşturmaktadır. Bu çalışmada TAA 'nın kalp ve böbrek üzerinde oluşturduğu oksidatif stres ve artan lipit peroksidasyonu antioksidan özellikteki nerolidol ile geriye döndürülmesi amaçlanmıştır. Nerolidol biyoflavanoid yapıda bir seksiterpendir ve literatür çalışmalarında antioksidan etkileri gösterilmiştir.
Bu çalışma kapsamında kullanılan sıçanlar dört gruba ayrıldı (kontrol, TAA, NRL, TAA+NRL). Her grupta sekiz sıçan olmak üzere toplam 32 erkek Wistar Albino üzerinde çalışma yapıldı. Kontrol grubuna sadece gavajla mısır yağı verildi. TAA grubuna üç hafta boyunca haftada iki kez olacak şekilde 200 mg/kg ip olarak TAA uygulandı. NRL grubuna- 100 mg/kg dozunda gavajla her gün NRL uygulandı. TAA ve NRL grubuna ise 200 mg / kg TAA haftada iki kez ip olarak verildi. NRL mısır yağı içinde süspansiyon haline getirilmiş 100 mg / kg dozunda her gün gavajla verildi. Bu uygulamalar 21 gün boyunca yapıldı. Bu doz uygulamaları sonucunda TAA kalp ve böbrek dokularında serbest oksijen radikalleri oluşturarak oksidatif stres meydana getirdi. TBARS seviyeleri, SOD, CAT, GSH, GPx seviyeleri belirlendi. Nerolidol uygulaması TBARS seviyelerini düşürerek antioksidan enzimlerin artmasını sağladı. Organlarda meydan gelen histopatolojik değişiklikler TAA+ NRL uygulamasından sonra anlamlı olarak değişmiştir.
Çalışma sonucuna göre NRL uygulaması TAA kaynaklı lipit peroksidasyonunu ve oksidatif stres parametrelerinin düzelmesini sağlamıştır. Nerolidol antioksidan savunma sistemini arttırmanın yanı sısa TAA'nın dokularda meydana getirdiği histopatolojik değişiklikler üzerinde anlamlı düzelmeler sağlamıştır. Sonuç olarak çoklu organ hasarı yapan TAA' nın nerolidol gibi bir antioksidan biyoflavonoid ile tedavi edildiği gösterilmiştir.

Anahtar kelimeler: Nerolidol, TAA, oksidatif stres, lipit peroksidasyonu

➢ *ORAL PRESENTATION*

Polyvinylalcohol/polyethyleneimine hydrogels: preparation and swelling kinetic study

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Abstract

Hydrogels are polymeric structures with 3-D network prepared via crosslinking. Hydrogels show unique swelling characteristics due to their high hydrophilic nature and their crosslinking degree. They present reversible phase transitions with environmental conditions such as pH, temperature etc. and thus utilizing them as significant alternatives for various applications. Polyvinylalcohol (PVA) hydrogel with high biocompatibility, high hydrophilicity and good film-forming properties is one of the widely used biopolymer for biomedical applications. Polyethyleneimine (PEI) is a branched polymer with high hydrophilicity of amine groups in its structure. In this work, PVA/PEI hydrogels were prepared via chemical crosslinking using glutaraldehyde. The chemical characterization of the PVA/PEI hydrogels were performed with FTIR measurements. The effects of glutaraldehyde amount, PEI amount and PVA percentage on the swelling characteristics were investigated. The pH-responsivity of the resultant hydrogels was performed using pH 4 and pH 7. The kinetic parameters and the diffusion coefficients of the hydrogels were also calculated.

Keywords: Hydrogel, swelling, polyvinylalcohol, polyethyleneimine

➢ *ORAL PRESENTATION*

Bazı aktif bileşiklerin Enoil-Acp-FAS I-II üzerindeki biyokimyasal inhibisyon etkisi

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Abstract

Son zamanlarda antibiyotik kullanımı hususunda mikroorganizmaların ilaçlara karşı direnç kazanması ve immune sistemin adapta olup savunma sisteminin zayıflaması neticesi olarak sınırlama yapılması gerekliliğini ortaya koymuştur. Günlük hayatta besinlerimizin içinde yer alan ve zaman zamanda ek gıda olarak tüketilen ve geleneksel olarak birçok hastalığın tedavisinde kullanılan geleneksel tıbbi ilaçların içindeki aktif maddelerin bakteriyel yağ asidi sentezinde görev alan en önemli enzimlerden birisi enoil-ACP redüktaz üzerindeki inhibe etkisinin ne derece öneminin farkına varılmalıdır. İnsan sağlığına tehdit eden ve birçok hastalığa neden olan mikroorganizmaların çoğalmasının durdurulması alınan gıdalardaki aktif maddelerin özellikle hücre duvarı sentzine etki eden bu bileşiklerin inhibe etkisinin bilinmesi ile mümkündür ve kısa zamanda maliyeti az olan, doğru, güvenilir sonuçlar veren doking çalışmaları ile burada olduğu gibi bazı aktif bileşiklerin inhibe etkileri karşılaştırılmıştır.

Keywords: Enoil-ACP-FAS I-II, doking

Bu çalışma Kırıkkale Üniversitesi BAP-2020/062 ile desteklenmiştir.

➢ *ORAL PRESENTATION*

Assessment of Adulteration Identification Based on Elucidation and Comparison of Honey and Apis mellifera L. Hypopharyngeal Gland Protein Profiles

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Abstract

Adulteration using Invert Sugar Syrups (ISS) are common at honey fraudulent. β-fructofuranosidase (BFF) enzyme is being used for the cleavage of the sucrose to gain fructose/glucose mixture at ISS production. We thought that differences in proteome profiles of honey samples can indicate the adulteration due to the non-straightforward addition of BFF. α-glucosidase and α-amylase are the most important enzymes in the maturation of honey. The origin of enzymes in honey is attributed to the bee's hypopharyngeal glands (HPG). This study was aiming to develop an effective method to detect ISS adulteration via proteomic approach and to compare the enzyme profiles of authentic and fraudulent honey along with HPG protein extracts. HPG protein profile was investigated for the purpose of validation of natural proteins that should be seen at honey. We also conducted a targeted purification for α-glucosidase and α-amylase for bulk enzyme identification at protein profiles.
As research materials, we collected ISS samples, honey from different origins, and HPG samples. For HPG samples, European honeybees were chosen randomly and anesthetized. The glands were dissected using a binocular microscope. We prepared adultered samples by addition ISS to the authentic samples. Samples were concentrated and cleaned-up using ultrafiltration and centrifugal ultradiafiltration. Extracts were purified by FPLC. The fractions were combined and transferred to gel electrophoresis. In parallel, non-purified bulk extracts were also diluted and loaded. Protein concentrations, specific activities, and sugar contents were measured for keeping the track of the performance at pretreatment steps.
Molecular weights and activities of bulk enzymes from honey and HPG were identical. The enzyme electrophoresis profiles were distinguished from those adultered with the addition of ISS. Adultered samples gave additional protein bands representing BFF and hence ISS addition. Thus, enzyme electrophoresis profiles can be considered in terms of ISS detection.

Keywords: Adulteration, Honey, Protein, Enzyme, SDS-PAGE, Hypopharyngeal

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➢ *ORAL PRESENTATION*

Involvement of endometrial IGF-1R/IGF-1/Bcl-2 pathways in experimental polycystic ovary syndrome: identification of the regulatory effect of melatonin

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Abstract

Introduction: The etiology and pathogenesis of endometrial cancer in Polycystic Ovary Syndrome (PCOS) are based on several reasons, so the underlying mechanisms between endometrial changes, hyperplasia and PCOS have been a subject of research that has not yet been clarified and the use of agents that improve PCOS patients' life quality has become important. The involvement of endometrial IGF-1R/IGF-1/Bcl-2 pathways and the potential regulatory effects of exogenously administrated melatonin on this expression is investigated in the experimental PCOS model in the present study.
Methods: Thirty-two 6-8 week Sprague Dawley rats were divided into four groups: the Sham Control (1% CMC/day by oral gavage [o.g.]); the Melatonin (2 mg/kg/day melatonin by subcutaneously [s.c.]); the Experimental PCOS (1 mg/kg/day Letrozole by o.g.); and the Experimental PCOS+Melatonin (1 mg/kg/day Letrozole by o.g. and 2 mg/kg/day melatonin by s.c.). Vaginal smear samples were taken from the 14th day to the end of the experiment for colpocytological measurements. At the end of the 21 day experimental period, uterine tissues were taken; Hematoxylin-Eosin histochemical, IGF-1R/IGF-1/Bcl-2 immuno-histochemical staining and western blot analyses were performed for related antibodies. All of the data was supported statistically.
Results: The epithelium of endometrium lost its single-layer structure in some parts, seperation was observed between the epithelium and the basal membrane junction, intracellular edema was found in the uterine glands by the polycystic ovary-induction. Also this induction increased the expression of IGF-1R/IGF-1 and Bcl-2 proteins. Morphological degenerations returned to its normal appearance generally by the melatonin administrations and melatonin also regulated the increased expression of endometrial IGF-1R/IGF-1/Bcl-2 pathways.
Conclusion: It is concluded that through further study, using melatonin as a supporting agent may be appropriate in cases of PCOS.

Keywords: Polycystic ovary syndrome, Endometrium, Melatonin, IGF-1R, IGF-1, Bcl-2

Funding: We express thanks to the Independent Scientific Research Projects of Gazi University that supported this study with the project code 01/2017-28.

➢ *ORAL PRESENTATION*

Lithospermum arvense L. bitkisi ve $AgNO_3$ kullanılarak elde edilen gümüş nanopartiküllerinin antimikrobiyal aktivitesi

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Özet

Günümüzde sağlık sorunları giderek artış göstermektedir. Yeni hastalıkların ortaya çıkması ve hastalık yapan mikroorganizmaların mevcut ilaçlara karşı direnç kazanması, tedavi sürecinde yeni etken maddelere olan ihtiyacı artırmaktadır. Bu durum daha gelişmiş yöntemleri gerekli kılmıştır. Nano düzeyde parçacık üretimi bu ihtiyacın karşılanmasında önemli bir yer tutmaktadır. Son yıllarda bitkisel kaynaklı nanopartikül kullanımı ile ilgili birçok çalışma yapılmıştır. Özellikle dezenfeksiyon özelliği nedeniyle nano biyoajan olarak gümüş kullanımı çok fazla tercih edilmiştir. İlaç endüstrisinde etken madde üretim aşamalarından biri de antimikrobiyal aktivite analizleridir. Yapılan çalışmada *Lithospermum arvense* L. bitkisi ve gümüş nitrat kullanılarak Ag NPs/La nano kümeleri elde edilmiştir. Gümüş nanoparçacıklarının bazı patojen mikroorganizmalara karşı antimikrobiyal aktivitesi incelenmiştir. Elde edilen sonuçlara bakıldığında Ag NPs/La yapılarının insanlarda hastalık etmeni olan *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25952, *Staphylococcus aureus* ATTC 29213, *Pseudomonas aeruginosa* ATCC 27853 ve *Candida albicans* ATTC 90028 patojenlerine karşı 9.10 ve 12.20 arasında değişen oranlarda inhibisyon zonu oluşturduğu ve antimikrobiyal etkisinin olduğu görülmüştür.

Anahtar Kelimeler: Nanopartikül, *Lithospermum arvense*, antimikrobiyal, $AgNO_3$

➢ *ORAL PRESENTATION*

Kükürtlü Bileşiklerin Gideriminde Biyoteknolojik Yöntemlerin Uygulanması

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Özet

Dünya çapında başlıca enerji kaynaklarından biri kükürt içeren fosil kökenli yakıtlardır. Bu yakıtların tüketilmesi asit yağmuru oluşumuna ve çevre kirliliğine neden olabilmektedir. Yakıtlardaki kükürtün uzaklaştırılması için ise mikrobiyal desülfürizasyon (biyodesülfürizasyon) olmak üzere çeşitli çalışmalar yürütülmektedir. Bu çalışmada, *Paenibacillus* 32O-Y suşu kullanılarak kükürtlü bir bileşik olan dibenzotiyofenin (DBT) gideriminin arttırılması amaçlanmıştır. Bu kapsamda, *Paenibacillus* suşu kalsiyum aljinat yöntemi ile immobilize edilmiş ve 0.1 mM DBT ortamında 96 saat süre boyunca inkübe edilmiştir. 32O-Y suşunun desülfürizasyon aktivite değeri en fazla 72. saatte belirlenmiştir. İmmobilizasyon ile elde edilen bu verimin, serbest form ile elde edilen desülfürizasyon aktivitesinden %30 daha fazla olduğu bulunmuştur. Elde edilen sonuçlar ile, *Paenibacillus* 32O-Y suşunun desülfürizasyon çalışmalarında kullanılabileceği ve desülfürizasyon veriminin arttırılmasında da immobilizasyon yönteminin kullanılabileceği belirlenmiştir.

Anahtar Kelimeler: Kükürtlü bileşikler, kükürt giderimi, degredasyon, immobilizasyon.

➢ *ORAL PRESENTATION*

Recent combinational therapy approaches targeting DNA damage in breast cancer patients

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Abstract

Breast cancer is attracting enormous attention from clinical scientists and surgeons due to the uncertainty of occurrence and developmental causes of the disease, puzzling combinations of treatment options, and the drastic variations in the individual patient response to the selected therapies. Accurate detection of the causes of breast cancer still presents the most challenging step to research attempts to discover therapies for the treatment of the disease. Excessive or uncontrolled production of reactive oxygen species (ROS) and free radicals in the body cause oxidative stress that ultimately leads to the formation of pathogenic diseases such as cancer. In addition, the extent to which the ROS and free radical formation are controlled in cancer patients determines the fate of the molecular mechanisms triggering the anti-breast tumor drug resistance. In this study, the role of these oxidative stress components and the most advanced treatment options targeting these molecules have been covered in the case of breast cancer. The roles of oxidative stress induced by free radicals and ROS on the initiation, progression, and maintenance of breast tumors have been investigated thoroughly. The current study also details the very exciting therapy approaches that enhance the formation of free radicals and ROS in breast cancer cells. With these targeted therapies, the anti-tumor efficacy of the administered drugs can be enhanced. Furthermore, DNA repair mechanisms in response to the treatment modalities have been fully detailed. The purpose of the current study is to further explore the mono- and combinational-therapy approaches targeting DNA damage in cancer forming cells in breast cancer patients. It is envisioned that the current methodologies and therapies would make a valuable impact on the on-going efforts to prevent the spread of and cure this deadly disease.

Keywords: Breast cancer, Anti-tumor therapies, Free radicals, Reactive oxygen species, DNA damage, Oxidative stress

➢ *ORAL PRESENTATION*

The Utilization of Microalgal Wastes in Mass Production of Medicinal Plants

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Abstract

Biotechnological advancements led to many developments in agriculture and food sector, yet these are insufficient to meet food demands of Global human population. Studies focusing on improving biomass, yield and productivity in agricultural crops has become of great interest for the societies suffering from food shortage. Beyond that, most naturally growing medicinal plants are almost endangered due to mass production. Their tissue culturing practises are thus started to be performed to meet rising demands. *Ocimum basilicum* L.(Common basil) and *Thymus vulgaris* L. are two traditionally essential plants, both having variety of different uses in food industry, cosmetics and medicine. *O.basilicum* is considered as the most commercially important basil herb worldwide. It's essential oil reveals high antioxidant, antimicrobial, antihypertensive, anticancer, and antiinflammatory activities. *T.vulgaris is the key producer of thyme compound displaying* antiseptic, antibiotic, and antifungal properties and widely used to treat cough, diabetes, inflammation, cold and chest infections. The environmentally-friendly mass production of both plants require establishment of optimal growth conditions. *Chlorella vulgaris* (*Cv*) microalga is currently utilized as industrially crucial feedstock for production of biofuels, cosmetics, pharmaceuticals, etc. Wastes from these industries, however, do not have common utilization as biofertilizers. Therefore, we decided to examine the effectiveness of microalgae wastes to improve growth conditions of both medicinal plants towards mass production under chemical fertilizer-free conditions. For this goal, the seed germination and seedling growth promotion tests on both plants were performed upon soil application of two different *Cv* preparations (sonicated biomass and usual microalgae culture, ddH_2O:control). *Cv* waste treated seeds from both plants germinated earlier than control groups. Moreover, soil application of sonicated biomass were more effective than live *Cv* cultures to promote vegetative growth, biomass accumulation and pigmentation in both plants. In conclusion, microalgal wastes from different industrial plants could be utilized as efficient plant growth promoting biofertilizers.

Keywords: *Ocimum basilicum, Thymus vulgaris, microalgal waste, biofertilizer*

➢ *ORAL PRESENTATION*

***Chlorella vulgaris* is a Promising Biofertilizer for *Camellia sinensis* Organic Farming**

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Abstract

Due to rapid growth in world population and decrease in agricultural lands, more food production demand is soaring. Growers and governments thus try to use more chemical fertilizers in agriculture. Although intensive use of chemicals in fields may increase yield and production amounts, yet it diminishes soil fertility, increases soil and drinking water pollutions and minimizes microbial diversity. To address this problem, novel approaches have started seek alternatives to chemical fertilizers such as different wastes and/or plant growth promoting (PGP) microorganisms as biofertilizers. As parallel, organic agriculture as a form of bioproduction aiming to improve the quality of crops through environmentally-friendly practices has emerged. The search for alternative fertilizers that are applicable in organic farming has notified that photosynthetic microalgae could be a favorable candidate. Photosynthetic microalgae are single-celled organisms inhabit in marine and freshwater waters where they naturally perform the fixation of atmospheric CO_2; in return, producing about more than half of atmospheric oxygen. Microalgae cells accumulate high amounts of different organic substances (carbohydrates, proteins, fatty acids) only utilizing sunlight and inorganic substances. We hypothesized that de-oiled microalgae biomass waste (DMBW) from biofuels production could be used in organic farming. Thus, we tested the PGP effects of *Chlorella vulgaris* (Cv:CCALA_211/11J) culture effluents on *Camellia sinensis* production. *Cv* was cultured in 4L bioreactor system and was prepared in 4 different identical forms (ddH_2O-control, cell-free supernatant, sonicated cells in ddH_2O, non-sonicated microalgae culture) before application onto green tea heaps through soil and foliar parts. Upon application, all 4 *Cv* preparations improved shoot development, chlorophyll pigmentation and biomass amount compared to control plants. Furthermore, soil application of sonicated cells-culture medium and non-sonicated microalgae culture revealed the best PGP effects. Current results denote that microalgae wastes, in particular DMBWs could be utilized as highly promising biofertilizers in organic tea farming.

Keywords: *Camellia sinensis, Chlorella vulgaris*, biofertilizer, organic farming

➢ *ORAL PRESENTATION*

Identification of Post-Harvest Candidate Genes by Rna-Seq for Longer Shelf Life in Tomato

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Abstract

Fruit ripening is the part of a complex developmental process which is highly coordinated includes changes in colour, texture, taste and flavour. During ripening, texture of fruit changes, resulting in a decrease in firmness accompanied by an increase of ethylene release and up-regulated expression of genes in climacteric fruits. During ripening and softening, various cell wall modifying enzymes take responsibility in the degradation of pectic polymers. Down-regulation some of ripening related genes resulted in a variety of shelf-life characteristics.
During the process of domestication, the majority of valuable genetic materials has been lost due to several rounds of domestication and intense breeding activities. The natural variation existing in the 12 wild tomato relatives is a great potential source for the improvement of cultivated tomato varieties. In this study, we screened the firmness and shelf-life characteristics of the *Solanum pennellii* ILs, each of IL contains a chromosomal fragment from cultivated tomato *Solanum lycopersicum*, to evaluate the best and worst ILs based on their firmness (measurement of firmness for all ILs) and integrity during storage at room temperature. With the help of RNA-seq technology, additional and novel ripening/softening candidate genes was identified. Some of the selected candidate genes were analysed by qPCR at Br7 and Br14 ripening stages to evaluate the expression trends of the genes during postharvest period.

Keywords: shelf life, postharvest ripening, tomato, cell wall, candidate gene

➢ *ORAL PRESENTATION*

Experimental Investigation of the Effect of Four Different Cortisol Derivatives on Routine Spectrophotometric Biochemical Tests

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Abstract

Objective: Methylprednisolone, triamciolone acetonate, betamethasone disodium phosphate, dexamethasone are routinely used parenteral cortisol derivatives. Taking samples under intensive working conditions in services and intensive care units after the administration of these drugs may affect routine biochemistry tests. The aim of this study was to investigate experimentally the effect of methylprednisolone, triamciolone acetonate, betamethasone disodium phosphate, dexamethasone on albumin, ALP, ALT, calcium, amylase, total cholesterol, creatinine, GGT, glucose, phosphorus, magnesium, total protein, triglyceride, uric acid, urea test results.
Methods: Methylprednisolone, triamciolone acetonate, betamethasone disodium phosphate and dexamethasone were added to the control material. Four separate mixtures were prepared by taking 20µL of each drug solution and 180µL of the control solution. Albumin, ALP, ALT, calcium, amylase, total cholesterol, creatinine, GGT, glucose, phosphorus, magnesium, total protein, triglyceride, uric acid, urea tests were studied in the biochemistry autoanalyzer. The same work was repeated by adding 20uL of distilled water. Deviation amounts were calculated with bias%.
Results: Due to methylprednisolone and betamethasone disodium phosphate, phosphorus concentration was exposured positive interference with a rate of 158.06% and 385.48%, respectively. After adding Triamciolone acetonate, creatinine concentration was affected at rate of -16.31%.
By addition of dexamethasone, negative interference was observed in total cholesterol, triglyceride, uric acid at the rate of -20% -30.61%, -14.46%, respectively. The highest deviation occurred in creatinine due to dexamethasone at the rate of 526.72%. Deviation rates in other tests remained minimal.
Conclusion: This study showed that phosphorus, creatinine, total cholesterol, triglyceride and uric acid concentrations may be affected through cortisol derivatives. The results of these biochemistry tests to be performed after cortisol drug infusions should be evaluated carefully. Blood samples should be taken prior to cortisol injection.

Keywords: Methylprednisolone, triamciolone acetonate, betamethasone disodium phosphate, dexamethasone, creatinine, interference

➢ *ORAL PRESENTATION*

Hepatit B virüs aktivasyonunda *HLA* gen polimorfizmlerinin etkisi

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Özet

Amaç: Hepatit B virüsü (HBV) karaciğerde inflamasyona neden olarak kronik hepatite, karaciğer sirozuna ve karaciğer kanserine neden olabilmektedir. HBV ile enfekte olan bireyler doğal bağışık, inaktif taşıyıcı ya da aktif kronik hepatit B hastası olarak yaşamlarına devam etmektedir. Bazı inaktif taşıyıcılar aktivasyon fazına girerek ilaç kullanmak zorunda kalmaktadır. Kişilerin virüse yanıtındaki bu farklılıkların nedeninin genetik faktörler olduğu birçok çalışmada belirtilmektedir. İnsan lökosit antijen (HLA) proteinleri antijenleri bağlayan ve T hücrelerine sunan proteinlerdir. HLA-DPA1 ve HLA-DPB1 genlerinin 3'-kodlanmayan (UTR) bölgesinde bulunan rs3077 ve rs9277535 polimorfizmlerinin mRNA ekspresyonunu etkilediği, ayrıca post-translasyon sürecinde mikro-RNA-mRNA etkileşimini olumsuz yönde etkilediği buna bağlı olarak HLA moleküllerinin HBV antijenlerini T hücrelerine yeterince tanıtamadığı bildirilmektedir. Bu çalışmanın amacı HLA rs3077 ve rs9277535 polimorfizmlerinin HBV aktivasyonu üzerine etkisinin olup olmadığını araştırmaktı.
Metot: Çalışmaya 123 inaktif HBV taşıyıcı ile 136 aktif kronik hepatit B hastası alınarak HLA genlerindeki rs3077 ve rs9277535 polimorfizmleri gerçek-zamanlı polimeraz zincir reaksiyonu (RT-PCR) yöntemi kullanılarak genotiplendirildi.
Bulgular: HLA geni rs3077 A/G polimorfizmi resesif modelde AA genotipi HBV aktivasyon riskini 1.71 kat artırarak istatistiksel olarak anlamlıydı (P=0.046 OR=1.71 (1.01-2.91)). Ayrıca, overdominant modelde TC genotipi aktivasyona karşı anlamlı bir koruyucu etki gösterdi (P=0.03 OR=0.54 (0.31-0.94)). HLA geni rs9277535 A/G polimorfizminin A allel ve AA genotipi HBV aktivasyon riskini arttırmasına rağmen istatistiksel olarak anlamlı değildi (P=0.22 OR=1.32 (0.85-2.04); P=0.16 OR=1.47 (0.86-2.51)).
Sonuç: Mevcut çalışma, HLA genlerindeki rs3077 ve rs9277535 polimorfizmleri ile HBV aktivasyonu arasındaki ilişkiyi araştıran ilk çalışmadır. HLA-DPA1 geni rs3077 polimorfizminin HBV aktivasyonu üzerinde istatistiksel olarak önemli bir etkiye sahip olduğu görülmektedir. HLA-DPB1 geni rs9277535 polimorfizminin ise riski arttırmasına rağmen istatistiksel olarak etkisinin olmadığı belirlenmiştir. Bu konunun aydınlatılması için daha geniş örneklem büyüklüğü ile çalışmanın tekrar edilmesi gerekmektedir.

Anahtar Kelimeler: HLA gen polimorfizmleri, Hepatit B enfeksiyonu.

➢ *ORAL PRESENTATION*

Effects of carbon tetrachloride and crocin applied to rats on neurotoxicity

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Abstract

The present study aimed to investigate the effects of carbon tetrachloride and crocin, which was considered as a protective agent, on rat brain tissues. This study was conducted after Inonu University ethics committee approval was obtained. Fifty rats were randomly divided into 5 groups with 10 rats each. These groups were Control, Corn oil, Crocin, Carbon tetrachloride, Crocin + Carbon tetrachloride groups. After 2 weeks of administration, rats were decapitated under anesthesia and rat brain tissues were removed and biochemically analyzed. It was determined that there were no differences between the control and corn oil groups. It was observed that malondialdehyde (MDA) and total oxidant status (TOS) levels increased in the carbon tetrachloride group, while catalase (CAT) and total antioxidant status (TAS) levels decreased. In the Crocin group, TAS and CAT levels increased, and MDA and TOS levels decreased when compared to all other groups. In the Crocin + Carbon tetrachloride group, biochemical parameters improved when compared to the Carbon tetrachloride group ($p \leq 0.05$). Crocin was able to exhibit neuroprotective properties against carbon tetrachloride, reportedly a lipophilic and highly toxic substance. Crocin could maintain the antioxidant/oxidant balance by exhibiting a strong antioxidant effect and suppressed oxidative stress formation. During the tested period and dose, crocin may have protective properties against carbon tetrachloride neurotoxicity.

Keywords: Carbon tetrachloride, crocin, brain, oxidative stress, rats

➢ *ORAL PRESENTATION*

The Determination of Reproductive Biology of European Pilchard, *Sardina pilchardus* (Walbaum, 1792) Distributed in The Aegean Sea

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Abstract

In order to examine the reproduction biology of the species, the specimens were obtained in the known spawning period indicated by the current literature obtained from fishers who were commercially fishing in December 2018 and January-February 2019. After the total length together with their weight measurements, the specimens were dissected in the laboratory. The adult females' gonads were then fixed in 10% formalin solution for fecundity. A total of 53 individuals was examined and the values of total length and weight were computed as 11-14 cm (mean: 12.57±0.70), 9.04-22.72 g (mean: 14.33±3.0), respectively. 26 of the supplied specimens were male (49.06%), 27 female (50.94%), thus, the female: male ratio was determined as 1:0.96. According to χ^2 test results, a statistically no significant difference was observed among individuals. For the specimens examined, the total length-weight relationship was calculated as $W = 0.0013L^{3.67}$ (R^2=0.92). In order to calculate the fecundity, sub-samples were taken from the anterior, median and posterior parts of 11 ovaries in the ration of 2 - 5% of the ovary weight and the mature oocytes were counted. As a result, the species' fecundity was found to be between 108-341 (175.45±66.92). The relationship between total length and fecundity was computed as W= 62.154L-607.69 (R^2=0.75) and a linear relationship was determined.

Keywords: European Pilchard, *Sardina pilchardus*, Reproduction, Aegean Sea.

➢ *ORAL PRESENTATION*

Protective Effects of Beta Glucan on Oxidative Damage Caused by 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in the Brain

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Abstract

Aim: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a common environmental contaminant that causes serious toxic effects. TCDD toxic effects on some tissues such as heart, kidney, liver, and brain are mediated by oxidative stress. Beta-glucan is a powerful antioxidant that has beneficial health effects. Beta glucans are commonly found in cellulose of plants, bran in cereal seeds, cell wall of sourdough, some fungi and bacteria. Beta glucan fibers obtained from oat or bread yeast, is water-soluble and derived from the inner cell wall. We investigated the impact of Beta-glucan on brain tissue of rats with TCDD induced toxicity.
Methods: 32 Sprague-Dawley female rats of 8-12 weeks old and weigh 190-250 gr were divided into four groups. (a) Control, (b) TCDD was administered via gavage a dose of 2µg/kg/week for three weeks. (c) Beta-glucan was given at a dose of 50 mg/kg/day for three weeks via gavage. (d) TCDD and Beta-glucan treated group were assigned 2 µg/kg/week TCDD and 50 mg/kg/day Beta-glucan for three weeks. TBARS (Thiobarbituric acid reactive substances), SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase) and GSH (reduced glutathione) levels were determined spectrophotometrically. Hemotoxilen-eozin staining was performed for histopathological analysis.
Results: The results were shown that TBARS levels, which is a marker of oxidative stress in the TCDD treated group, increased significantly compared to the other groups. Furthermore GSH, SOD, CAT, and GSH-Px levels decreased in the TCDD group. However, GSH, SOD, CAT, GSH-Px levels increased in the group treated with Beta-glucan while TBARS levels decreased. Histopathological data were convenient with the biochemical results.
Conclusion: Oxidative stress and histopathological changes caused by TCDD were improved with Beta-glucan treatment. Beta-glucan can prevent brain toxicity caused by TCDD. The results of the present study show that beta-glucan can be considered as an alternative anti-TCDD toxicity agent.

Keywords: TCDD, Beta glucan, Oxidative damage

➢ *ORAL PRESENTATION*

Biyolojik delme işleminin ladin diri ve olgun odununun kimyasal özellikleri üzerine etkisi

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Özet

Bu çalışmada ülkemizin Doğu Karadeniz Bölgesinde yayılış gösteren ve permeabilitesi düşük ağaç türlerinden biri olan Doğu ladini (*Picea orientalis* L.) odununun emprenye edilebilirliğini artırmak amacıyla uygulanan ve biyoteknolojik yöntemlerden biri olan biyolojik delme (bio-incising) işleminin odunun kimyasal özellikleri üzerindeki etkileri incelenmiştir. Bu kapsamda Doğu ladini diri ve olgun odun kısımlarından elde edilen odun örneklerine (10 x 2,5 x 2,5 cm) farklı ağırlık kayıpları (%5-10, %10-15, %20 ve %30) elde edilecek şekilde biyolojik delme işlemi uygulanmıştır. Bu uygulama neticesinde bir beyaz çürüklük mantarı olan *Physisporinus vitreus*'un ladin diri ve olgun odun kısımlarının kimyasal özellikleri üzerindeki etkilerini anlayabilmek için FTIR-ATR (Fourier transform infrared spectroscopy) analizi gerçekleştirilmiştir. Numaralandırma parmak izi bölgesi olarak adlandırılan ve odundaki karakteristik piklerin bulunduğu bölge olan 1800 cm^{-1} ile 650 cm^{-1} aralığı referans alınarak yapılmıştır. Bir beyaz çürüklük mantarı olan *P. vitreus* ile biyolojik delme işlemi uygulanan diri odunda ağırlık kayıplarının % 5 düzeyinde gerçekleştiği örneklerde bozunmaların ihmal edilecek kadar az olduğu, % 10 ağırlık kaybının gerçekleştiği örneklerde ise piklerde deformasyonların olduğu ama asıl önemli değişikliklerin % 20 ve üzeri ağırlık kaybına uğrayan örneklerde meydana geldiği görülmüştür. Olgun odunda FTIR spektrumlarına bakıldığında yine en büyük değişikliklerin % 30 ağırlık kaybına uğrayan örneklerde olduğu görülmektedir. Bu durumu % 20 ağırlık kaybının meydana geldiği örnekler izlemektedir. Özellikle % 5 ve % 10 ağırlık kayıplarının meydana geldiği örneklerde pik absorbanslarında düşüşler ya da ortadan kalkmalar yerine piklerin sağa ya da sola kayarak yer değiştirdikleri gözlenmektedir. Bu da odunda tüm odun polimerlerinin % 10 ağırlık kaybına kadar kendini koruduğunu, ağır ve şiddetli bozunmaların ise % 20 ağırlık kaybı ve sonrasında meydana geldiğini göstermektedir.

Anahtar Kelimeler: Doğu ladini, Permeabilite, Biyolojik delme, FTIR-ATR.

➢ *ORAL PRESENTATION*

Optimization of Calcium Carbonate Crystallization in the presence of Threonine Using Response Surface Methodology

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Abstract

This objective of this study was to investigate the effect of threonine as a crystal modifier on $CaCO_3$ crystallization. The structure, functional groups, crystal morphology, and size of the crystals were investigated experimentally using X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy, and a particle size analyzer. The characterization results show that $CaCO_3$ crystals obtained in pure media were in the form of calcite structure with cubic shape, however, the smaller ellipsoidal vaterite crystals were created in the presence of the additive media. Moreover, central composite design (CCD) with response surface methodology was used to determine how temperature, stirring rate, and threonine concentration influenced the $CaCO_3$ crystals in terms of the amount of vaterite produced and particle size in this study. The results indicate that the data sufficiently fit the second-order polynomial model. Among the three investigated variables, threonine concentration was the most efficient for creating small sized vaterite crystals. Consequently, the detailed information about the characterization and optimization could provide a reference for the $CaCO_3$ crystallization for scientific and industrial purposes.

Keywords: Calcium carbonate, optimization, crystallization, central composite design.

Acknowledgement: Authors would like to acknowledge the Marmara University Scientific Research Projects Commission for financial support. (Project number: FYL-2020-10025)

➢ *ORAL PRESENTATION*

Colorimetric method for ovalbumin detection using a new magnetic nanozyme

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Abstract

A new magnetic nanozyme was developed for colorimetric determination of glycoproteins. Ovalbumin (OVA) was selected as a model glycoprotein. Peroxidase like activity of 4-Mercaptophenylboronic acid (MPBA) immobilized-Au nanoparticle attached-silica shell coated magnetic SiO_2 (MPBA@Au@SiO_2@MagSiO_2) microspheres was investigated to determine OVA concentration in buffer media. O-phenylenediamine (OPDA) was used as a synthetic substrate. The pseudo-specific interaction for the target molecule (i.e. OVA) occurred via the reversible borate ester formation reaction between the cis-diol groups of glycoprotein and phenylboronic acid (PBA) groups linked to microspheres, based on borate affinity chromatography. Magnetic and monodisperse-porous silica microspheres were synthesized 6.5 μm in size, by "staged sol-gel templating protocol". Au nanoparticles (Au NPs) obtained by Turkevich method were attached to magnetic, monodisperse-porous silica microspheres (SiO_2@MagSiO_2). MPBA was directly attached to Au NPs immobilized on the magnetic nanozyme. The colorimetric response (measured at 416 nm) is linear in the OVA concentration range of 0.1-20 μg/mL. Maximum substrate consumption rate and Km were determined as 19.9 μM/min and 207.7 μM, respectively, according to Michaelis-Menten model. In this study, the new, selective and easily separable magnetic nanozyme may be useful for the colorimetric detection of glycoproteins.

Keywords: Colorimetric detection, peroxidase like activity, nanozyme

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➢ *ORAL PRESENTATION*

Computational Design of Doped Metal Oxide Nanoarticles with Antimicrobial Activities

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Abstract

Microbial contamination on inanimate objects and surfaces, particularly in public places (hospitals, schools, public transport, etc.), is an important problem that leads to the spread of bacterial and viral diseases. Surface disinfection with chlorine or other chemical liquids is inadequate due to the difficulty of systematic application. Coating materials with inherent antimicrobial properties, i.e. with long-term bactericidal, virucidal, fungicidal effects, are emerging as a need in the health sector. These materials should also be cheap, broad-spectrum to be applicable to hospitals and fast effective to avoid resistance development in microbes.
The antibacterial / viral effect of molybdenum oxide nanoparticles is reported previously. Antimicrobial effect of molybdenum oxide is due to the creation of an acidic environment under the presence of water. pH sensitivity is not microbe-specific; It is known that the majority of microorganisms die at pH 3.5-4.0. So, by tuning the acidic properties of molybdenum oxide, it may be possible to obtain a cost-efficient material suitable for coating applications in highly contaminated surfaces such as hospitals. In nanochemistry, it is common to manipulate the physical and chemical properties of materials by doping with a second material. Here, we tested metals that were reported to have antimicrobial effects in the literature, such as Ag,Cu,Pt, etc. as dopants to molybdenum oxide and evaluated the doping-induced changes in acidity.
The scanning of materials to identify the best dopant for molybdenum oxide was performed in the »virtual laboratory«, e.g. using computational modelling. The acidity of nanoparticles at different compositions were calculated with density functionals. The most promising candidate materials found in this way will be synthesized and used in antimicrobial experiments in the future. This integrated experimental–theoretical approach increases the possibility of technical success while minimizing the time, raw material and workforce requirements.

Keywords: antibacterial, nanoparticles, computational modelling

➢ *ORAL PRESENTATION*

Evaluation of the catalytic and antimicrobial effects of greenly synthesized silver nanoparticle decorated magnetic nanoparticles

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Abstract

In this study, magnetic nanoparticles decorated with silver nanoparticles were synthesized using completely biocompatible and naturalistic methods and their catalytic and antimicrobial effects were investigated. The effects of harmful chemicals released into the nature in synthesis processes have recently become more evident. For this reason, scientists have focused on green synthesis methods with which they can achieve the same performance. For this purpose, magnetic Fe_3O_4 nanoparticles (MNP) were synthesized using *Camellia sinensis* extract in this study. Whether these nanoparticles are magnetic or not was determined by examining their behavior under external magnetic field. Then, these nanoparticles were coated with stabilizing and reducing polyphenols contained in *Rosmarinus officinalis* extract. MNPs treated with *R. officinalis* extract were coated with silver nanoparticles (Ag NP) after drying. As a result, , field emission scanning electron microscope (FESEM), energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction analysis (XRD), and FTIR analyzes were recorded for each stage for the structural characterization of the MNP @ PF @ AgNP hybrid nanostructure obtained. After the hybrid nanostructure was found to be synthesized successfully, its catalytic effect on 4- Nitrophenol removal as a heterogeneous catalyst was examined using UV-VIS spectrophotometer and it was found that it had a good catalytic effect. Finally, the antimicrobial effect of these nanostructures has been examined for *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* bacteria and indicated its antibacterial activities against gram negative *E. coli* bacteria and gram positive *S. aureus*.

Keywords: Magnetic nanoparticles, *Rosmarinus officinalis,* Silver, 4-Nitrophenol, Antibacterial.

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➢ *ORAL PRESENTATION*

An electrochemical Carcino Embriyonic Antigen immunosensor based on titanium IV oxide /polyacrylonitrile nanofibers modified carbon electrodes

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Abstract

An electrochemical immunosensor for the determination of Carcinoembryonic antigen (CEA) was presented. The titanium(IV) oxide nanoparticle (TiO_2np) loaded polyacrylonitrile nanofibers (PANnf) were prepared by electrospinning at the surface of the discharged battery coal electrode (DBC) and loaded with CEA antibodies (Anti-CEA) as CEA receptor. Fabrication steps were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) in the presence of $[Fe(CN)_6]^{3-/4-}$ probe. PANnf and TiO_2nps exhibited a very fine network for immunosensing. The DBC/PANnf+TiO_2np/Anti-CEA immunosensor exhibited high sensitivity toward CEA biomarker in the low concentration range 0.01–10 ng mL^{-1}, with the detection limit of 0.01 ng mL^{-1} and relative standard deviation of 4.51 (n=4). The results indicated that even very small changes in CEA concentration can be sensed with the presented system. Also, recovery of the immunosensor was found as %99.42±1.41 in the real sera samples. It has great potential in the clinical screening of divergent cancer biomarkers.

Keywords: Carcinoembryonic antigen**,** Titanium (IV) oxide, Polyacrylonitrile, Immunosensor, Electrospinning, Electrochemistry.

➢ *ORAL PRESENTATION*

Escherichia Coli ATCC 25922 suşunun tükürük içerisinde QCM sensör ile direkt tespiti

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Özet

Escherichia coli (*E. coli*), insan intestinal mikroflorasının doğal bir üyesi olan gram negatif bir bakteridir. Geniş ve çeşitli bir bakteri grubunun alt grubunda bulunan E.coli suşlarının bazıları zararsız olsa da bazı suşları hastalığa neden olabilmektedir. Patojenik bakterilerin erken teşhisi uygun tedavi seçimi ve hızlı tedavi uygulanabilmesi adına büyük öneme sahiptir. Bu nedenle, patojenik E. coli suşlarını, bulaştırma ortamlarından biri olan tükürük içerisinde tespit etmek için hızlı, basit ve hassas yöntemler geliştirilmesi önem arz etmektedir. Kuvars kristal mikro terazi (QCM) tabanlı biyosensör, konseptindeki basitliği, kullanım kolaylığı, düşük maliyeti, daha kısa analiz süresi ve etiketsiz ölçüme uygunluğu nedeniyle patojenlerin ve toksinlerin hızlı tespiti için yeni bir immünolojik tespit yöntemi sunmaktadır. Ayrıca ölçüm yönteminin basit ve kolay adımlar içermesi nedeniyle ek bir avantaja sahiptir. QCM, sensör yüzeyine bağlanan bir kütle nedeniyle oluşan rezonans frekansındaki değişim prensibi ile çalışan bir biyosensördür. Ek olarak, çalışma ortamı olan tükürük, invaziv olmayan numune alma metotları ile kolay ve ucuz bir şekilde elde edilebildiği için en ideal tanı ortamlarından biridir. Bu çalışmada,tükürük içerisinde Kuvars Kristal Mikroterazi (QCM) sensör ile Escherichia coli ATCC 25922 suşunun direkt tespiti gerçekleştirilmiştir. Ölçüm sistemi E. coli bakterisi, anti E. coli antikor ab25823 ve QCM sensörden oluşmaktadır. QCM sensör yüzeyinin modifikasyonu, 11-MUA (11-Mercaptoundecanoic acid) kendiliğinden oluşan tek katman (SAM) ile gerçekleştirildi. Bu aşamadan sonra, EDC/NHS (N-(3-Dimethylaminopropyl)-N′- ethylcarbodiimide hydrochloride) / (N-Hydroxysuccinimide), MUA molekülündeki karboksilat grubu ile anti-antide amin grubu arasında bir amid bağı oluşumuna aracılık etmek için kullanıldı. QCM sensör yüzeyi, spesifik olmayan bağlanmayı önlemek için BSA (Bovine Serum Albumin) bloke edici proteine maruz bırakıldı ve yüzey doyuruldu. Son olarak, gerçek tükürük numunesine 10^7 CFU/mL konsantrasyonunda eklenen E. coli ile QCM sensörün ortalama rezonans kayması –120 (±46) Hz olacak şekilde sonuç vermesi sağlandı. İleride yapılacak çalışmalarda, gerçek tükürükteki Escherichia coli bakterisi üç farklı konsantrasyonda(10^7, 10^6 ve 10^4 CFU,/mL) en az 3 tekralı uygulanarak tespit sisteminin ölçüm sığası belirlenecektir.

Anahtar Kelimeler: QCM; Biyosensör; Bakteri Tespiti; Tükürük

➢ *ORAL PRESENTATION*

Antimicrobial activity of different concentration of PdCoAg/C nanoparticles.

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Abstract

There has been an increasing trend towards nanocompozite material in research and development. They are superior to bulk materials and have become very interesting because they have outstanding properties Chaturvedi and et al., (2012). As the size of the nanoparticles decreases, the ratio of the surface to the volume increases and this is a real advantage to wide usage area in new technologies such as electronics, materials science and nanomedicine. Bar et. al., (2009). For example, silver nanoparticles are used for anti-microbial applications Liangpeng et al. (2014). The properties of the metal nanoparticles do not resemble the characteristics of the bulk structure, thanks to these new features, metal nanoparticles, especially silver nanoparticles,
In this paper, different concentration of PdCoAg/C nanoparticles were used to find antifungal activities. Morphological characterization of these particles to be used in experimental studies was determined using Scanning Electronic Microscope (SEM). Antifungal activities were observed against *C. albican* In addition the inhibitions zone's diameter of nanostructures was obtained. It was determined that PdCoAg/C nanoparticles has effective antifungal activity for C. Albican. As a result this feature of the relevant was introduced into the literature as a antifungal activity and it would have an important place in the practical applications of medical, food and etc. fields.

Keywords: Antifungal activity; Nanotechnology; Silver-nanoparticles

➢ *ORAL PRESENTATION*

Meme kanseri hücre hatları üzerine *Ecballium elaterium* meyve özsuyunun sitotoksik etkisinin araştırılması

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Özet

Çağımızın en önemli hastalıklarından olan kanser türlerine karşı tedavi yöntemleri ve bu yöntemler arasında bitkisel kökenli kimyasallarla tedavi olanaklarının araştırılması güncel bir konudur. Her yöntemin kendine özgü avantaj ve dezavantajlarının bulunması, kanserin kişiye özgü bir hastalık olması, tedavilerin kişiden kişiye farklılık gösterebilmesi nedeniyle kesin bir tedavi yönteminin varlığından bahsetmek imkansızdır.
Bitkiler antineoplastik özelliği bilinen birçok ilacın ham maddesini oluşturmaktadırlar. Bitki özütlerinin bu yönüyle araştırılmaları konusunda ülkemizde büyük bir açık bulunmaktadır. Türkiye'de de kolaylıkla bulunabilen *Ecbalium elaterium,* cucurbitaceae familyasına ait otsu bir bitkidir. Birçok biyolojik ve farmakolojik aktiviteye sahip olan cucurbitasinlerin bazı türlerinin anti-kanser aktivitesine sahip olduğu düşünülmektedir.
Diğer taraftan meme kanseri, kadınlarda en sık görülen kanser tipidir. Sık görülmesi, sıklığının giderek artması, erken evrelerde teşhis ve tedavi edilebilir olması, meme kanserinin önemini daha da arttırmaktadır.
Bu çalışmada; tedavi amacıyla kullanılmak üzere *Ecballium elaterium* meyve öz suyunun, farklı konsantrasyonlarının meme kanseri MDA-MB-231 hücre hatları üzerine sitotoksik etkisi araştırıldı. Bitkinin olgunlaşmış meyve öz sularının kullanıldığı çalışmada, meme kanseri hücre kültürlerinde büyütülen MDA-MB-231 hücre hatlarına sitotoksisite analizi yapıldı. Sitotoksisite analizleri için XTT cell proliferation kiti (BioInd, USA) kullanıldı. Çalışma yöntemi, üretici firmanın prosedürü doğrultusunda gerçekleştirildi. Deneysel prosedürler 3 kere tekrarlandı. Elde edilen sonuçlara göre bitki öz suyunun kanser hücrelerinin %50'sini (LD50-Lethal Dose 50) öldüren dozu 98 µg/ml olarak bulundu.
Bu çalışmada; *Ecballium elaterium*'un meme kanseri MDA-MB-231 hücre hatları üzerine umut vaat edecek düzeyde sitotoksik aktivite gösterdiği tespit edildi. Daha ileri çalışmalar için başlangıç niteliğindeki bu çalışma, *Ecballium elaterium*'un anti kanser ajanı olarak bir potansiyele sahip olduğunu ortaya koymaktadır. Anti kanser ilaç geliştirmede, *Ecballium elaterium*'un moleküler etki mekanizmalarının daha ayrıntılı araştırılması gerekmektedir. Böylelikle, meme kanseri tedavisi için yeni moleküler hedeflerin tanımlanmasına olanak sağlanmış olacaktır.

Anahtar Kelimeler: *Ecballium elaterium*, meme kanseri, MDA-MB-231 hücre hatları, sitotoksisite analizi

➢ *ORAL PRESENTATION*

Determination of the effects of phenylalanine on phenolic compound production and antioxidant activity in basil callus culture

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Abstract

Callus culture is a biotechnological method suitable for the production of valuable plant secondary metabolites used in many sectors such as medicine, food and cosmetics. Phenolic compounds constitute an important part of plant secondary metabolites and have many biological activities such as antioxidant, antimicrobial and anticancer. The production of these valuable compounds with callus culture is extensively investigated and many different strategies are applied to increase metabolite yield. The aim of this study was to determine the effect of phenylalanine application, which is the precursor of phenolic compounds, on total phenolic compound content, total flavonoid content and antioxidant activity in basil (*Occimum basilicum* L.) callus culture. The calluses were obtained from hypocotyl explants in a nutrient medium containing 4,4 g/L MS (Murashige and Skoog), 2 mg/L naphthaleneacetic acid, 0,25 mg/L benzylaminopurine, 30 g/L sucrose and 2 g/L phytagel. The calluses were subcultured for 10 days on a nutrient medium containing 0, 5, 10 and 50 mg/L phenylalanine, then harvested and their antioxidant activity and metabolite contents were analysed. Total phenolic and flavonoid contents were determined by Folin-Ciocalteu and aluminium chloride methods, respectively. The antioxidant activity of the callus was evaluated by measuring free radical scavenging activity (DPPH), cation radical scavenging activity (ABTS) and ferric reducing antioxidant power (FRAP). Total phenolic compound content increases with an increase in phenylalanine concentration and the highest phenolic compound content was determined as 2,12 mg gallic acid equivalent /g FW (fresh weight) in callus treated with 50 mg/L phenylalanine. Flavonoid content of 5 and 10 mg/L phenylalanine applied callus was lower than the control group. The highest flavonoid content was determined as 0.224 mg quercetin equivalent /g FW in callus treated with 50 mg/L phenylalanine. According to DPPH, ABTS and FRAP methods, the highest antioxidant activity was determined in callus treated with 50 mg/L phenylalanine. As a result, the increase in phenylalanine concentration increased the phenolic compound content and antioxidant activity in basil callus culture.

Keywords: Antioxidant activity, basil, callus culture, phenylalanine, phenolic compound

➢ *ORAL PRESENTATION*

A novel microheater integrated microelectrode enhances the sensitivity of conventional electrochemical nucleic acid biosensors

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Abstract

Electrochemical Impedance Spectroscopy (EIS) is highly sensitive tool and mostly used in biosensors [1] and its performance enhance localised heating. The aim of the study was therefore to develop a microheater surrounding Au-made electrodes and sensing the genes of *Mycobacterium tuberculosis.* Design of the chip was performed by AUTOCAD Software (Figure 1a) and heat transfer was simulated by using COMSOL Multiphysics (Figure 1b). The chip was fabricated via thermal evaporation (Figure 1c). The concept of the biosensor is based on self-assembled monolayer (probe DNA) formed on the electrode surface, its interaction with a target DNA (heat (50 °C) applied with microheater) and thereafter EIS detection which measures charge transfer resistance (Rct) upon DNA hybridisation. Therefore, the Rct values increased about 160% as opposed to non-heating experiments. We conclude from this the microheater enhances the efficiency of the sensor and paves the way for the development of highly-sensitive integrated label-free biosensors.

Keywords: Electrochemical impedance spectroscopy, nucleic acid biosensor, microheater, gold electrode, *Mycobacterium tuberculosis*

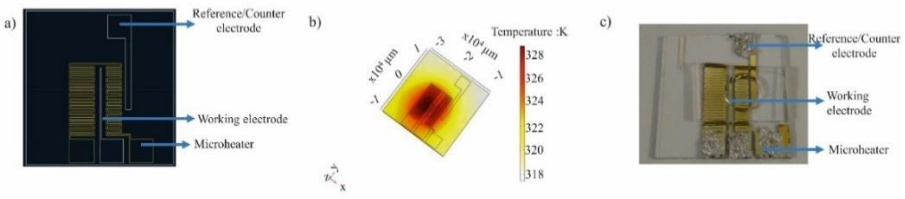

Figure 1 (a) Design of chip by using AUTOCAD Software, (b) Heating simulation of microheater at 50°C by using COMSOL Multiphysics, (c) a figure of fabricated chip.

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➢ *ORAL PRESENTATION*

Genetic diversity and population structure of Upland (*Gossypium hirsutum* l.) cotton germplasm through SSR markers

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Abstract

Cotton is a very important crop cultivated under biotic and abiotic stress conditions worldwide. To avoid the harmful effects of chemicals used to combat these stresses, it is the best way to develop tolerant or resistant varieties in plant breeding programs. In the present study, genetic diversity 17 upland cotton varieties were analyzed with 20 SSR primers, and their population structure was examined. As a result of the amplification, a total of 55 scorable bands were obtained. A total of 80% of the alleles were found to be polymorphic, and an average of 2.2 alleles were determined per locus. The mean PIC value of the markers was measured to be 0.4655. The Genetic Distance (GD) values of the markers varied between 0.0 and 0.49. The highest GD values were between Sure Grow 96 and Carmen, Sealand-542 and Siokra ¼, and between Sphinx V and Stoneville-453 cultivars. As a result, the genetically far distant genotypes may be used in the MAS as parents for breeding programs for the desired purpose after their other characteristics are taken into consideration.

Keywords: Genetic diversity, SSR, Upland cotton, PIC, MAS

➢ *ORAL PRESENTATION*

Sekanslama Yoluyla Genotipleme (GBS) ile İlişkilendirme Haritalaması Yapılarak Pamukta Verticillium solgunluk hastalığına dayanıklılık ile ilişkili DNA markörlerinin belirlenmesi

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Özet

Pamuk (*Gossypium spp*), Gossypiyum Cinsi, Malva-ceae (Ebegümecigiller) familyasındandır ve dünyanın en iyi doğal bitkisel lif kaynağı olarak dünyada kullanılan lifin %35'ini tek başına karşılamaktadır. Pamuk bitkisi geniş bir istihdam olanağı yaratması açısından önemli bir endüstri bitkisidir. Dünya çapında yaklaşık 20 milyon çiftçi pamuğun yetiştiriciliğini yapmakta ve yaklaşık 180 milyon insan geçimini pamuktan sağlamaktadır. Pamukta verim ve kaliteyi negatif yönde etkileyen 20 kadar hastalık ve zararlı arasında en tahripkâr olanı *Verticillium dahliae* Kleb.' in neden olduğu Verticillium solgunluğu hastalığıdır. Toprak kökenli olan *Verticillium dahliae* Kleb. fungusu patojeninin neden olduğu solgunluk hastalığı 40 familyadan 400'den fazla bitki türüne bulaşabilmektedir. Etkin ve ekonomik bir mücadelesi bulunmayan bu hastalığın kontrolünde, hastalığa karşı dayanıklı/tolerant çeşit geliştirmenin en etkili yol olduğu bilinmektedir. Bunun için hastalığın genetik mekanizmasının bilinmesi gerekir. Patojenin yaprak döken (T-1) ile yaprak dökmeyen (SS-4) patotipleri yapay inoküle edilerek ve tarla koşullarında hastalığın pamuk bitkisindeki reaksiyonlarının %50-60 koza açımı döneminde yapraklarda görülen sararma, kloroz, nekroz (0-5 scalasına göre) ile gövde kesitinde ksilemin tıkanma durumuna göre (0-4 scalasına) alınan verilerin hastalık şiddeti ortalamaları hesaplanır ve JMP 7.0 istatisik programıyla varyans analizi yapılır. Pamuğun Verticillium solgunluk hastalığı ile DNA markör arasındaki ilişki ilişkilendirme haritalaması (association mapping) (AM) TASSEL 5.0 programında belirlenir. Bu amaçla öncelikle GBS metodu sonucu elde edilen SNP markörlerinin minör allel frekansı 0.05'den küçük olanlar (MAF<0.05) silinir ve analiz yapılır. Filtrelemeden sonra TASSEL programında bağlantı denksizliği (LD: linkage disequilibrium), STRUCTURE programında popülasyonun genetik yapısını temsil eden Q matrix verileri hesaplanır. Fenotip, genotip dataları ve Qmatriks dataları kullanılarak TASSEL programında genel linear modelde (GLM) ilişkilendirme yapılır. Genel linear modelin (GLM) doğrulaması olan karışık linear modelde (MLM) ilişkilendirmede fenotip, genotip, Qmatriks ve kinship değerleri kullanılır. Karakter-lokus arasındaki ilişki belirlenmeye çalışılırken LOD scoru ≥3, P değeri ≤0.001 ile r^2 değeri ≤0.1 olan markörler hastalığa karşı dayanıklılıkla güçlü bir ilişkisi olan markörler olarak belirlenmiş olacaktır.

Anahtar Kelimeler: Association mapping (AM), Sekanslama yoluyla genotipleme (GBS), Pamuk, *Verticillium dahliae* Kleb., Tek nükleotit farklılığı (SNP), Markör

➢ *ORAL PRESENTATION*

Benzoin based diimine molecule and its mononuclear Cu(II) and Mn(II) complexes: Synthesis, characterization, catalase-like and catecholase-like enzymatic activities

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Abstract

One of the most important factors in the development of coordination chemistry is the activities of Schiff bases and their complexes. For many years, a wide variety of Schiff bases have been synthesized and their metal complexes have been obtained. In recent years, important activities of synthesized ligands and complexes in different areas have been investigated. One of the important activities shown by the synthesized Schiff base metal complexes is enzyme activity [1].
In this study, a novel diimine ligand and its mononuclear Cu(II) and Mn(II) complexes were synthesized and characterized by different physical techniques. Elemental analysis, ICP-OES, FT-IR, UV-vis, molar conductivity, magnetic moment measurements and thermal analysis studies were used for the characterization of the complexes. The free ligand was also characterized by ^{1}H- and ^{13}C-NMR spectra. Elemental analyses, stoichiometric and spectroscopic data indicated that the metal:ligand ratio of the synthesized metal complexes was found to be 1:1. Furthermore the synthesized complexes were tested as catalyst for the disproportionation of hydrogen peroxide to the water and molecular oxygen in the presence of 1-methylimidazole (catalase-like activity) and catalytic oxidation of 3,5-di-tert-butylcatechol to 3,5-di-tert-butylquinone at aerobic medium (catecholase-like activity) [2,3]. The synthesized complexes showed good levels of both catalytic activities. Besides, it was concluded that the Mn(II) complex had better activity in the selected enzymatic reactions.

Keywords: Benzoin, Schiff base, complex, catalase-like activity, catecholase-like activity.

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➢ *ORAL PRESENTATION*

Peptide theraupetics from microalgal sources as versatile bio-factories for mining novel theraupetic compounds

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Abstract

Peptide therapeutics are novel emerging sources to design various bioactive pharmaceutical fine chemicals and feedstocks. Peptides can be incorporated into therapeutic agent, biosensor applications and as a growth factor for tissue engineering constructs. They can be mediators to activate certain signalling pathways to control metabolic regulation and act as a transducer in bio/mechanical stimuli. Thus, peptide therapeutics are emerging as versatile mediators for the regulation of vital bio-signals in living organisms. Even though customized chemical synthesis of certain short sequence oligopeptides is available in the market, biological sources has tremendous machineries to produce natural and unnatural peptides which could be evaluated in food, cosmeceutical and pharmaceutical industries. Mimicking the peptide production strategies can be challenging via synthetic paths. In this case, it is certain that there is a great requirement to obtain sustainable peptide production from natural sources. Microalgae and blue-green algae (cyanobacteria) species are emerging aquatic sources, promising novel peptides through their non-ribosomal peptide synthesis (NRPS) machineries and high protein content later to be applied *in vitro* enzymatic hydrolysis for obtaining bioactive peptides from bulk hydrolysis. In this study, common microalgae and cyanobacteria species are evaluated by their protein content and potential bioactive peptides via enzymatic hydrolysis. Besides chemical and biological methods to mine peptides, novel methods as computational design of peptides with susceptible bioactivities using in silico tools and screening resources. With these techniques and combinatorial approaches, novel peptides showing anthelmintic, anticancer, antioxidant, immunostimulant and melanogenesis regulatory properties can be defined and selected.

Keywords: microalgae, peptide, bioactive peptides, bio-mining, pharmaceuticals, theraupetics

➢ *ORAL PRESENTATION*

Yüksek *DNAJC9* ifadesi meme kanserinde olumsuz sağ kalım sonucu ile ilişkilidir

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Özet

Meme kanseri, dünyada kadınlar arasında insidansı en yüksek kanser türüdür. Heterojen ve kemo-direnç mekanizmalarına sahip bir kanser olması sebebiyle, meme kanseri tedavisine, tanısına ve prognozuna yönelik hedeflerin belirlenmesi hala önemlidir. Isı şoku proteinleri (HSP) son yıllarda sıklıkla kanserlerle ilişkili gösterilmektedir ve birçoğu biyobelirteç ve anti-kanser ilaç hedefi olarak önerilmiştir. DNAJC9, HSP ailesi üyesidir ve önceki çalışmalarda DNAJC9 ifadesi kanserleşme ve radyoterapi direnci ile ilişkili bulunmuştur. Bu çalışmada, UALCAN, KM-plotter ve Sanger-COSMIC in siliko araçlarını kullanarak, DNAJC9 geninin meme kanseri klinik örneklerindeki ifadesi, sağ-kalım değerleri, promotor metilasyon düzeyi ve genetik alterasyonları incelenmiştir. DNAJC9 mRNA ifadesi, meme kanseri klinik örneklerinde (n=1097), normallere (n=114) kıyasla anlamlı olarak daha yüksek bulunmuştur (*P*<1e-12). KM-plotter analizi sonuçlarına göre, *DNAJC9* mRNA ifadesi yüksek olan meme kanseri hastalarında tam sağ kalım (OS), nüksetmeden sağ kalım (RFS), uzak metastazsız sağ kalım (DMFS) ve post-progresyon sağ kalım (PPS) oranları anlamlı olarak düşüktür (sırasıyla; *P*=1.5e-07, *P*<1e-16, *P*=9.2e-07, *P*=0.00014). UALCAN in siliko aracı, DNAJC9 promotorunun normal (n=97) ve kanser (n=793) örneklerinde hipometile olduğunu göstermiştir (beta value<0.25). SANGER-COSMIC veri tabanı verilerine göre, meme kanserinde *DNAJC9* genetik alterasyonları (nokta mutasyon ve kopya sayısı değişimi) nadirdir (sırasıyla %0.47 ve %0.87). Sonuç olarak, Yüksek DNAJC9 ifadesi meme kanserinde olumsuz sağ kalım sonucu ile ilişkilidir. *DNAJC9* geninin regülasyonunda genetik alterasyonların ve promotor hipometilasyonunun etkisinin az olduğu öngörülebilir. Ancak, *DNAJC9*'nun genetik/epigenetik regülasyon mekanizmalarının daha iyi anlaşılması için fonksiyonel çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *DNAJC9*, İfade, Meme Kanseri, Metilasyon, Sağ kalım

➢ *ORAL PRESENTATION*

Fermente gıdalar, meyveler ve ticari ürünlerden *S. boulardii* suşlarının izolasyonu ve fenotipik karakteristiklerinin belirlenmesi

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Özet

Saccharomyces cerevisiae var. *boulardii* fırıncılık mayası olarak bilinen *Saccharomyces cerevisiae*'nın yakın akrabasıdır. *S. boulardii* gastrik koşullarda canlı kalabilme, optimum gelişme sıcaklığının insan vücut sıcaklığı olması, normal mikrobiyotayı olumsuz yönde etkilememesi ve antibiyotikler tarafından inhibe edilmemesi gibi özellikleri nedeniyle eşsiz bir probiyotiktir. *S. boulardii* hem göstermiş olduğu probiyotik özellikler hem de galaktozu karbon kaynağı olarak kullanamaması ve askospor oluşturamaması gibi özelliklerinden dolayı *S. cerevisiae*'den ayrılmaktadır. Bu çalışmanın amacı boza, tarhana ve zeytin gibi fermente ürünler ile üzüm, nar, muz, kavun, karpuz, Trabzon hurması gibi şeker içeriği yüksek meyveler ile *S. boulardii* içeren farklı ticari ürünlerden izolasyon yapılarak elde edilen izolatların fenotipik ve genotipik olarak kıyaslanmasıdır. Fenotipik analiz için galaktoz fermentasyonu ve askospor oluşturma formasyonu, genotipik analiz için ise polimorfik mikrosatellit lokus geni üzerinde tanımlı olan YLR177w geni için uygun olan primer çiftleri seçilmiştir. 11 farklı meyve ve 4 farklı fermente üründen 127 izolat elde edilerek *S. boulardii*'ye uygun primerler ile kontrol edilmiştir. Bu izolatlardan 12 tanesi ve ticari ürünlerden izole edilen *S. boulardii* suşları PCR (+) olarak belirlenmiştir. Ticari ürünlerden izole edilen *S. boulardii* suşları ile diğer 12 izolat fenotipik olarak değerlendirilmiştir. Ticari ürünlerden izole dilen *S. boulardii* suşlarının hem galaktozu karbon kaynağı olarak kullanamadığı hem de askospor oluşturmadıkları gözlemlenmiştir. Ancak, fermente ürünler ve şeker içeriği yüksek meyvelerden izole edilen mayaların galaktozu karbon kaynağı olarak kullanabildiğini ve askospor oluşturdukları belirlenmiştir. Elde edilen sonuçlar *S. boulardii*'nin izolasyonu için polimorfik mikrosatellit analizi ile birlikte fenotipik karakteristiklerinde belirlenmesi gerektiğini göstermiştir.

Anahtar Kelimeler: *S. boulardii*, izolasyon, fenotipik karakteristikler, polimorfik mikrosatellit

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➢ *ORAL PRESENTATION*

The antioxidant capacity of nanofiber dressings loaded with AV and HPO on STZ induced diabetic rat wound model

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Abstract

Diabetic wounds have a slow healing process and easy to be infected. In addition to current drug treatments, plant extracts have been used for their healing effects on different types of wound treatment. Aloe Vera (AV) and Hypericum perforatum oil (HPO) are two important plants that have benefits for the healing of wounds. In this study we aimed to compare the antioxidant capacity of AV extract and HPO on Streptozotocin (STZ) induced diabetic rats' wounds, using plants loaded innovative nanofiber dressings which were polymeric and biodegradable. Wistar albino male rats weighing (n = 20) 250 – 300 g were randomly divided into two main groups: sham-operated (n = 5) and STZ induced (n = 15) group. STZ group was further divided into subgroups as vehicle (n = 5), AV gel, PCL/gel (n = 5) and HPO treated (n=5) groups. STZ induction was repeated for seven days. On the seventh day, a surgically incisional diabetic wound was performed on the back of animals as in the sham group with similar operative procedures. Animals were treated for three days with nanofiber dressings and wound tissue was obtained to evaluate the topical antioxidant capacity of nanofiber dressings. We used biochemical assays such as total antioxidant status (TAS), total oxidant level (TOS), oxidative stress index (OSI) and as a pro-inflammatory mediator, tumor necrosis factor-alpha (TNF-α) levels of the treated skin were studied. Also, we measured the wound area in the process of the experiment. Our results showed, that both HPO and AV have antioxidant capacity on the surgical diabetic wound. However, Hypericum perforatum oil gives better results for bio-nano applications.

Keywords; Aloe vera extract; Hypericum perforatum oil; Oxidant status; Antioxidant status: Polymeric nanofibers dressings; Diabetic wound healing

➢ *ORAL PRESENTATION*

Synthesis of Novel Anticancer Compounds by Ultrasonic Sonication and Molecular Docking Studies

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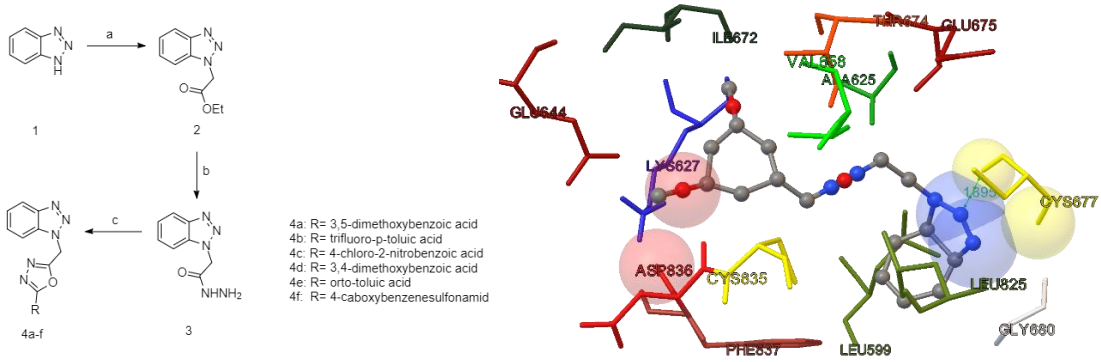
Abstract

Cancer is a major health problem involving numerous spatiotemporal changes in cell physiology in both developed and undeveloped countries. According to the World Health Organization (WHO) report, in 2015, 8.8 million people died globally from cancer [1]. While 20% of all diagnosed cancers are associated with obesity, physical inactivity, excessive alcohol consumption and / or malnutrition, some cancers include human papilloma virus (HPV), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and Helicobacter pylori (H. pyroli). The hybrid anticancer drug approach is an novel synthetic strategy that is an innovative synthetic synthetic method based on either combining the hepatophoric portions of different drugs in a new molecular structure, or directly linking two or more potential anticancer pharmacophores via cleavable / non-cleavable linkages based on the ability to retain affinity and activity for biological targets in the newly synthesized molecular hybrid. It is believed that two or more pharmacophore groups in a single structure not only synergize with their biological effects, but also increase their ability to inhibit multiple biological targets. Recently, the molecular hybrid approach has resulted in the synthesis of new chemical compounds with improved anticancer activity and selectivity with reduced side effects. Hydrazones containing the azomethine (- NH - N = C) group form an important class of compounds for new drug development. In many research areas, these compounds have been synthesized as target structures and their biological activity has been studied. Hydrazones have been reported to have antimicrobial, antitubercular, anticonvulsant, analgesic, anti-inflammatory, antiplatelet, anticancer, antiviral, antitumoral and antimalarial activity [2-4].



Scheme 1. a: Ethylbromoacetate, THF, TEA, b: Hydrazine hydrate, EtOH, c: $POCl_3$, substituted aryl acid.

Keywords: Anticancer activity, Ultrasonic Sonication, Benzotriazole, Oxadiazole, Computational study.

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➢ *ORAL PRESENTATION*

Evaluation of the case with a new mutation in the PTPN11 gene in the light of the literature

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Abstract

Metahondromatosis is a rare, autosomal dominant tumor syndrome with incomplete penetrance accompanied by exostosis and enchondromatosis. It has clinical and genetically different features from other multiple exostosis or multiple enchondromatosis syndromes. The disease is not associated with EXT1 and EXT2, which are genes responsible for autosomal dominant multiple osteochondromas. Heterozygous mutations in the PTPN11 gene on chromosome 12q24 cause the disease. Metahondromatosis is a disease characterized by multiple exostoses and multiple enchondromas of the long bone metaphyses and iliac crests, usually in the hands and feet. In this case report, a patient who was diagnosed with metachondromatosis in the light of clinical and radiological findings and with a novel mutation in the PTPN11 gene that was not previously described in the literature is presented. In addition, it was aimed to discuss the case with the previously reported metahondromatosis cases in terms of genotype and phenotypic findings in the light of the literature.

Keywords: metahondromatosis; PTPN11; exostosis; enchondromatosis

➢ *ORAL PRESENTATION*

Farklı solventlerle ekstrakte edilen Mazı meşesi (*Quercus infectoria*) gal tohumlarında antioksidan kapasitesinin araştırılması

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Özet

Giriş ve Amaç: Güney Doğu Anadolu Bölgesi fiziksel ve farklı iklimsel özellikleri sebebiyle büyük bir genetik bitki çeşitliliğine sahiptir. Bu bitkiler, oksijen ve fotonların sebep olduğu oksidatif stresi önleyen potansiyel antioksidan kaynaklarıdır. Son yıllarda gıdalarda ve biyolojik sistemlerde doğal olarak bulunan birçok molekülün antioksidan kapasitesinin çalışılması önem kazanmıştır. Bunun sebebi, antioksidanlarca zengin gıdaların tüketme miktarı artırıldığında farklı dejeneretif hastalıklara yakalanma riskinin azaltılacağına inanılmasıdır. Bu çalışmamızda, Güney Doğu Anadolu Bölgesinde yetiştirilen Quercus infectoria gal tohumlarında antioksidan kapasitesinin ölçülmesi amaçlandı.
Materyal ve Metot: 30 tane Quercus infectoria gal tohumları, su, etanol ve metanolle ekstrakte edilmiş ve daha sonra ekstraktlarda antioksidan enzim aktiviteleri (katalaz ve süperoksit dismutaz) ve oksidatif stresin indikatörü olan malondialdehit düzeyleri spektrofotometrik yöntemlerle saptanmıştır.
Bulgular: Etanol ve metanolden elde edilen ekstraktların sudan elde edilen ekstraktlara göre antioksidan kapasitesi (katalaz ve süperoksit dismutaz aktiviteleri) daha yüksek ($p<0,05$) ve malondialdehit düzeylerinin istatiksel olarak daha düşük olduğu saptanmıştır ($p<0,05$). Bununla birlikte, metanolden elde edilen ekstraktların etanolden elde edilen ekstraktlara kıyasla antioksidan kapasitesi ve malondialdehit düzeyleri arasında istatiksel olarak anlamlı bir farklılık olmadığı saptanmıştır ($p>0,05$).
Sonuçlar: Quercus infectoria gal tohumunun güçlü antioksidan etkiye sahip olduğu sonucuna varılmıştır. Ayrıca etanol ve metanolden elde edilen ekstraktların sudan elde edilen ekstraktlara göre daha yüksek antioksidan kapasiteye sahip oldukları gözlenmiştir.

Anahtar Kelimeler: Quercus infectoria gal, katalaz, süperoksit dismutaz, malondialdehit

➢ *ORAL PRESENTATION*

Evaluation of the genotypic distribution and population genetic structure of *MyoD1* g.782G>A polymorphism in Turkish Grey Steppe cattle breed

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Abstract

Myogenic determination factor 1 (*MyoD1*) gene is a member of the myogenic differentiation gene family which plays a crucial role in growth and muscle development. There is a lack of information about the genetic variants of this gene in Turkish Grey Steppe (Boz) cattle which is an important native cattle breed in Turkey. The present study aimed to determine the genotypic/allelic frequencies and population genetic indices of *MyoD1* g.782G>A polymorphism in Turkish Grey Steppe cattle. A total of 142 purebred bulls were used. Genomic DNA was isolated from whole blood using the standard phenol-chloroform extraction method. The polymerase chain reaction-restriction fragment length polymorphism technique was used for genotyping of the g.782G>A polymorphism in exon 1 of the *MyoD1* gene. Estimation of genotypic/allelic frequencies and the Hardy–Weinberg equilibrium (HWE) testing (α=0.05) were performed by using Cervus v3.0 software. Moreover, the population indexes including heterozygosity (He), the number of effective alleles (Ne), and the polymorphism information content (PIC) were calculated on the basis of allelic distribution. The fixation index (F_{IS}) was estimated from the values of theoretical (H_{the}) and experimental (H_{exp}) heterozygosities as follows: $F_{IS}=(H_{the}-H_{exp})/H_{the}$. Results revealed that BB was the preponderant genotype (%41.55) but the AA genotype exhibited a remarkable close frequency (% 39.44). Accordingly, allelic frequencies were very close to each other (A:0.49; B:0.51). The $\chi 2$ test showed that the corresponding *MyoD1* locus did not conform to Hardy-Weinberg equilibrium ($\chi 2$=54.52; P<0.001). He, Ne, and PIC values were 0.4998, 1.9992, and 0.3749, respectively. Moreover, F_{IS} value was found to be 0.6195. These results indicated that *MyoD1* g.782G>A polymorphism is a mildly informative genetic marker for Turkish Grey Steppe cattle. *MyoD1* gene has been previously identified as an important constituent of the genetic control of cattle growth. The present genotypic evaluation may be a precursor study of *MyoD1* g.782G>A polymorphism in Turkish native cattle breeds.

Keywords: Turkish Grey Steppe cattle, *MyoD1*, single nucleotide polymorphism, PCR-RFLP.

➢ *ORAL PRESENTATION*

Gastrik kanserde ANGPT2 ile korele diagnostik ve prognostik kullanım potansiyelleri olan moleküler biyobelirteç araştırılması

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Özet

Gastrik kanser günümüz teknolojik gelişmelerine karşın mortalitesi tüm kanserler içinde üçüncü sırayı alan önemli bir kanser türüdür. Hastalık sıklıkla erken evrede semptom vermediği için tanısı geç evrede koyulmakta, bu durum da tedaviyi güçleştirmektedir. Bir diğer ifade ile hastaların yaklaşık %40'ı teşhis edildiklerinde metastazlara sahiptir ve hastalığın takibinde kullanılan karsinoembriyonik antijen (CEA) ve karbohidrat antijeni (CA 19-9) hastalığın duyarlılığı ve özgüllüğü açısından yetersiz kalmaktadır (CEA duyarlılık ; %58.82, negatif öngörü değeri (NPV) yaklaşık% 30'dur, CA 19-9 duyarlılık; %26, NPV %57.47). Bu nedenle, gerek hastalığın takibi gerekse teşhisi açısından yüksek duyarlılık ve özgüllükteki biyobelirteçlere ihtiyaç vardır.
Endotel tabakasına spesifik tirozin kinaz reseptörü Tie-2 için ligandlar olduğu bilinen anjiopoietinlerin tümör gelişimi ve progresyonu ile bağlantıları yapılan pek çok çalışma ile ortaya konulmuştur. Bu protein ailesinden "Angiopoietin-2"ise gastrik kanserde bir prognostik faktör olarak kabul edilmektedir. Tekli prognostik faktörden ziyade kombinasyonel faktörler diagnostik ve prognostik açıdan daha faydalı olarak görüldüğünden mevcut çalışmamızda Angiopoietin-2 ile yüksek korelasyonu olan biyobelirteçlerin taranarak toplam sağkalım ve hastalıksız sağkalım üzerine olan etkilerinin incelenmesi, ayrıca bu genlerin ifadelerine bakılarak sağlıklı ve tümörlü dokuları ayırmadaki etkinlikleri değerlendirilmiştir. Bu amaçla GEPIA veritabanından mide kanseri hastalarından normal dokuları ile kıyaslandığında tümör dokularında en fazla ifade artışı olan üç yüz gen Angiopoietin-2 ile korelasyonu açısından değerlendirilmiştir. Korelasyon açısından anlamlı bulunan genler seçilerek bu genlerin toplam sağkalım ve hastalıksız sağkalım grafikleri oluşturulmuştur. Seçilen genlerin ayrıca normal doku ve tümörlü doku ifade oranları kıyaslanarak diagnostik kullanımları incelenmiştir.
Elde edilen analizler CLRN3, CKS2, ASF1B, CEP55, INHBA, ECT2, COL1A1, RP11-284F21.7, ASCL2, SPARC genlerinin ANGPT2 ile yüksek korelasyonuna işaret etmektedir. Bu genlerin sağkalım üzerine olan ileri analizi toplam sağkalım analizlerinde tümünün anlamlı olduğunu, hastalıksız sağkalımda ise CLRN3, CKS2, ASF1B, ASCL2 genlerinin anlamlı olduğunu göstermektedir. Sağlıklı ve tümörlü dokuları ayırmada ise özellikle CLRN3, INHBA, ASCL2 genlerinin anlamlı olduğu görülmektedir. Ancak özellikle kombinasyonel biyomarker tespiti için sonuçların ıslak laboratuvar çalışmalarıyla doğrulanması gerekmektedir.

Anahtar Kelimeler: Gastrik kanser, biyomarker, Angiopoietin-2, ANGPT2

➢ *ORAL PRESENTATION*

Kayısı posasından biyoaktif madde ekstraksiyon koşullarının optimizasyonu

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Özet

Ülkemizde önemli bir yere sahip olan kayısı, dünyanın pek çok yerinde yetişen bir ılıman iklim meyvesidir. *Rosaceae* (Gülgiller) familyasının *Armeniaca* cinsine ait olan kayısının botanik adı *Prunus armeniaca* L.'dir. Ülkemizde kayısıdan meyve suyu ve konsantresi, konsantre ürünler, pestil vs. üretimi gerçekleşmektedir. Bunun yanında, kayısı suyunun işlenmesi önemli çeşit ve miktarda yan ürün (kayısı posası, kabuk, çekirdek, kabuk, çekirdek ve çekirdek kabuğu) açığa çıkmaktadır. Kayısı posası; esas olarak kayısı meyvesinin pres sonrası kalan deri ve kabuk kısmının bileşiminden oluşmaktadır. Son yıllarda, kayısı posasında olması muhtemel değerli bileşenler, antioksidan özellikleri ve kronik hastalıklardan korunma kabiliyetleri gibi çeşitli biyoaktiviteleri sebebiyle büyük ilgi görmüştür. Bu çalışmada, kayısı posasından biyoaktif bileşenlerin ekstraksiyonu amacıyla, çevreci ve verimli bir yöntem olan ultrasonikasyon kullanılmış olup ekstraksiyon koşulları (sıcaklık ve süre) Yanıt Yüzey Yöntemi kullanılarak optimize edilmiştir. Ekstraksiyon işleminde, kayısı posası (1:10) örneklerinde çözgen olarak %50 etanol kullanılmış, sıcaklık (30°C -50°C) ve süre (30-90 dk) aralığında ayarlanıp, yanıt yüzey yöntemine göre modelleme oluşturulmuştur. Elde edilen deneme noktalarında yanıt olarak seçilen ekstrakt verimi, toplam fenolik ve toplam flavonoid miktarları ile antiradikal (DPPH radikali süpürme) aktiviteleri analiz edilmiştir. Analiz sonucunda, kayısı posası örneklerinden yapılan ekstraksiyonda optimum noktalar 50°C sıcaklık ve 90dk süre olarak bulunmuştur. Bu noktada ölçülen toplam fenolik ve flavonoid madde miktarı sırasıyla 1.206 mg GAE /g DM ve 1.015 mg CE/g DM olarak tespit edilmiştir. Yine aynı deneme noktasındaki antiradikal aktivite değerinin %79.85, ekstraksiyon veriminin ise %7.86 olduğu belirlenmiştir. Sonuç olarak, kayısı posasının gıda endüstrisinde kullanım potansiyeline sahip katma değeri yüksek alanlarda kullanılabilecek biyoaktif bileşenlerce zengin olduğu anlaşılmıştır.

Anahtar Kelimeler: kayısı posası, fenolik bileşenler, yanıt yüzey yöntemi, ultrasonik destekli ekstraksiyon

➢ *ORAL PRESENTATION*

Applications of 3D printed microfluidics in analytical chemistry

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Abstract

Microfluidic systems have widely used in chemistry, medical and environmental areas. These provide precise control of decreasing liquid volumes and miniaturization of the size of the liquid transport system. Three-dimensional (3D) printing is become an alternative to traditional methods by enabling fast design iterations during the development phase, while also reducing costs associated with corporate infrastructure, equipment installation, maintenance and physical space. Microfluidic systems constitute the basic platform of on-chip laboratory applications. By using microfluidic systems, the amount of reagents needed in chemical or biological reactions is reduced from milliliter levels to femtoliter levels and reaction time drops to less than one second. 3D printing systems are the main applications of microfluidics today. 3D printing refers to a set of additional manufacturing techniques that can build solid 3D objects layer by layer under precise digital control Of these techniques, the most relevant for microfluidic device manufacturing are: stereolithography, multi-jet modelling and fused deposition modelling. Many microfluidic technologies allow making devices containing multiple components with different functions, so many laboratory operations can be done in a single device. Microfluidic systems used for the determination of biological materials, food analysis, and quantification of drugs will be discussed in this study.

Keywords: Microfluidics, 3D printing, on-chip laboratory, analytical chemistry.

➢ *ORAL PRESENTATION*

Reusable halophilic bacterium attached electrospun nanofibrous webs for chromium removal in wastewater

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Abstract

Since chromium use in industries and wastewater discharge to environment, community health is endangered and necessity to removal from wastewater is arose.
In this research, halophilic bacterium *Citricoccus* sp. attached to cellulose acetate nanofiber web (CA-NFW) and used for chromium removal from wastewater. Firstly, optimum parameters were studied and as seen in Fig.1, they were found as pH 9, 24h, 30 °C, 100 rpm and 3.5% NaCl concentration.

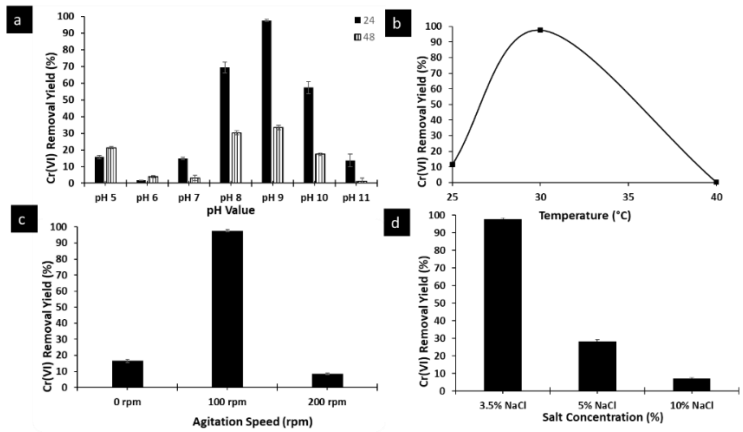

Figure 1: Optimization of a) pH, b) temperature, c) agitation speed and d) salt concentration

Fig.2 shows the effect of Cr(VI) concentration on the removal yields of the (a) bacteria, (b)CA-NFW and bacteria attached CA-NFW.

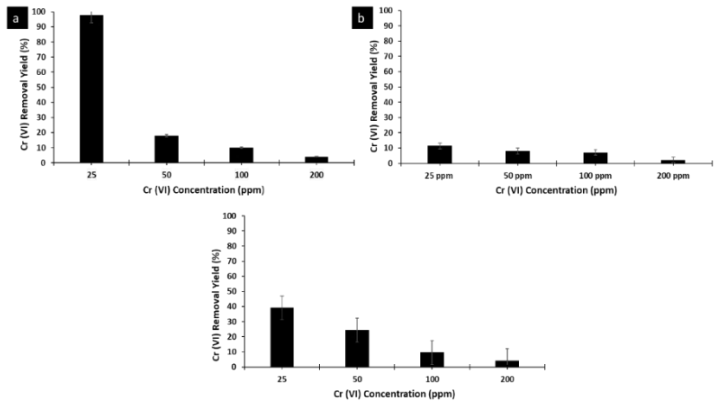

Figure 2: The effect of Cr(VI) concentration on the removal yield of the a)bacteria, b)CA-NFW and c)bacteria attached CA-NFW.

Bacteria attached CA-NFW was used three cycles for reuse that showed in Fig.3. After 3 cycles, removal yield was observed as 5.63%.

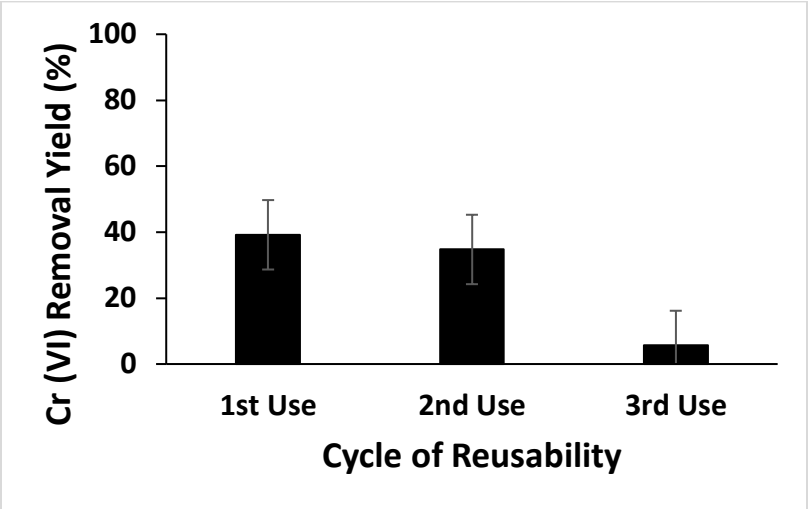

Figure 3: Reusability results at 25 ppm Cr(VI) concentration

Keywords: Nanobiotechnology, hexavalent chromium, electrospun cellulose acetate

➢ *ORAL PRESENTATION*

Determination of 3-*O*-caffeoylquinic acid in *Coffea arabica* L. beans by HPLC: Evaluation of the effect of roasting

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Abstract

Coffee is one of the most commonly consumed beverages all around the World. Roasted fruits of *Coffea* trees are used to prepare different types of beverages. The genus *Coffea* belongs to the Rubiaceae family. It has over sixty species but only *C. arabica* L., *C. robusta* L. and *C. liberica* L. have economical value. Generally, the beverages which contain mostly *C. arabica* are preferred because of their higher quality.
The green (unroasted) coffee has a chemical composition consisting of saccharides, lipids, sterols, fatty acids, alkaloids, terpenes, phenolics. Roasting which is essential to generate the flavour of coffee beans changes the chemical composition. Phenolics partially degrade and/or form polymer structures and many other compounds form in Maillard reactions, carbohydrate caramelization and pyrolysis of organic compounds with this process.
Chlorogenic acids are produced by esterification of hydroxycinnamic acids and quinic acid. Six to ten percent of the dry weight of the green coffee beans comprises of chlorogenic acids, and ~50% of the chlorogenic acids is 3-*O*-caffeoylquinic acid (3CQA). Chlorogenic acids and their derivatives have various biological activities as antioxidant, neuroprotective and cardioprotective effects. Also, they are potent α-glucosidase inhibitors and participate in the regulation of plasma and liver concentrations of cholesterol, triacylglycerol and minerals, prevention of septic arthritis caused by *Candida albicans* and inhibition of lipopolysaccharide-induced cyclooxygenase-2 expression.
In the present study an HPLC-UV method was developed and validated for determination of 3CQA content of *C. arabica* beans. The proposed method was validated in terms of linearity, precision, accuracy, limits of detection and quantification. The effect of roasting degree on 3CQA content was evaluated by the analysis of green and roasted (for 12, 20, 22 and 25 minutes at 140-160°C) samples. The green beans were found to contain the highest 3CQA concentration and the 3CQA concentration was decreased as the roasting time increased.

Keywords: 3-*O*-caffeoylquinic acid, *Coffea arabica* L., HPLC

➢ *ORAL PRESENTATION*

Self-emulsifying drug delivery systems (SEDDS) for siRNA delivery

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Abstract

Since RNAi has been found to occur in mammalian cells, it has been extensively researched as a novel therapeutic strategy. RNAi can be triggered by the addition of synthetic sequence-specific small interfering RNA (siRNA) that can target and separate complementary mRNA to achieve specific gene silencing. EpH receptors are the largest receptor member of the tyrosine kinase family, which can carry signals from the external environment to the inner compartments. Overexpression of EpH genes contributes to tumor growth by inducing tumor progression and metastasis. Self-emulsifying systems are isotropic and thermodynamically resistant systems that consist of oil, surfactant, co-solvent / co-surfactant and drug components, and can form water/oil emulsion when mixed with water at low speed. In this study, it was aimed to develop a self- emulsifying drug delivery system that can carry siRNA/EpH. For this purpose different formulations were prepared using Labrafac lipophile WL 1349 as oily phase, Tween 80® as surfactant; Capryol 90® were used as co-surfactants and distilled water was used as the aqueous phase. In this study, the determination of droplet size, physical appearance, morphological analysis, rheological analysis, stability and siRNA loading and release properties, evaluation of toxicity of systems in various cancer cell lines and determination of transfection properties were carried out. According to results, w/o emulsions were formed and the siRNA was allowed to remain in the aqueous phase. While the droplet size varied between 125-285 nm during the 6-month stability period. And also, the zeta potential values of the systems with Newtonian flow characteristics were determined to be around -37mV and, after adding cationic agent was +40mV. On the other hand, it has been observed that the successfully loaded siRNA can release extended up to 100 hours. As a conclusion, it is thought that they may be candidate systems for oral transport of genetic material.

Key words: siRNA, SEDDS, gene therapy, cancer prevention

➢ *ORAL PRESENTATION*

Pichia pastoris: A powerful and versatile heterologous protein expression system

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Abstract

Pichia (*Komagataella*) *pastoris* expression system is widely accepted as one of the most efficient and versatile platforms using for the production of recombinant protein in molecular biology. This methylotrophic yeast has many advantages such as stable gene expression with integration into the genome, efficient secretory expression, which enables easy-purification of the heterologous proteins, high cell-density with rapid growth rate and posttranslational modifications. Over the past three decades, *P. pastoris* has also been improved as a versatile cell factory for producing of thousands of biomolecules both on industrial scale and a laboratory. Up to now, over 5000 recombinant proteins have been produced which can comprise approximately 30% of the total cell protein or 80% of the total secreted protein in this system. In addition to over 300 licensed industrial processes using this yeast, more than 70 commercial products are produced in *P. pastoris*. Some of them are, in the field of biopharmaceuticals, such as hepatitis B surface antigen, insulin, epidermal growth factor and human serum albumin. The others are valuable enzymes in the field of industrial biotechnology, such as lipase, phytase, xylanase and mannanase. This yeast is also considered a unique host for the production of subunit vaccines that have recently been replaced by other forms of vaccines such as killed/inactivated and live attenuated vaccines relative to other expression systems. Multi-level optimization strategies including codon bias, promoters, gene dosage, signal peptides and environmental conditions provide efficient production of recombinant proteins. Therefore, determination of optimal conditions which differ according to the target protein for the maximum production of a recombinant protein is required even if *P. pastoris* expression systems are effective and easy to use with well-defined process protocols. Eventually, *P. pastoris* will continue to contribute as a powerful expression system in both industrial applications and research areas.

Keywords: Expression system, *Pichia pastoris*, Recombinant protein

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➢ *ORAL PRESENTATION*

Minyatürize biyosensör sistemi tasarımında yeni yaklaşımlar ve teknolojiler

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Özet

Biyosensörler, çeşitli transducer (akım iletici) türleri üzerine farklı biyokomponentlerin immobilize edilmesi ile oluşturulan ve kanser biyobelirteçleri, hormonlar, ilaçlar, patojenler, metabolitler gibi birçok molekülü hızlı, tutarlı ve güvenilir bir şekilde tayin etmemize olanak sağlayan sistemlerdir. Leland C. Clark'ın yaklaşık 60 yıl önce tasarladığı ilk biyosensörden günümüze dek biyosensör teknolojisi, çok hızlı bir değişim ve gelişim göstermiştir. Bu bağlamda ilk 20-25 yılda biyokomponent türleri ve onların farklı immobilizasyon stratejileri üzerine bir gelişim olurken; son 20 yılda –bilgisayar teknolojisinin de gelişmesi ile- değişim daha çok transducer/sinyal iletici, yükseltici ve gösterim biçimlerinde olmuştur [1]. Bu gelişim sonucunda geliştirilen aptasensorler, microarrayler, kullan-at elektrotlar gibi yeni nesil biyosensör sistemleri, günümüzde, tıbbi tanı, adli tıp, gıda teknolojisi, biyoproses ve kirletici izlemi gibi öncelikli alanlarda yaygınca kullanılmaktadırlar. Özellikle çip teknolojisi ve mikroakış sistemlerinin gelişmesi ile öne çıkan mikroarrayler (mikrodiziler), birden fazla örneği aynı anda analiz etme becerilerinden ötürü tıbbi tanı alanında rutin olarak kullanılmaya başlanmıştır. Ayrıca yüksek spesifiklerinden dolayı DNA-RNA veya aptamer yapıları ile kombine edilen mikroarrayler, birçok genetik hastalığın, nokta mutasyonunun, ilaç ve zehrin belirlenmesinde yaygınca kullan bir olmuştur[2]. Bunlarla birlikte, esnek, taşınabilir, giyilebilir, implant edilebilir ve/veya kullan-at elektrotlar ile hazırlanan minyatürize biyosensör sistemleri üzerine son yirmi yılda birçok çalışma yapılmış ve bunlardan bazıları gündelik hayatta kullanılmaya başlanmıştır. Özellikle de taşınabilir ve implant edilebilir biyosensörler, diyabetes mellitus ve koroner kalp yetmezliği gibi hastalıklara ilişkin belirteçlerin ve metabolitlerin anlık olarak izlenip rapor edilmesi noktasında başarılı olmuşlardır. Ayrıca bu biyosensörlerin, intravenöz ilaç uygulaması yapan çeşitli cihazlarla kombine edilip; kandaki metabolit-belirteç miktarı değişimlerine göre belirli periyotlarla hastaya ilaç uygulamasının yapıldığı çalışmalar da "bakım noktası" (point of care) konusunda ümit vaat etmektedir [3].

Anahtar Kelimeler: Minyatürize Biyosensörler, Mikroarrayler, Biyoçipler,

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➢ *ORAL PRESENTATION*

Baklagillerde *SOS1* Geninin *In Silico* Biyoinformatik Analizi

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Özet

Bitkiler büyüme ve gelişme evrelerinde yaşam döngülerini olumsuz yönde etkileyecek birçok stres faktörüne maruz kalmaktadırlar. Biyotik ve abiyotik stres faktörleri bitkilerin hücresel işlevlerini etkileyerek fizyolojik ve biyokimyasal zararlar oluşturabilmekte ve bunun sonucunda ürünlerin verim ve kalitesinde kayıplara neden olabilmektedir. Abiyotik streslerden biri olan tuzluluk ve buna bağlı olarak bitkilerdeki tuz stresi, mevcut verimli tarım alanlarını tehlikeye atarak besin ürünlerinin üretimini önemli düzeyde kısıtlamaktadır. Baklagiller diğer türlerle karşılaştırıldığında, tuz stresine karşı en hassas grup içerisinde bulunmakta ve tuzluluğa karşı tolerantlık seviyesinin oldukça az olduğu bilinmektedir. Tuzluluğun bitkilerde zararlara neden olması yüksek oranlarda sodyum birikmesinden kaynaklanmaktadır ve bu durum bitkilerin organ ve hücrelerinde su yetersizliğine ve enzimlerin işlevsizliğine neden olmaktadır. Bitkilerde, plazma ve vakuol membranda bulunan Na^{+}/H^{+} antiporterler (*NHX*), aktif olarak aşırı Na^{+} 'yı sitozolden uzaklaştırabilir veya Na^{+} toksisitesini gidermek için tonoplastta bölümlendirebilir. Bitkilerde tuz stresine karşı toleransınoluşmasında görev alan en önemli spesifik gen ailesi ise *SOS* (salt overly sensitive)'tur. *SOS* genlerinden *SOS1(NHX7)* bitkilerde tuz stresinin kontrol edilmesinde en önemli role sahip olduğu bilinmektedir. Bu çalışmada önemli baklagil bitkilerinden (*Cicer Arietinum, Glycine Max, , Medicago Truncatula, Phaseolus Vulgaris, Trifolium Pratense vb.)* veri tabanlarından elde edilen *SOS1* genlerine amino asit dizileri kullanılarak *in silico* biyoinformatik analiz yapılmıştır. Biyoinformatik analizler sonucunda SOS1 protein dizilerinin birbiriyle benzerlikleri saptanmıştır. Seçilen Baklagillerde SOS1 protein dizilerinin MEGA7 programında hizalaması yapılmış, hizalama verileri kullanılarak filogenetik ağaç oluşturulmuş ve dizi bilgileri kullanılarak SWISS-MODEL veri tabanında üç boyutlu yapıları oluşturulmuştur.

Anahtar Kelimeler: Baklagiller, *NHX7*, *SOS*, Tuz stresi, Biyoinformatik

➢ *ORAL PRESENTATION*

Geleneksel yoğurtlardan izole edilen *S. thermophilus* ve *L. delbrueckii* subsp. *bulgaricus* türlerinin tanımlanması ve MLST yöntemi ile alt suşların belirlenmesi

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Özet

Yoğurt, eski dönemlerden günümüze kadar geleneksel olarak üretilen fermente bir süt ürünüdür. Yoğurt üretiminde kullanılan *S. thermophilus* ile *L. delbrueckii* subsp. *bulgaricus* yoğurt starter kültürü olarak bilinmektedir. Endüstriyel yoğurt üretimi ticari starter kültürler ile gerçekleştirilirken, geleneksel üretimde ise yörelere özgü geleneksel yoğurt kültürleri kullanılmaktadır. Ülkemizde geleneksel olarak üretilen ve yüksek besin değerine sahip olan yoğurt zengin bir mikrofloraya sahiptir. Endüstriyel olarak üretilen yoğurtlar *S. thermophilus* ve *L. delbrueckii* subsp. *bulgaricus* türlerini içerirken geleneksel yöntemlerle üretilen yoğurtlar bu iki bakterinin yanı sıra, farklı laktik asit bakterileri de içermektedir. Ancak ticari starterler, zamanla geleneksel üretimde kullanılan yoğurt kültürlerinin yerini almakla birlikte geleneksel yoğurtlara özgü tat ve aromanın değişmesine sebep olmaktadır. Bu çalışmanın amacı, Türkiye'nin farklı bölgelerinde geleneksel olarak üretilen yoğurtlardan, ticari kültür olarak kullanılmasını sağlamak amacıyla *S.thermophilus* ve *L. delbrueckii* subsp. *bulgaricus* türlerinin farklı suşlarının belirlenmesidir. İzole edilen bakterilerin ilk olarak ilgili primerlerle kontrolü yapılmış daha sonra 16S rRNA gen bölgesi baz alınarak genotipik olarak tanımlanması gerçekleştirilmiştir. Sonuç olarak bu yoğurtlardan 32 tane *S. thermophilus* ve 20 tane *L. delbrueckii* subsp. *bulgaricus* tanımlanmıştır. Çoklu lokus dizilim analiz (MLST) yönteminde, farklı primerler kullanılarak birden fazla gen bölgesi tarandığı için analiz edilen genlerin sayısının artması ile ayrım gücünün de arttığı bilinmektedir. Tanımlanan bu türlerin farklı suşlar olduğunun tespit edilmesi amacıyla 3 farklı MLST primeri ile gen bölgeleri taranmış filogenetik sınıflandırma yapılarak suş bazında ayrım yapılmıştır. Elde edilen sonuçlara göre 14 tane *S. thermophilus* ve 6 tane *L. delbrueckii* subsp. *bulgaricus* suşunun ayrımı gerçekleştirilmiştir.

Anahtar Kelimeler: yoğurt, starter kültür, 16S, MLST

➢ *ORAL PRESENTATION*

Hesperetin'in adriamisin uygulanan sıçan akciğer dokusunda NF-κB, IL-1β ve TNF-α ekspresyonları üzerine etkisi

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Özet

Amaç: Kemoterapötik bir ajan olan adriamisin (ADR) hem hematolojik hem de katı tümörlerin tedavisi için yaygın olarak kullanılan bir antrasiklindir. Doza bağlı olarak akciğer ve diğer dokulardaki yan etkileri nedeniyle kemoterapideki etkinliği sınırlardırılmıştır. ADR kaynaklı pulmoner toksisitenin nedeni tam olarak ortaya koyulmamıştır, fakat artan serbest radikaller ile oksidan/antioksidan dengesizlikler suçlanmıştır. Ayrıca oksidatif stresle ilişkili inflamasyonun, ADR'nin neden olduğu doku hasarlarında rol oynadığı bilinmektedir. Nükleer faktör-kappa B (NF-κB), çeşitli stres tepkileri ile ilişkili genleri düzenleyen bir transkripsiyon faktörüdür ve inflamatuar sitokinlerin salgılanmasına katkıda bulunarak akciğer hastalıklarının patogenezinde rol oynar. Bu nedenle, NF-kβ aktivasyonunu inhibe eden ajanların pulmoner inflamasyonu ve doku hasarını azaltmada faydalı olduğu düşünülmektedir. Hesperetin (HES) bazı turunçgillerde bol miktarda bulunan doğal bir biyoflavonoiddir. HES'in NF-κB aracılı inflamasyonu inhibe ederek akut akciğer hasarlarını zayıflattığı gösterilmiştir. Çalışmada, ADR uygulanan sıçan akciğer dokularında HES'in koruyucu etkisinin NF-κB, IL-1β ve TNF-α ile ilişkisinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: Çalışmada 28 adet Sprague-Dawley cinsi erkek sıçan kullanıldı. Sıçanlar Kontrol, ADR, ADR+HES ve HES olmak üzere dört gruba ayrıldı (n=7). Kontrol grubuna deney süresi boyunca (28 gün) hiçbir uygulama yapılmadı. ADR grubuna, 15 mg/kg ADR i.p olarak tek doz uygulandı. ADR+HES grubuna, ADR verilmesinden sonra gün aşırı 50 mg/kg HES oral gavaj yolu ile verildi. HES grubuna ise gün aşırı 50 mg/kg dozunda HES oral olarak uygulandı. Deney sonunda akciğer dokularındaki NF-κB, IL-1β ve TNF-α genlerinin ifade düzeyindeki değişimlerini belirlemek için immünreaktivite ve kantitatif real-time PZR analizleri gerçekleştirildi.
Bulgular ve Sonuç: Akciğer dokusuna ait NF-κB, IL-1β ve TNF-α gen ekspresyonlarında kontrol grubuyla karşılaştırıldığında ADR grubunda anlamlı bir artış olduğu, ADR+HES grubunda ise ADR grubuyla karşılaştırıldığında istatistiksel olarak anlamlı bir azalma olduğu tespit edildi. Elde ettiğimiz bulgular ile NF-kB, IL-1β ve TNF-α 'nın ADR kaynaklı pulmoner toksisiteye karşı farmakolojik müdahalede potansiyel hedefler olduğunu ve HES tedavisinin ADR kaynaklı akciğer hasarlarında iyileştirici etkiyi inflamasyonla ilişkili genlerin fonksiyonu düzenleyerek sağlayabileceğini ortaya koyduk.

Anahtar Kelimeler: kemoterapötik, adriamisin, akciğer, hesperetin, NF-κB, IL-1β , TNF-α

➢ *ORAL PRESENTATION*

Siklomatriks polifosfazen nano/mikroküreler ve ilaç salımı uygulamalarındaki son gelişmeler

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Özet

Fosfazenler, yapılarında tekrarlayan –P=N– grupları içeren, fosfor atomlarına organik yapılı grupların bağlanması ile inorganik-organik hibrit yapılar oluşturan, düz zincirli, halkalı veya polimerik yapıda olabilen bileşiklerdir. Polifosfazenler ise düz zincirli, siklolineer, siklomatriks ve lineer matriks olmak üzere farklı yapılarda bulunabilmektedir. Özellikle son dönemde, siklomatriks polifosfazenler oldukça ilgi çekmektedir. Siklomatriks polifosfazenler inorganik-organik hibrit yapılı materyallerdir. Bu tip polifosfazenlerin sentezinde çapraz bağlayıcı olarak hekzaklorosiklotrifosfazen (trimer; $N_3P_3Cl_6$) veya oktaklorosiklotetrafosfazen (tetramer; $N_4P_4Cl_8$), monomer olarak ise iki veya daha fazla amin (3,3′-diaminobenzidin vb.) veya hidroksil (floroglusinol vb.) grubu içeren organik bileşikler kullanılmaktadır. Reaksiyon ortamına tuz tutucu ve aynı zamanda bir katalizör olarak trietilamin eklenmektedir. Uygulanan polikondenzasyon sentez yöntemi kolay, hızlı ve pratiktir. Ultrasonik güç kullanılarak, sadece birkaç saat içerisinde nano/mikro küreler elde edilebilmektedir.
Fosfazenler toksik olmayan, biyouyumlu ve biyolojik olarak fosfat ve amonyağa kadar parçalanabilen bileşiklerdir. Bu özellikleri sayesinde ilaç hedefleme ve kontrollü ilaç salımı, protein, gen taşıma, hücre/tümör görüntüleme, fototermal terapi gibi pek çok biyolojik ve medikal uygulamada biyomateryal olarak kullanılabilmektedirler. Bunların arasında kontrollü ilaç salımı üzerine yapılan araştırmalar son yıllarda hızlanmıştır. Kontrollü ilaç salımı uygulamaları için sentezlenen siklomatriks tip polifosfazen küreler nano veya mikro boyutlarda, solid veya içi boş/delik şekilde hazırlanabilmekte, ayrıca bu kürelere floresans özellik kazandırılarak ilaçların hedef organa iletimi takip edilebilmektedir. İlaç salımı amacıyla çalışılan model ilaçlara örnek olarak doksorubisin, B12 vitamini, Rhodamin 6G, resveratrol verilebilir. İlaçlar adsorpsiyon veya kovalent bağlanma yoluyla kürelere yüklenmekte, daha sonra uygun pH'lardaki salımları *in vitro* veya *in vivo* koşullarda incelenmektedir. Bu açıdan siklomatriks polifosfazen nano/mikro küreler kontrollü ilaç salımı ve taşınması uygulamalarında kullanılabilecek yeni nesil biyomateryaller olarak bilim insanlarına ışık tutmakta ve oldukça umut vadetmektedirler.

Anahtar Kelimeler: Fosfazen, siklomatriks polifosfazenler, ilaç salımı, polikondenzasyon, inorganik-organik hibrit yapılar

➢ *ORAL PRESENTATION*

Composition analysis and biological activities of different extracts of *Teucrium divaricatum* Kotschy ssp. *canescens* (Celak.) an endemic plant of Cyprus.

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver malignant tumour. It is categorized as the fifth most common cancer and the third cause of cancer-related deaths globally. There are different treatment strategies for HCC, however, the approaches are not successfully enough. In the last two decades, herbal medicines have become popular and there are many pharmaceutical researches for studying plants as a main source for new medicines or herbal treatments which some have been proven to be effective against HCC. *Teucrium divaricatum* ssp. *canescens* (*TDC*), which belongs to the Lamiaceae family, is an endemic plant in Cyprus.

In this study, chemical composition and various effects of 70% methanolic extract of *TDC* and its sequentially prepared sub-fractions (hexane, ethyl acetate, butanol, and water) were studied on the proliferation, motility, oxidative state on the SK-HEP-1 cells. For this purpose MTT, DCFH-DA, and wound healing assays were carried out and composition analyses were done using LC-MS/MS.

Aqueous methanolic extract and all sub-fractions decreased cancer cell viability significantly in a concentration dependent manner. Additionally, they were effective at inhibiting the 2D motility. Even though, methanolic extract and all sub-fractions successfully decreased the basal oxidative stress, the only sub-fraction which was effective on induced oxidative stress was water sub-fraction.

The main compounds were identified as chlorogenic acid Na derivative, 5-caffeoylquinic acid, forsythoside b, verbascoside, luteolin apiosylglucoside, luteolin-glucoside, luteolin glucuronide, apigenin glucoside, luteolin glucoside, apigenin glucuronide, naringenin, luteolin, cirsiliol, cirsimaritin within the samples by LC/MS/MS.

Our results conclude that *TDC* can have novel molecular candidates for anticancer chemotherapeutic approaches.

Keywords: *Teucrium divaricatum* ssp. *canescens*, HCC, luteolin, LC-MS/MS, cytotoxicity

➢ *ORAL PRESENTATION*

Determination and distribution of biofilm forming cervicovaginal polymicrobial cultures

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Abstract

The health of the female reproductive tract depends on healthy cervicovaginal microbiota and some factors may lead to vaginal colonization with potentially biofilm forming microorganisms. Our aim was to determine biofilm formation of polymicrobial cultures in cervicovaginal samples (n=223) and to estimate the distribution of biofilm forming polymicrobial cultures according to age and bacterial/fungal infections. Cervicovaginal samples were obtained by a cytobrush, smeared to a slide, stained by Papanicolaou method and examined cytologically. Cytobrushes were immersed in Brain Heart Infusion Broth media in order to grow microorganisms. Biofilm formation (BF) of polymicrobial cultures were assessed by "Crystal Violet Binding" assay. BF was determined as, low (LBF), intermediate (IBF) and high (HBF) with a rate of 25.56%, 5.83% and 5.38%, respectively. Among four different age groups (0-20; 21-40; 41-60; 61-80), the frequency of BF in 61-80 year-old women was the highest (63.64%). The frequency of BF in fungal infections was higher than the healthy and bacterial vaginosis diagnosed cervicovaginal samples. Our findings show that, biofilm forming polymicrobial colonization in vaginal microbiota increases in parallel with the increase in age of a woman and women with fungal infections are at higher risk for biofilm associated cervicovaginal infections.

Keywords: Biofilm formation, Cervicovaginal infections, Bacterial vaginosis, Fungal vaginitis.

➢ *ORAL PRESENTATION*

Analysis of Mitochondrial *ATPase-6* Gene Mutations and Telomere Length in Blood Dna of Breast Cancer Patients

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Abstract

Breast cancer (BCa) is the most common cancer type in women. The mitochondrion has a significant role in cellular energy production, carcinogenesis and tumor progression. Telomeres constitute the end of human chromosomes, and they keep the genome. The goal of this study was to invesitigate the *ATPase-6* gene mitochondrion DNA (mtDNA) mutation and telomere length in BCa patients.
mtDNA *ATPase-6* gene alterations and clinical data for 73 blood samples with BCa who had Sanger DNA sequencing achieved. Relative telomere length (RTL) was analysed with qPCR.
The detected most common mtDNA *ATPase-6* gene alterations in the 73 patients were A8860G (%27.4), G8697A (6.85%), A8701G (4.1%) and C8684T (2.7%). We found that telomere length was significantly shortened in healty samples as compared with tumour samples (p=0.0001). We determinated that telomere length was significantly shortened in wild-type samples as compared *ATPase-6* mutation samples (p=0.0285). Our results demonstrated that *ATPase-6* mutation has a significant association with histologic grade (p=0.0068) and age (p=0.0058). Also, we found a significant relation between telomere length and age of patients (p=0.0120).
ATP-ase-6 mutations and telomere length might be used to diagnose the BCa patients. There is also a need to discover whether other mtDNA mutations and telomere length have an effect on prognosis of BCa patients.

Keywords: *ATPase-6*, telomere length, breast cancer, mtDNA, Sanger sequencing

➢ *ORAL PRESENTATION*

Investigation of miR-485-3p and miR-4728-5p expression levels in colorectal cancer

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Absract

Colorectal cancer is one of the most common cancers and a leading cause of death worldwide. MicroRNAs (miRNAs) are small single-strand non-coding RNAs of approximately 18-22 nucleotides that are involved in the post-transcriptional regulation of gene expressions. miRNAs play an important role in biological processes such as cell growth, cell differentiation, cell proliferation, metastasis and cell death. In previous studies, it has been shown that various dysregulations in expression levels of miRNAs are associated with the colorectal cancer carcinogenesis. The aim of this study is to investigate expression levels of miR-485-3p and miR-4728-5p in the tissues of colorectal cancer patients. This study has received approval by local ethical committee of Gaziantep University, and all participants signed the informed consent to participate in this research. Tumor and adjacent healthy tissues of a total of 59 patients with colorectal cancer were collected from Department of General Surgery, Gaziantep University-Turkey. In the present study, RNA from both tumor and adjacent healthy tissues of colorectal cancer patients were extracted and synthesized the cDNA. Then, the expression levels of miR-485-3p and miR-4728-5p were determined by quantitative Real-Time PCR. As a result, the expression levels of miR-485-3p and miR-4728-5p were found significantly downregulated in colorectal cancer tissues compared to adjacent healthy tissues ($p<.001$.). It has been concluded that miR-485-3p and miR-4728-5p may be important biological markers as tumor suppressors in the colorectal cancer carcinogenesis.

Keywords: Colorectal cancer, expression, miR-485-3p, miR-4728-5p, Real Time PCR

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➢ *ORAL PRESENTATION*

Lipidome profiles in hepatocellular carcinoma for Wnt/β-catenin signaling pathway

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Abstract

Wnt signaling has essential roles during vertebrate development and also in adult tissue homeostasis. Aberrant Wnt signaling are associated with hereditary and degenerative diseases and cancer.Canonical Wnt signaling is the most well-characterized Wnt signaling pathway. Signaling is initiated by binding of the secreted Wnt ligand to its receptors at the cell surface. The cytoplasmic events relevant with this pathway have been largely revealed. However, the little is known about the mechanistic role of the nano environment where Wnt-receptor complex forms.
In our previous study, we show that Wnt-receptor complex is formed in ordered nanodomains of plasma membrane. Results show that signaling activity decrease in response to inhibition of lipid structures in this nanodomains (Sezgin et al., FEBS Journal, 2017). In currently, we focus on the nano environment where this complex is formed in cancer cells. We focus on specifically hepatocellular carcinoma (HCC) cell lines because it is the fourth cause of cancer deaths in the World and there is misregulated signaling actvity and components of Wnt signaling pathway. HCC cell lines with abnormal Wnt signaling activity were used for this purpose.
Our aim is that the lipid content of the nano environment where Wnt-receptor complex forms is characterized by lipidome profiling using specific isolation methods. Following step, we will focus on specific lipids and their ihibitors that are assoiated with Wnt levels. Decipher to role of the specific membrane structures of the cancer cells depending on canonical Wnt signaling activity, our study will contribute to the specific targeting of cancer cells for therapeutic purposes.

Keywords: Canonical Wnt, HCC, lipidomics, plasma membrane

➢ *ORAL PRESENTATION*

Sodium borate decahydrate affects the fractal dimension of SH-SY5Y cells in an *in vitro* amyloid-beta toxicity model

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Abstract

Boron containing compounds are usually found as borates in nature. Borate is metabolized as boric acid in body fluids and tissues of animals including human beings. Borates have important physiological functions in central nervous system, endocrine system and mineral metabolism. Amyloid-beta toxicity is one of the neuropathological hallmarks of several neurodegenerative disorders, and it is also known that boron containing compounds have pro-survival effects in the context of amyloid-beta toxicity. Neurite outgrowth is one of the basic processes of neuronal network formation, plasticity and development of the nervous system; it is also one of the indicators of neural regeneration. The fractal dimension analysis of neurite branchings or neurite-like extensions provide useful information about the the degree of informational complexity and health status of the neuronal networks. The aim of this study is to investigate the possible effects of two boron containing compounds on the fractal dimension of SH-SY5Y cells in an in vitro amyloid-beta toxicity model. The cultured SH-SY5Y cells were treated with 10µM amyloid-beta and 200 µg/ml sodium borate decahydrate (SBD) and boric acid were applied before and after amyloid-beta application. The light microscope images were taken and neurite-like extensions of the cells in each experimental group were analysed by using the FracLac plugin of ImageJ software. Data were statistically assessed by using one-way ANOVA test, p value lower than 0.05 was considered as statistically significant. The results revealed that amyloid-beta application significantly reduced the fractal dimension of neurite-like extensions of the cells, whereas SBD pre-treatment increased the fractal dimension of the neurite-like extensions significantly. Our findings show for the first time that a boron containing compound (SBD) increases the fractal dimension of neurite-like extensions of SH-SY5Y cells in the presence of amyloid-beta and this might also provides the neuroprotective role of SBD in the context of fractal dimension.

Keywords: Boron, fractal dimension, SH-SY5Y, amyloid-beta

➢ *ORAL PRESENTATION*

Novel bacterial strains associated with wheat roots

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Abstract

Fertilization plays a vital role in the production of wheat as in many agricultural products. The presence and amount of the sufficient nutrients in the soil are the necessary parameters for growth of plants. Microbial fertilizers are the alternative and sustainable counterpart of chemical fertilizers to improve the quality and the quantity of agricultural plants. The microbial fertilizers cover the microorganisms that are competent to colonize on plant roots. They promote the growth and development of the host plant by supplying macromolecules such as nitrogen, phosphorus etc.
The aim of this study is isolation, purification, and identification of the natural bacterial stains from the rhizosphere of the local wheat grown in Seben, Bolu. Root associated bacteria were isolated by sonication of wheat roots. Conventional techniques were applied for purification bacteria isolates. Bacterial species were identified by using different methods: Maldi-TOF analysis, and comparative analysis 16S rDNA, and 16S-23S rDNA intergenic regions. We compared the data collected from three approaches to increase the confidence for species names. Our data suggested that among several species, we were able to verify species name for four bacterial strains: *Pseudomonas poae, Pseudomonas thivervalensis, Bacillus subtilis,* and *Microbacterium foliorum.*
For six species we were able to verify the genus names and referred them according to their laboratory stock barcode codes: *Arthobacter spp. ESU164, Arthobacter spp. ESU193, Pseudomonas spp. ESU131, Pseudomonas spp. ESU141, Pseudomonas spp. ESU1531, Microbacterium spp. ESU121.* Our preliminary analysis of six species suggest that those bacteria are potentially new species.
The bacteria presented in this study are promising for agricultural application to promote plant growth by contributing atmospheric nitrogen fixation, indole-3-acetic acid production and phosphate solubilization.

Keywords: Microbial fertilizers, Plant Growth Promoting Bacteria (PGPB).

➢ *ORAL PRESENTATION*

Erciyes Dağı'na endemik *Taraxacum farinosum* türünün DNA barkodlama tekniği ile belirlenmesi

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Özet

DNA Barkodlama genomun küçük parçalarının (mitokondri ve kloroplast) DNA sekans verilerini kullanarak türlerin kimliklerinin belirleyen bir tekniktir. DNA barkodlama, biyolojik çeşitliliğin bir envanterini oluşturulması, hızlı bir şekilde, tanımlanamayan türlerin tanımlanması, koruma altındaki türlerin belirlenmesi, insan hastalık vektörlerinin tanımlanması, tarım zararlılarının belirlenmesi, biogüvenlik çalışmaları, yasa dışı ticaretin önlenmesi gibi yaşamın birçok aşamasında kullanılmaktadır. Canlılarda en uygun ve güvenilir DNA barkodlama amacıyla farklı gen bölgeleri belirlenmiştir. Bitkiler için kloraplast DNA bölgelerinden biri olan matK gen bölgeleri çoğunlukla barkodlama için standart gen bölgesi olarak kullanılmaktadır. Bu çalışmada Kayseri (Erciyes Dağı)' ye endemik olan Taraxacum *farinosum* türünün DNA barkodunun belirlenmesi amaçlanmıştır. Bu kapsamda, Taraxacum *farinosum* türüne ait farklı bireyler Erciyes Dağ'ından toplanıldı ve kurutularak herbaryum materyali haline getirilen örnek Erciyes Üniversitesi Herbaryumunda muhafaza edilmektedir. Taraxacum *farinosum* türünün *MatK* gen bölgesi spesifik primerler kullanılarak amplifikasyonu başarılı bir şekilde yapılmış ve türün matK gen bölgesinden elde edilen gen dizileri kullanılarak diğer yakın türlerden ayırt edilebildiği gözlemlenirken, ayrıca aynı türe ait bireyleri de bir arada gruplandırdığı görülmüştür. Sonuç olarak, *matK* gen bölgesinin Taraxacum *farinosum* türü için potansiyel bir barkod olarak kullanılabileceği ve bu yöntemle türün teşhis edilmesi ve taksonomik olarak sınıflandırılmasının mümkün olduğu öngörülmüştür.

Anahtar Kelimeler: Asteraceae, DNA barkodlama, *matK*, *Taraxacum*.

➢ *ORAL PRESENTATION*

Immobilization of L-ASNase on clay minerals for biocatalysts applications

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Abstract

L-asparaginase (L-asparagine amino hydrolase, E.C 3.5.1.1) is a chemotherapeutic enzyme used in the treatment of many cancer diseases such as leukemia [1]. It hydrolyzes the L-asparagine amino acid necessary for the growth of leukemic cells. Thus, leukemic cells cannot provide the amino acid necessary for protein synthesis and die. [2] L-asparaginase is also an enzyme explored in food industry and sensor technology. Although L-asparaginase plays an important role in the effectiveness of therapy, one of the biggest challenges in therapy is the short half-time of L-asparaginase due to its continuous use. In addition, it causes serious side effects due to those isolated from foreign sources. Immobilization is a straightforward strategy that enables recycling of enzymes.
A variety of solid materials (organic or inorganic) have been widely used as supports for enzyme immobilization. Immobilization of enzyme on solid materials may result in improved activity, stability and recyclability of the enzyme in a broader working pH and temperature range than the native enzymes [3]. Therefore, the aim of this work was to characterize and use clay minerals for the immobilization of L-asparaginase. The clay minerals were characterized by Fourier-transform infrared spectroscopy, scanning electron microscopy, thermal analysis, X-ray diffraction, and energy dispersive X-ray spectroscopy. Afterward, L-asparaginase was immobilized via adsorption on the clay minerals. As a result, the immobilized L-ASNase exhibited better activity at various temperatures and broader pH values. In addition, immobilized L-asparaginase displayed high reusability, thermal and storage stability compared to the free L-ASNase.
In conclusion, the study demonstrated that the clay minerals can be utilized as promising solid supports for the immobilization of L-asparaginase aiming to biotechnological applications.

Keywords: L-Asparaginase, Enzyme immobilization, Clay, Enhanced stability

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➢ *ORAL PRESENTATION*

Simultaneous Determination of Cefdinir and Clavulanic Acid in Tablets by using High-Performance Liquid Chromatographic Methods

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Abstract

In this study, a new, simple and fast high performance liquid chromatograpic method has been presented for the simultaneous determination of cefdinir and clavulanic acid in tablets. The chromatographic separation was carried out on reverse phase C18 colum by using as mobile phase 20.0 mM phosphate buffer (pH 3): methanol (65:35, v/v). The mobile phase flow rate was 1.0 mL/min and the substances were detected at 210 nm. The linearity ranges were found as 18.0-84.0 µg/mL and 7.5-35.0 µg/mL for cefdinir and clavulanic acid, respectively. The limits of detection and quantification were found to be 0.63 and 1.91 µg/mL for cefdinir and 0.44 and 1.33 µg/mL for clavulanic acid, respectively. The mean recoveries for cefdinir and clavulanic acid were calculated as 96.60-98.52% and 96.045-104.26% respectively. The proposed methods were successfully applied to the determination of commercially available tablets and the obtained results were compared statistically with pharmakope methods of Student-t and Fischer-F tests. It was found that there is no significant difference between methods in terms of the mean values and standard deviations at %95 confidence levels. The developed method is simple, fast, selective, reproducible and reliable can be used safely routine simultaneous determination of cefdinir and clavulanic acid in tablets.

Keywords: Cefdinir, Clavulanic Acid, HPLC, Simultaneous Determination, Drug Analysis

This study was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project numbers: 39303).

➢ *ORAL PRESENTATION*

Mitochondrial DNA variations of the populations of *Mus domesticus* and *Mus macedonicus* (Rodentia: Muridae) distributed in the Marmara Region

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Abstract

"The Turkish strait system" (Istanbul and Çanakkale Straits, and the Marmara Sea) located in the Marmara Region has been considered to constitute a geographic barrier for the allopatric differentiation of the species living in Thrace and Anatolia. Also, the lack of connection of the islands in this region with the mainland has been known to be an absolute enhancer effect on the interruption of the gene flow between populations as it was in general evolutionary perspective. In line with this direction, it was aimed to investigate the effects of "the Turkish strait system" on the genetic differentiation of *Mus domesticus* and *Mus macedonicus* populations from Thracian and Anatolian parts of the Marmara Region, and to determine the genetic characteristics of island populations of those two species. In addition to them, the reconstruction of the phylogenetic relationships between and within these species living in the Marmara Region was intended. Genetic diversity and the phylogeny were described by using a 1089 base pair length of cytochrome *b* gene sequences of mitochondrial DNA. A total of 100 samples from Thracian and Anatolian parts of the Marmara Region, Gökçeada, Bozcaada, and Marmara Island were used. The genetic variations in the populations were determined by two model-based (ML and BI), one distance-based (NJ) phylogenetic analyses, and network analysis. DNA polymorphism, the number of haplotypes, haplotype, and nucleotide diversity, and standard deviations of them were determined. Genetic distance was calculated using the Kimura-2 parameter (K2P) model with 1000 bootstrap replicates. According to the results of the study, it was determined that *M. macedonicus* lives in only Gökçeada, while, *M. domesticus* find in Marmara Island and Bozcaada. On the mainland, it was detected that both species live in both Thrace and Anatolian parts of the Marmara Region. A total of 33 *M. domesticus* haplotypes and 19 *M. macedonicus* haplotypes were identified by the genetic analyses. The populations of both species were clearly split into two main clades in all phylogenetic trees. However, the intraspecific phylogenies were more complex than interspecific phylogeny. The mean genetic distance (Kimura-2 Parameter) value between two species was at the level of 0,073±0,008 (K2P = 7.3%) representing the separate biological species. Contrary to this, the level of intraspecific genetic differentiation between subpopulations (grouped according to their geographic distribution) within both species was low (K2P = 0.5% or 0.6% in *M. domesticus*, K2P = 0.4%, 0.5% or 0.6% in *M. macedonicus*). Also, both species had haplotype values at moderate level and nucleotide diversity values at low level ($h = 0.924$, $\pi = 0.00486$ for *M. domesticus*, $h = 0.843$, $\pi = 0.00377$ for *M. macedonicus*). Due to the low level of genetic differentiation detected between the subpopulations of both species, it was thought that the "Turkish strait system" does not constitute a strong geographical barrier in terms of the phylogenetic evolution of these two species. The population of *M. macedonicus* in Gökçeada had completely island-specific haplotypes, whereas, gene flow was continuous between the island populations (Marmara Island and Bozcaada) and the mainland populations of *M. domesticus*. It was observed that the populations of *M. domesticus* from Bozcaada and Marmara Island were genetically similar to Thracian and Anatolian populations. The Gökçeada population of *M. macedonicus* was found to be closer to Thracian populations. The Kocaeli population of *M. macedonicus* was different from the other populations of the species in that it always forms a separate group on phylogenetic trees and had unique haplotypes.

Keywords: *Mus macedonicus, Mus domesticus, mitochondrial DNA, phylogeny, Marmara Region, Turkey*

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➢ *ORAL PRESENTATION*

Döl tutmayan (repeat breeder) yüksek verimli süt ineklerinde pgf2α ve metricure uygulamasının gebelik oranı üzerine etkisinin araştırılması

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Özet

Bu çalışmada yüksek verimli döl tutmayan (repeat breeder) ineklerde (1. laktasyon) metricure uygulamasının gebelik oranlarındaki başarısı üzerine etkisinin araştırılması amaçlandı. Çalışma materyalini, 2-3 yaşlarında, en az 3 kez tohumlandığı halde gebe kalmayan ve düzenli östrüs siklusu gösteren aynı ırktan (holstein) 87 baş inek oluşturdu. Klinik muayeneler sonucu ineklerin genital organlarında makroskopik bir bozukluk teşhis edilmedi. Hayvanlar PGF2α (kontrol) ve PGF2α + Metricure uygulanan hayvanlar olmak üzere iki gruba ayrıldı. Birinci grup (41 hayvan) hayvanlara ultrasonografik muayene yapılarak +3 CL (corpus luteum) tespit edilenlere tek doz PGF2α enjeksiyonu yapıldı. İkinci gruptaki (46 hayvan) hayvanlara ise aynı şekilde ultrasonografik muayene yapılarak +3 CL tespit edilenlere tek doz PGF2α enjeksiyonu ve bununla birlikte metricure uygulaması yapıldı. Her iki gruptaki hayvanlar bu uygulamalardan sonra görülen ilk östrüslerinde tohumlandı. Tohumlamalardan 30 gün sonra yapılan gebelik muayenesi sonucunda birinci gruptaki 41 hayvandan 17 tanesinin, ikinci grupta bulunan 46 hayvandan ise 30 tanesinin gebe olduğu tespit edildi. Yapılan çalışma sonucunda döl tutmayan yüksek verimli hayvanlarda PGF2α uygulamasıyla birlikte yapılan metricure uygulamasının olumlu etkilerinin olduğu görülmüştür.

Anahtar Kelimeler: Repeat Breeder, Metricure, Pgf2α, Gebelik Oranı

➢ *ORAL PRESENTATION*

The antimicrobial activity of *Melissa officinalis* L. encapsulated in liposomes

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Abstract

Melissa officinalis L. (lemon balm) is an aromatic perennial herb, growing in the Mediterranean region, western Asia, southwestern Siberia, and northern Africa. The leaf of *Melissa officinalis* contains a lot of bioactive compounds such as flavonoids, polyphenolic compounds, terpenes, and tannins. The aim of this study was to investigate the antimicrobial activity of extracted bioactive compounds of *Melissa officinalis* encapsulated in liposomes against selected bacterial strains, considering advantages of liposomes such as carrying large and both hydrophilic and hydrophobic drug payloads, controlled release, capacity for self-assembly. Briefly, liposomes was prepared by ultrasonic homogenizer using soybean phospholipid under 75% amplitude and constant ph for 5 minutes and then loaded with lyophilized extract at 1:1 ratio and characterized. This formulation was used for evaluating antimicrobial activity against *S.aureus* ATCC 25923 and *E.coli* ATCC 25922 strains by Kirby-Bauer disc diffusion method and broth microdilution method offered by CLSI. Also, Folin-Ciocalteu assay was conducted to determine the total phenolic content in the extract of *Melissa officinalis*. Antimicrobial tests showed that the formulation has potential antimicrobial activity against tested bacterial strains. Replicates made at least 3 times for statistics and the formulation will be tested for cytotoxicity.

Keywords: Liposomes, Antimicrobial, Extraction, Encapsulation, Homogenization

➢ *ORAL PRESENTATION*

İnek sütünden üretilen Edirne tipi Beyaz peynirin depolama boyunca ACE inhibitör aktivite değişiminin belirlenmesi

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Özet

Anjiyotensin Çevirici Enzim (ACE) -inhibitör maddeler günümüzde hipertansiyon tedavisi için en iyi stratejilerden biri olarak düşünülmektedir. Sütün ana bileşini kazein ve peynir altı suyu proteinleri, temel biyoaktif peptit öncüleridir. Süt proteinlerinden elde edilen biyolojik aktif peptilerin çoğu ACE-inhibitör etki göstermektedir. ACE, substratın sonundaki karboksil terminalı temizleme kapasitesine sahiptir böylece anjiyotensin I'i kuvvetli kan damarı daraltıcı olan aktif bir peptid hormonu anjiyotensin II' ye dönüştürerek kan basıncındaki artışı düzenler. Yüksek tansiyon, koroner kalp hastalığı ve felç gibi kalp-damar hastalıklarının tedavisinde ve korunmasında önemli rol oynadığında için antihipertansif aktiviteli gıdaların gelişimine ilgi giderek artmaktadır.

Bu çalışmada, Edirne ilinden temin edilen inek sütünden tekniğine uygun olarak üretilen Edirne Tipi Beyaz peynirin ACE inhibitör aktivite özelliğinin depolama süresince değişiminin belirlenmesi araştırılmıştır. Bu amaçla, %11,84 kuru madde, %3,6 yağlı ve %0,17 titrasyon asitliğine sahip inek sütü 67±2℃ de 10-15 dakika pastörizasyon işlemine tabi tutulmuş, 30±2 ℃ de starter kültür eklemesi yapılmadan 1/16000 maya kuvvetine sahip rennin enzimi ile pıhtılaşma süresi 90 dk olacak şekilde mayalanmıştır. Pıhtı kesimi, peynir altı suyunun ayrılması ve baskılama aşamasından sonra 13 bome salamurada ön olgunlaştırma (pH 5,2-4,8) için 1 gün bekletilerek tenekelere yerleştirilen peynirler hava sızdırmayacak şekilde kapatılarak ve 4±2 ℃'de depolanarak 180 gün olgunlaştırmaya bırakılmıştır. Olgunlaştırmanın 1., 30., 90., 150. ve 180. günlerinde peynirlerin suda çözünebilir ekstralarının ACE inhibitör aktivitesi hippuril-L-histidil-L-lösin (HHL)'den serbest kalan hippurik asitin 228 nm'de spektrofotometrik olarak belirlenmesi ile tespit edilmiştir. Yapılan analizler sonucunda, olgunlaşma sürecinde peynirin proteoliz derecesine bağlı olarak ACE-inhibitör aktivitenin 150. güne kadar arttığı, olgunlaşma süresinin ilerlemesi ile ACE inhibitör özelliğe sahip peptitlerin ileri derecede proteolizinin bir sonucu olarak ACE inhibitör aktivitenin azalma yönünde değişim gösterdiği tespit edilmiştir.

Anahtar Kelimeler: ACE inhibitör aktivite, proteoliz, biyoaktif peptit

➢ *ORAL PRESENTATION*

Nigella sativa ve başlıca bileşeni olan timonkinonun kanserleri önlemede ve tedavideki yeri

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Özet

Timokinon, *Nigella sativa* olarak da bilinen çörek otu bitkisinin aktif bileşeni olup yüzyıllardır bronşit, baş ağrısı, egzama, grip gibi çeşitli hastalıkların tedavisinde kullanılmaktadır. Bunun yanında; *Nigella sativa* ve timokinonun antiinflamatuvar, antibakteriyel, antitümor, antioksidant, antiproliteratif ve antimetastazik etkileri yapılan bilimsel çalışmalarla kanıtlanmıştır. Kanser ise, çağımızın vebası olarak da bilinen, hücre proliferasyonunun bozulması ya da apoptoz mekanizmasının hasara uğramasıyla ortaya çıkan kontrolsüz hücre çoğalmasıdır. Timokinon, kanser hücrelerine farklı yolaklar üzerinden etki eder. Çörek otu ekstraktı, doza bağımlı olarak, meme kanseri hücre hatlarında AKT/ PI3K yolağı ile hücre proliferasyonunu inhibe etmiş veya kaspaz-3, kaspaz-8, kaspaz-9 ve p53 ekspresyonunu arttırarak apoptozu indüklemiştir. Ayrıca; çörek otu özütü, radyasyon tedavisi ile birlikte kullanıldığında meme kanserine karşı sinerjik etki göstermiştir. Hepatoselüler karsinomada kullanılan metanolik çörek otu özütü, karaciğer enzim parametrelerinin önemli ölçüde normale yakın aralıkta kalmasını sağlamıştır. Timokinon, hepataselüler karsinomada hücre döngüsü progresyonunu düzenleyerek tümörün büyümesini baskılamıştır. *Nigella sativa* veya metanolik özütünün, hepatoselüler karsinomonun önlenmesinde kullanılması önerilmiştir. Kolon kanseri üzerinde uygulanan timokinon, apoptozu arttırıcı etki göstermiştir. Timokinon; over kanseri, meme kanseri, pankreas kanseri ve prostat kanseri gibi çeşitli kanser türlerinin tedavisinde besin desteği olarak kullanılabilmektedir. Lösemili ratlara timokinon uygulanması ile löseminin ilerlemesi yavaşlamıştır. Timokinon ile yapılan doz çalışmalarında 10-100 mg/kg aralığında her hangi bir toksik etki ya da mortalite gözlenmemiştir. Timokinonun kapsül halinde nanopartikül olarak kullanımı, serbest halde kullanımından daha etkili bulunmuştur. Bu çalışma ile *Nigella sativa* ve başlıca bileşeni olan timokinonun kanserlerin önlenmesi ve tedavisindeki rolü güncel literatur eşliğinde incelenmiştir.

Anahtar Kelimeler: *Nigella sativa*, Timokinon, Kanserler

➢ *ORAL PRESENTATION*

Effect of flunixin meglumine treatment on pregnancy rate in embryo transfer in different breed cattle

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Abstract

The aim of the present study was to investigate the effect of flunixin meglumine treatment on pregnancy rate during and after the transfer of embryos obtained in vivo to beef and dairy cattle.
In this study, 3-4 aged Holstein cows with a body condition score of 3-3.25 were used as donors. Progesterone-based estrus synchronization and superovulation protocol was applied to the donor cows included in the study. Double dose PGF2α was administered to the recipient animals for synchronization. Uterine flushing was performed on the 7th day after artificial insemination. A total of 295 transferable embryos were obtained. The obtained embryos were transferred to Angus cows (n = 85), Holstein heifers (n = 80) and Holstein cows (n = 130). After the transfer, these animals were divided into 3 subgroups. 1.1 mg / kg flunixin meglumine was applied to the first group (ET) during embryo transfer, and to the second group (ETS) flunixin meglumine was applied both during the embryo transfer and on the 8th and 9th days after the transfer. No application was made to the third group and it was determined as the control group. Pregnancy examinations of the recipients were performed with real-time ultrasonography on the 23rd day after the transfer. After embryo transfer, the pregnancy rate in Angus cows, Holstein heifers and Holstein cows were 43.52%, 42.5 and 24.61%, respectively ($P < 0.05$). When the animals were not classified according to their breeds, pregnancy rates in ET, ETS and control groups were determined as 29.29%, 45.10 and 29.79%, respectively ($P < 0.05$). However, conception rates in the ET and ETS subgroups were higher in Angus cows and Holstein heifers than in Holstein cows ($P < 0.05$).

As a result, it was determined that the pregnancy rate obtained after embryo transfer was higher in Angus cows and Holstein heifers compared to Holstein cows, and flunixin meglumine treatment during and after transfer increased the rate of conception. In addition, it was concluded that the flunixin meglumine treatment during embryo transfer and during / after transfer in Angus cows and Holstein heifers may be effective in increasing the conception rate.

Keywords: Embryo transfer, flunixin meglumine, conception rate.

➢ *ORAL PRESENTATION*

Kinetics Analysis for Calcium Carbonate Prepared using Threonine

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Abstract

Thermal decomposition behavior of calcium carbonate crystals obtained in the absence and the presence of threonine used as an additive was explored at different heating rates at a temperature ranging from 25 to 1000 °C in a thermogravimetric analyzer (TGA). Thermal decomposition kinetics of calcium carbonate was investigated using the model-fitting and model-free methods. According to the model-fitting method (Coats–Redfern), the thermal decomposition kinetics of the calcium carbonate in pure media estimated by the R_3 type model showed the best agreement with the experimental data out of the tested models with high accuracy (R^2 =0.9954). In addition to kinetic analysis, the thermodynamic parameters of the calcium carbonate crystals were calculated in this study. Finally, the evolved gases released during the thermal decomposition of calcium carbonate were determined by means of TGA-FTIR.

Keywords: Calcium carbonate, thermal decomposition, kinetic modelling, TGA-FTIR.

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➢ *ORAL PRESENTATION*

Fetal amelia: Case report

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Abstract

Introduction:Examination of the extremities is one of the important components of midtrimester ultrasonography. However, due to structures such as the heart and brain, which can be more difficult to evaluate and require more experience, sometimes the necessary importance is not given to the evaluation of the extremities. However, the high functional importance of limb reduction defects in the postnatal period causes them to be one of the most important medicolegal issues.

Case: 35-year-old, multiparous pregnant was referred to our hospital for detailed ultrasonography at 20 weeks of gestation. The combined test trisomy 21 risk was 1/186, but the patient refused invasive diagnostic test. There was no consanguinity, no genetic or syndromic disease in the family, and no problem was experienced in pregnancy follow-up.

During the ultrasonographic evaluation; the left upper extremity was not observed, there was strangulation at the left lower extremity and still there was still blood flow. No additional structural anomalies and amniotic band sign were detected (Figure 1).

After giving the detailed information, amniocentesis was performed and it was resulted as 45X*. On the basis of the ultrasound findings, the couple opted for termination of the pregnancy. Postnatal examination demonstrated left auricular anomaly, total absence of the left upper extremity and strangulation at the left lower extremity (Figure 2).

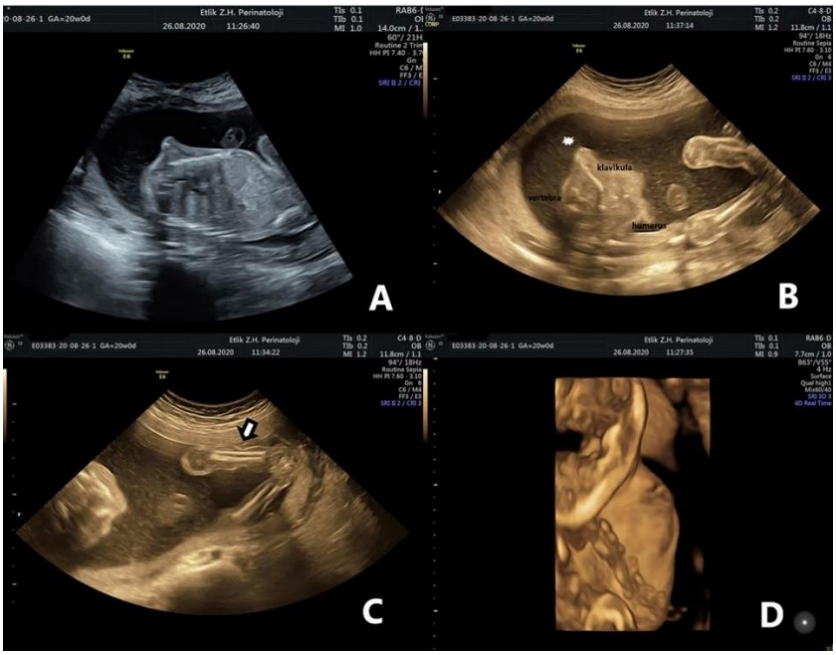

Figure 1: Antenatal ultrasonographic images

A: It is observed that the left upper extremity is not arising at the level of the scapula in the coronal section of the fetal trunk. **B:** The left upper extremity could not be observed in the axial section of the clavicle level. **C:** strangulation at the left lower extremity. **D:** The left upper extremity cannot be seen in 3D examination.

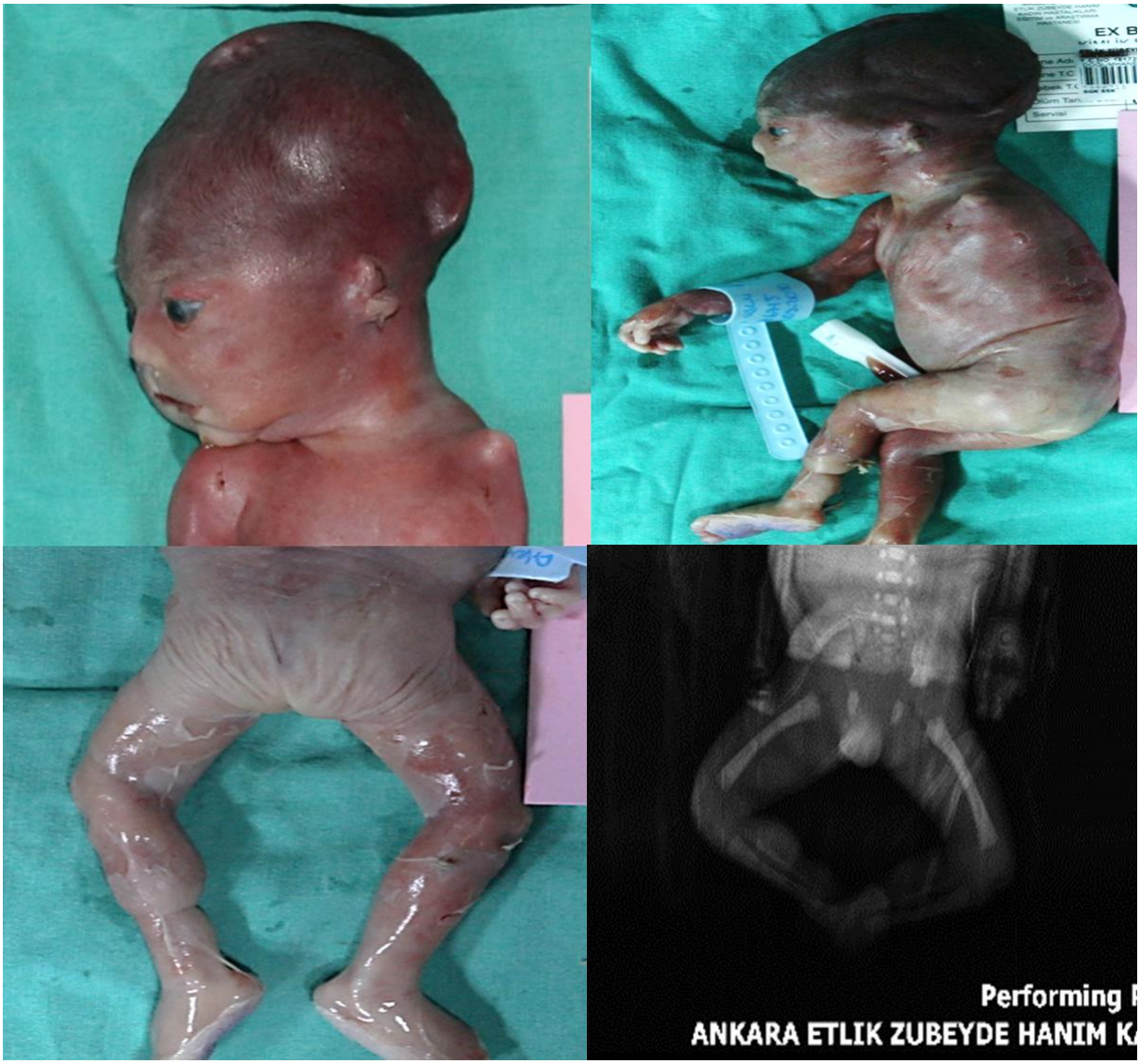

Figure 2: Postnatal examination findings and x-ray image

Postnatal examination images confirmed left auricle anomaly, absence of left upper extremity and strangulation of the left lower extremity. In the x-ray image, there is no bone defect in the left lower extremity.

Discussion

Numerous conditions associated with the absence of extremities have been described in the literature. However, amelia can also be seen as an isolated anomaly. The rate of prenatal ultrasonographic detection of the amelia varies according to the pregnancy follow-up policies of the countries. This rate is about 24% in isolated cases and 64% in cases with concomitant anomalies. In our case, although the amniotic band was not detected, the asymmetric findings and the constriction ring in the lower extremity was suggested amniotic band syndrome in diagnosis.

Keywords: Amniotic band syndrome, Absence of limbs, Fetal amelia, Fetal ultrasound, Prenatal diagnosis.

➢ *ORAL PRESENTATION*

Caving impacts on cave bacterial diversity: A case study of camping in Morca cave, Turkey

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Abstract

Caves are characterized by extreme conditions such as low constant temperature, and restricted nutrients under which, extreme organisms secrete bioactive compounds, like enzymes and antimicrobial substances. In this context, conservation of this diversity is economically important. However, to explore such ambiguous areas cavers camp inside, depending on the cave structure. Revealed the impacts of caving activities is important for the cave protection. In this study, such impacts were investigated by comparing bacterial diversity observed before (T1) and after (T2) 4 days camping inside Morca cave. DNA of the samples were isolated and sequenced for Next Generation Sequencing (NGS). Alpha-diversity analysis of the samples was determined using the OTU table with the QIIME software. Chao1, Shannon, Simpson and observed OTUs indices indicating the diversity levels were calculated. Total observed OTUs was 3131 at T1 while at T2 it was 3739. Chao1 index shown considerable community richness augmentation in both B and C areas (2.9% and 6.1%). Contrary in area D where the observed OTUs has considerably decreased (-3.4 %). Considering Shannon index, microbial diversity in area E has been 2 times increased at T2, indicating the appearance of new bacterial members in this point. Taxonomic results show that bacteria belonging *Proteobacteria* dominated all the samples at T1 (64%) and T2 (42%). Further, members of *Firmicutes* augmented considerably in all T2' samples with a rate of 33.6%. In total, 124 genera were identified in T1' samples, and *Acinetobacter* was the most dominant (17.2%). Despite its dominance at T2, its rate decreased. Our results do not show any appearance of human related microorganisms like *E. coli* and *Staphylococcus*. However, the increasing of *Firmicutes* members and the decreasing observed on the *Proteobacteria* members and *Actinobacteria* members, could be related to caving activities during that period.

Keywords: Bacterial diversity, Camp, Cave, Cavers, Next Generation Sequencing-NGS.

➢ *ORAL PRESENTATION*

Denetimsiz makine öğrenmesi yöntemlerinin Covid-19 hastalığının belirlenmesindeki Performansı

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Özet

Koronavirüs hastalığı (COVID-19) ani salgından bu yana, hızla yayılan önemli bir küresel sağlık sorununa dönüşmüştür. COVID-19'un patogenezi ve spesifik tedavi hakkında yapıcı bilgi eksikliği nedeniyle, erken teşhisi ve zamanında tedavisi önemlidir. Bu nedenle, alternatif, daha hızlı ve daha erişilebilir testlere ihtiyaç vardır. Tıpta geleneksel teşhis yöntemleri hala kullanılmaktadır. Ancak bugün gelişen yapay zekâ teknolojileri sayesinde hastalık teşhis, tanı ve tedavi süreçlerinde hekimlere destek olacak güçlü araçlar sunulabilmektedir. Bu çalışmada yapay zekânın bir alt alanı olan denetimsiz makine öğrenme algoritmalarından K-Means ve Agglomerative Hiyerarşik Kümeleme yönrtemleri Covid-19 hastalarının sınıflandırılması için kullanılmıştır. Covid-19 teşhisi ile yoğun bakım ve diğer servislerde yatan 556 hastanın rutin yapılan biyokimyasal, hemotoljik ve immünolojik toplam 40 parametre laboratuvar sonuçları toplanmıştır. Bu kapsamda rutin laboratuvar bulgularına göre Covid 19 hastalığının şiddetli geçirenler ile hafif geçirenlerin sınıflandırılmasında, çalışmada kullanılan modellerin başarıları karşılaştırılmıştır. Yöntemlerin parametreleri küme sayısı, mesafe eşiği, bağlantı tipi ve mesafe ölçütü dikkate alınarak en verimli model elde edilene kadar iterasyon devam ettirilmiştir. Başlangıç parametreleri random seçilmiştir. K-means kümeleme yönteminde optimum k değerinin hesaplanmasında WCSS hesaplaması dikkate alınmıştır. Agglomerative Hiyerarşik kümeleme yönteminde ward’s metodu uygulanarak optimum homojen küme sayısının belirlenmesi için otomatik-entropi kullanılmıştır. Yöntemlerin doğruluk değeri ve tanı kriterlerine göre başarıları karşılaştırılmıştır. Sonuç olarak, % 82.73 doğruluk oranı ve % 85.21 specificity değeri ile K-Means yöntemi COVİD-19 hastalığını daha hafif geçiren yoğun bakım ünitesi dışındaki servis hastalarının tespitinde Agglomerative Hiyerarşik Kümelemeye göre daha başarılı bulunmuştur.

Anahtar Kelimeler: Covid-19, Makine öğrenmesi yöntemleri, Denetimsiz makine öğrenmesi, Denetimsiz öğrenme

➢ *ORAL PRESENTATION*

The effect of flunixin meglumine supplementation on embryo development and quality in *in vitro* embryo production in cattle

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Abstract

The aim of the present study was to evaluate the effect of the flunixin meglumine supplementation, which is a nonsteroidal anti-inflammatory drug, to the culture medium on embryo development and quality during in vitro embryo production.

Cumulus oocyte complexes obtained from ovaries of Holstein breed cattle collected from slaughterhouses were used as material in the study. A and B type cumulus oocyte complexes obtained according to the International Embryo Technology Society (IETS) criteria were taken into a medium containing TCM-199 + 5% Calf Serum for maturation. After maturation, oocytes were transferred to the BO (Bracket and Oliphant) solution together with the sperm prepared by Percoll technique for fertilization. The probable zygotes were then divided into two groups before being transferred to culture. 5 ng / ml flunixin meglumine was added to the culture medium of the first group (FM, n = 110). Any supplementation was not applied to the control group. (C, n = 110). Probable zygotes were cultured for 7 days with CR1aa culture medium. The developmental stages and quality grading of the embryos obtained at the end of the culture process were made according to IETS criteria. After culture, the rates of reaching blastocyst in FM and control groups were 28.18% (31/110) and 19.09% (21/110), respectively. However, the number of Code 1 quality blastocysts was also higher in the FM group compared to the control group.

As a result, it was concluded that the flunixin meglumine supplementation to the culture medium during in vitro embryo production in cattle had a positive effect on the rate of reaching the blastocyst, embryo quality and development.

Keywords: Embryo production, Flunixin meglumine, Blastocyst reaching rate.

➢ *ORAL PRESENTATION*

The Effect of Salt on Hemolysin Transcription of Clinical and Environmental Strains of *Vibrio vulnificus*

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Abstract

Vibrio vulnificus is a highly fatal human pathogen which naturally resides in coastal marine environments worldwide and has been isolated in high numbers from a diverse range of seafood, mostly in oysters. It accounts for the highest seafood-related deaths in the USA. The most lethal infection caused by this pathogen is septicemia and its mortality rate is about 50%. It also causes wound infections with a mortality rate of 25% in immunocompromised patients. *Vibrio vulnificus* strains were isolated from oysters and seawater. Following biochemical tests, the isolates were confirmed with hemolysin/cytolysin-encoding strain-specific gene-*vvhA*. Hemolysin/cytolysin is a potent exotoxin which causes several pathological effects and is also responsible for cytotoxic activity in animal models. Genetic distinction between clinical and environmental strains was made based on 16S rRNA *type A/B* and *vcg E*/*C* genes. Alkaline Peptone Water (APW) supplemented with 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% sodium chloride (w/v) was inoculated with strains having clinical and environmental origin. Relative changes in expression of the hemolysin/cytolysin-encoding gene were determined based on the $2^{-\Delta\Delta CT}$ method. The highest *vvhA* transcription was found at the salinity of 1.0 and 1.5% (Bonferroni *post hoc* test; $P<0.05$). All strains revealed almost similar expression patterns except two clinical strains originated from oysters. These two strains expressed higher *vvhA* than the others at 1% NaCl. The current study revealed that salinity appears to be very significant in *vvhA* expression by *Vibrio vulnificus*.

Keywords: *Vibrio vulnificus*, hemolysin, salinity, pathogen.

➢ *ORAL PRESENTATION*

Adropin and spexin hormones regulate matrix metalloproteinases in chronic kidney failure

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Abstract

Background: Chronic kidney failure (CKF) is indicated as a chronic and progressive deterioration of renal functions. Pathological conditions occur when the modulation of matrix metalloproteinase (MMP) is impaired in the tissue. It has been reported that the amounts of MMPs increased significantly, especially in cases of renal damage, as well as fibrotic damage developed in parallel. Spexin and adropin are protein hormones that regulate energy metabolism. Apart from this, it provides keeping the cardiorenal functions in balance.
Objective: Aim of this study, is to investigate how adropin and spexin hormones affect MMPs in chronic kidney failure.
Method: For this purpose, chronic kidney failure was induced in rats by administering adenine hemisulfate. Renal functions tests were measured by autoanalyzer. mRNA expressions of MMP-1, MMP-2, MMP-3 and MMP-13 genes in kidney tissue were measured by qPCR.
Results: We observed an increase in 24-hour urine volume, serum creatinine, BUN and urine protein levels significantly higher in the CKF group. Urine protein and 24-hour urine volume were decreased with adropin and spexin treatments. mRNA expressions of MMP-2 were significantly increased by CKF group compared to vehicle group, however MMP-13 levels were significantly decreased by CKF. In addition MMP-2 gene expression was significantly decreased by spexin treatment compared to CKF group. Also MMP-2 and MMP-3 gene expression levels significantly downregulated by adropin treatment compared to CKF group, conversely MMP-13 level upregulated by adropin treatment. Moreover MMP-1, MMP-2 and MMP-3 levels significantly decreased by adropin+spexin combined treatment compared to CKF.
Conclusion: Our findings indicate that renal functions and MMPs were modulated by adropin and spexin peptides. These peptides may have protective effects on renal failure progression.

Keywords: chronic kidney failure, adropin, spexin, matrix metalloproteinase

Acknowledgement: This work was supported by Amasya University Research Fund (Project no: FMB-BAP 20-0444) and Trakya University Research Fund (Project no: 201/118).

➢ *ORAL PRESENTATION*

Antibacterial activity of zinc incorporated silica aerogels against *Escherichia coli*

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Abstract

Bacterial infections are one of the biggest difficulties in the treatment of skin injuries. Rapid appearance of antibiotic resistant bacteria leads to growing treatment difficulties. Zinc has important role in wound healing, blood vessel growth and bone cell metabolism. Furthermore, zinc ions show antibacterial activity, which depend on contact time, concentration, particle shape and size of the zinc material. In this study, Zn was incorporated to the silica aerogel to obtain a material with slowed ion release that can have advantages such as long term antibacterial activity. In silica aerogel production, sodium silicate was used as precursor, and tetraethylorthosilicate (TEOS) was added at the aging step in the sol-gel synthesis method. Amine functionalized silica aerogels were produced using a similar method in which 3-aminopropyltriethoxysilane (APTES) is included as a surface modifier after the aging step. This method was slightly modified to produce Zn incorporated silica aerogels, briefly $Zn(SO_4)\cdot 7H_2O$ was added (1) in the gelation step, before APTES modification, and (2) in the solvent exchange step, after APTES modification. Aerogel samples were characterized by SEM, EDX, FTIR, and surface area measurements were performed. Zn release from silica aerogels in PBS was measured using ICP-MS. The antibacterial activity of aerogel samples were investigated on *Escherichia coli* (ATCC25922) via Standard Colony Count Method at 10, 20, 30, 40 and 50 mg/ml concentrations. Weighted amounts of aerogel samples were added into 1 ml of $1.0x10^6$ CFU/ml bacterial suspension in Mueller Hinton broth and incubated for 24 hours at 37°C. Thereafter, 10 µl of bacterial suspension was taken and plated on Mueller Hinton agar plates. The colonies formed were counted and, antibacterial activity was presented as viability %. Aerogel obtained by the addition of Zn in solvent exchange step showed antibacterial activity and higher zinc ion release in PBS.

Keywords: Silica aerogel, Zinc, Antibacterial activity.

➢ *ORAL PRESENTATION*

Genome wide identification of *OFP* gene family members in *Phaseolus vulgaris* and their expression under salt and drought stress

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Abstract

Ovate family protein (OFP), a class of plant-specific transcription factors, plays a role in regulating the growth and development of plants, including regulation of fruit shape, synthesis of secondary cell walls, ovule and vascular bundle development, DNA repair, and brassinolide signaling. *Phaseolus vulgaris* is the most widely grown legume worldwide. The goal of this study was to determine the expression levels of OVATE genes in *P. vulgaris* leaf tissue under salt and drought stress and to perform genome-wide analysis of OVATE gene family members using bioinformatics tools. Twenty-one Pvul-OFP genes with molecular weights between 20.4 kDa and 47.1 kDa, amino acid numbers between 180 and 405, theoretical isoelectric point between 5.26 and 9.85, and instable index between 31.15 and 69.69 have been identified in the common bean genome. Exon numbers estimated as a result of gene structure analysis were determined as 1 and 2. Some Pvul-OFP genes contain no intron, while other Pvul-OFP genes contain only one intron. Phylogenetic relationships among members of *P. vulgaris*, *Arabidopsis thaliana* and *Glycine max* OFP gene family were analyzed. Pvul-OFP-1/Pvul-OFP-12/Pvul-OFP-13, Pvul-OFP-2/Pvul-OFP-15, Pvul-OFP-4/Pvul-OFP-10, Pvul-OFP-7/Pvul-OFP-14/Pvul-OFP-21, Pvul-OFP-8/Pvul-OFP-20 and Pvul-OFP-9/Pvul-OFP-16/Pvul-OFP-17/Pvul-OFP-19 genes were segmentally-duplicated. Differences in the expression levels of Pvul-OFP genes were determined as a result of in silico analysis in the leaves of common bean plants exposed to salt and drought stress. While the expression level of Pvul-OFP-20 increased in common bean leaves under salt stress, the expression level of Pvul-OFP-13 and Pvul-OFP-9 decreased. On the other hand, while the expression level of Pvul-OFP-21 increased in common bean leaves under drought stress, the expression level of Pvul-OFP-16 and Pvul-OFP-18 decreased. No change was observed in the expression level of Pvul-OFP-7 under both stress conditions. The results of this study will guide future physiology and breeding studies on Pvul-OFP genes.

Keywords: abiotic stress, gene expression, OVATE family protein, phylogenetic analysis, transcription factor

➢ *ORAL PRESENTATION*

Multiple infections by bipartite dsRNA mycoviruses and an unassigned virus in the ascomycetous fungus *Caloscypha fulgens*

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Abstract

Virus populations of uncultivated fungi remain scarcely studied. In this presentation, we report on twelve new mycoviruses co-inhabiting an isolate of the ascomycetous spring orange peel fungus *Caloscypha fulgens*. Eleven of these identified viruses were defined as segments of the bipartite dsRNA mycoviruses from the family *Partitiviridae* and the unclassified virus group including the mutualistic Curvularia thermal tolerance virus (CThTV). The remaining one virus, designated as Caloscypha fulgens mycovirus A (CfVA) had a non-segmented RNA genome that encodes for a 3016 aa long polyprotein from a single ORF. Based on the BLASTp analysis and phylogeny of the RNA dependent RNA polymerase (RdRp) domain, the closest relatives of CfVA were found to be members of the recently proposed dsRNA virus family *Megatotiviridae*. Additionally, as a result of the sequence analyses, phlegivirus related 2A-like protease domain was identified in the N-terminal region of the CfVA polyprotein. This finding indicated a horizontal gene transfer event between these distant virus groups. The mycoviruses reported herein are the first viruses described in Caloscypha fulgens, and CfVA characterized in this study appears as a member of a yet unassigned virus family.

Keywords: mycoviruses; dsRNA; multiple infection; viral diversity

➢ *ORAL PRESENTATION*

Determination of the *In vitro* Enzyme Inhibitory Properties and Antioxidant Activity of *Moltkia coerulea* (Willd.) Lehm. (Boraginaceae) Medicinal Plant Growing in Batman Region, Turkey

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Abstract

Batman province is located in the Southeastern Anatolia region, and is important in terms of ecosystem and plant biological diversity. Recently, researching the economically important plant species has become a necessity in the province. In this context, while the members of the Boraginaceae genus have found a wide application area in traditional medicine in many countries from ancient times to today, they have been used for many purposes in our country. Most of the members of this family are medically important plants containing secondary metabolites such as flavonoids, terpenoids, alkaloids, fatty acids, glycosides, phytosterols, and various proteins. *Moltkia* genus (Boraginaceae) comprises of five species all growing in the Eastern part of the Mediterranean region. Among them, *Moltkia coerulea* (Willd.) Lehm. occurs in Anatolia, Lebanon and the Krimea; and *M. aurea* Boiss. is endemic to Anatolia. *Moltkia* species of Turkey known as "emzik çiceği, sancı out, sormuk, sarı kesen", are used against various health problems such as kidney disorders, diarrhea and abdominal pain traditionally. In this study, it was aimed to determine the antidiabetic and antioxidant activity of *Moltkia coerulea* (Willd.) Lehm, which has not been studied before, which grows in the untouched Mount Batıraman, Batman. The plant have been provided from Batıraman campus of Batman University. α-amylase and α-glucosidase inhibition results of methanol and water extracts of *Moltkia coerulea* were calculated as acarbose equivalent (mmol AKAE /g extract). α-amylase and α-glucosidase inhibition results are shows that the most effective extract on both amylase and glucosidase enzyme has been found to be methanol. In addition, the extracts were tested against the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free-radical for antioxidant activity. The highest antioxidant activity is MeOH % 61.2 for DPPH• method. These results shows that *Moltkia coerulea* could be used as potential natural antioxidant sources.

Keywords: Antidiabetic, antioxidant activity, *Moltkia* Lehm.

➢ *ORAL PRESENTATION*

Expressions of dopamine D2 Receptors and Neurofilament along to CA1 and DG regions of hippocampus as a result of using monosodium glutamate

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Abstract

Monosodium glutamate (MSG) is a flavor enhancer that is included in many ready-made foods and has a negative effect on neurons in the brain. The aim of the study is to investigate the possible toxic effect of MSG on neurons in the hippocampus regions of rats in childhood and the protective effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by immunohistochemically examining the expression of Neurofilament (NF) and Dopamine receptor D2 (D2R) in the brain. Wistar albino rats in childhood were used as 6 females in each group. Control (0,9% saline solution, 1.3.5.7.9. days, sc), MSG (4 mg/g 1.3.5.7.9. days, sc), MSG + EPA and MSG + DHA (4 mg/g 1.3.5.7.9. days sc + 300 mg/kg 9 day orally). At the end of the 9th day, the hippocampus regions of the brain tissues were fixed for immunohistochemistry staining. According to our findings, NF and D2R reactions in Cornu Ammonis (CA1) and Dentate Gyrus (DG) regions in the hippocampus area showed a strong reaction in memory neuron cytoplasms in the control group, while their expression was decreased in both regions in the MSG group. In the MSG-EPA and MSG-DHA groups, the NF and D2R immunoreactions in the neurons in the same region were similar to the control group. No significant difference was observed in expressions in hippocampal neurons between the MSG-EPA and MSG-DHA groups. As a result; We think that MSG may cause neuronal inhibition due to the decrease in NF and D2R neural signaling molecules in the brain hippocampus CA1 and DG regions of rats in childhood compared to the control groups. In addition, it was concluded that the use of EPA and DHA in addition to MSG, could be one of the solutions that can protect from the negative neurotoxic effects of MSG.

Keywords: MSG, EPA, DHA, Hippocampus, CA1, D2R

➢ *ORAL PRESENTATION*

Milk yield vs reproductive performance: The genetic dissection in dairy cows

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Abstract

Milk yield and reproductive performance are two major traits in dairy cattle breeding. In the great majority of countries, dairy cattle are under increased stress in their management for high milk production. Accordingly, genetic evaluation in dairy cattle focuses on the determination of the genotypic background influencing milk yield and composition. It is important to note that a negative relationship between milk production traits and cow's fertility has been continually declared in dairy cattle. Selection for improved milk yield performance has caused an eventual decline of cow fertility, especially for Holsteins. Recently, decreased profitability and sustainability as a consequence of decreased reproductive performance have gradually led to an increased interest in non-production traits, including reproductive efficiency, longevity, and health. Dairy cow fertility is a very complex trait and it consists of several sub-traits including age at first calving, first insemination to pregnancy interval, calving interval, days before the first insemination, number of inseminations required for conception, gestation length, and calving ease. Moreover, an unfavorable genetic association of reproductive disorders with milk production, insufficient records, management factors, and the impact of environmental conditions make the situation more complex and complicate the analysis of the traits. On the other hand, many reproduction traits have low heritabilities, usually less than 5%, and they are difficult to ascertain concerning parameter estimation and genetic evaluation. Contemporary genomic studies have enabled the development of effective and highly accurate selection methods but it is important to include all important aspects of fertility to achieve a good and expected selection response. Taken altogether, the present assessment aimed to perform a comprehensive comparison between cow milk production and fertility based on genomic approaches. There has been a growing interest in broadening selection indices to include functional traits, such as reproduction and health. The information represented here can provide important points for an adequate genotypic evaluation of dairy cattle reproduction performance.

Keywords: cattle, fertility, milk yield, genetic marker, genomic selection.

➢ *ORAL PRESENTATION*

Determination of Essential Oil Yield in Various Sweet Basil (*Ocimum spp.*) Species and Selection of Suitable Production Method

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Abstract

Basil (*Ocimum spp.*) essential oils are valuable oils because of their antifungal, insecticide, antioxidant and antibacterial properties. However, essential oils are susceptible to heat, light and air. As they tend to oxidation, they can easily decompose and lose their properties and a characteristic odor. For this reason, it is essential to choose the right production method. In this study, volatile components of two types of basils (*Ocimum b. and Ocimum m.*) grown in Yalova (Marmara) and Bayındır (Aegean) regions were obtained by water distillation and supercritical fluid extraction methods. Analysis of the obtained etheric oil and volatile components was carried out by the Gas Chromatography / Mass Spectrometry (GC-MS) method. Fifty components were found in the water distillation sample obtained from *Ocimum b.* belonging to Yalova region, and 33 components were found from *Ocimum m.*.50 components were determined on the water distillation sample obtained from *Ocimum b.* of Bayındır region, and 40 components were determined on *Ocimum m*. Fewer volatile component species are found in the basil extracts obtained by supercritical fluid extraction under different conditions.

Keywords: *Ocimum Basilicum*, *Ocimum Minimum*, Basil, Essential Oil, Hydrodistillation.

➢ *ORAL PRESENTATION*

Transcriptomic analysis of *Arabidopsis thaliana* plants treated with Cycloastragenol

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Abstract

Cycloastragenol (CAG), a molecule isolated from '*Astragalus membranaceus'*, significantly stimulates the telomerase activity and cell proliferation. It is proven that CAG has ability to prevent some diseases in human. In this study, we aimed to figure out the CAG effects on the different signaling mechanisms in plants and to broadly analyze the genome-wide transcriptional responses in order to demonstrate CAG as a new key molecule that can potentially help plants to overcome different environmental stresses. RNA-seq strategy was employed to assess the transcriptional profiles in *A. thaliana* roots calli. Our work primarily focused an overall study on the transcriptomic responses of *Arabidopsis* to CAG. A total of 22.593 unigenes have been detected among which 1045 unigenes were differentially expressed. The up-regulated genes are principally involved in cellular and metabolic processes in addition to response to stimulus. The data analysis revealed genes associated with defense signaling pathways such as cytochrome P450s transporter, antioxidant system genes and stress responsive protein families were significantly upregulated. The obtained results can potentially help in better understanding biotic and/or abiotic tolerance mechanisms.

Keywords: Cycloastragenol, Arabidopsis thaliana, RNA-seq.

➢ *ORAL PRESENTATION*

Eumelanin polymer biosynthesis and its *in vitro* antibacterial effects

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Abstract

Melanins are macromolecular polymers produced by different biochemical pathways in many different living organisms, from microorganisms to humans. These natural pigments produced by polymerization of phenolic and / or indolic compounds are negatively charged, amorphous and heterogeneous. It is known that melanins have many important biological activities such as anti-inflammatory, antioxidant, antimicrobial, antiviral, antitumor, antivenin and liver protective activity. In recent scientific studies, it is seen that melanin pigment has radioprotective, photoprotective and nano drug carrier properties. Because of these important properties, melanin pigments are used in medicine and pharmacology.

In this study, it was aimed to determine the antibacterial activity of eumelanin pigment obtained from the *Streptomyces parvus* BSB49 strain. For this purpose, the agar well diffusion method was used. In addition to these processes, minimum inhibition concentration (MIC) values and minimum bactericidal concentration (MBC) values of eumelanin pigment were determined using 96-well microtitre plates. According to the results, it was concluded that the eumelanin pigment has a bactericidal effect for the selected target pathogens (8 Gram positive and 8 Gram negative bacteria) at concentrations of 125 - 250 mg / mL.

In addition to these processes, scanning electron microscopy (SEM) images of eumelanin pigment and energy dispersive X-ray (EDX) analysis results were obtained. It has been observed that the obtained granular images are compatible with the literature. Additionally, as a result of EDX analysis, elemental identification and quantitative compositional information was obtained.

Keywords: Eumelanin, antibacterial effect, MIC, MBC, SEM, EDX.

➢ *ORAL PRESENTATION*

Effect of GA_3 on *Indigofera zollingeriana*'s growth under *in vitro* salinity stress

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Abstract

Salinity stress is one of abiotic stresses limiting growth of many crops in many areas including Indonesia which has approximately 0.44 million ha of saline land. Indigofera, a high nutritional forage legume is tolerant to moderate salinity stress. Seeds stored at 4^{o}C temperature for 45 months were sterilized using sulphuric acid for 5 minutes followed by 3 × rinsing with sterilized distillited water. Thereafter, the seeds were soaked in sterile distillated water and thermoshaked for 4 days. These seedling were transferred to MS medium containing 140, 180, 220, 260, 300 and 340 mmol/L NaCl. Various concentration of gibberalic acid (GA_3) were applied to find the best concentration of GA_3 for recovering seed damage at LD_{50}. More than 80% germinated seedlings were noted on the shaked medium. It was found that estimated LD_{50} as 228 mmol/L of NaCl. The plant showed 100% mortality after 4 weeks of treatment on the medium containing 300 and 340 mmol/L NaCl. Application of low concentration GA_3 can protect seed damage under salinity stress at Ld_{50}. High concentration of GA_3 had negative effects on the plant growth.

Keywords: indigofera, leguminosae, lethal dose, plant growth hormone, salt stress.

➢ *ORAL PRESENTATION*

The importance of elderberry (*Sambucus nigra* L.) in healthy nutrition

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Abstract

Sambucus nigra L. (elderberry) is a perennial plant species belonging to the Caprifoliaceae family in shrub or semi-shrub form. It is commonly known as elder, elderberry, black elder, European elder, European elderberry, and European black elderberry. In this review study, it was explained that elderberry is a healthy food by prioritizing its effects on health. Elderberry, which has an important place in the history of alternative medicine, is also very valuable in modern medicine. Elderberry is rich in protein content, free and conjugated forms of amino acids, unsaturated fatty acids, fiber fractions, vitamins, antioxidants and minerals. For this reason, it is used as a multi-purpose food and nutritional supplement. Many positive effects of elderberry on human health have been observed. It is used in alternative medicine for rheumatic symptoms, insect bites, wound / burn treatment and as an antidiuretic. In research in modern medicine, natural polyphenols extracted from elderberry have been reported to modulate specific and non-specific immune defense, inhibit LDL oxidation, and reduce pancreatic insulin in diabetes. It is known to significantly reduce the symptoms of upper respiratory tract diseases due to viral infections. Thanks to the phenolic compounds it contains, it increases the release of cytokines from monocytes and has an immunomodulatory effect. It has antioxidant properties with valuable flavonoids. Elderberry with important components regulates blood pressure, reduces oxidative stress, increases the activity of antioxidant enzymes in blood plasma and reduces uric acid levels. Considering the positive effects of elderberry, which has been the subject of many studies, on health, its consumption as food should be supported.

Keywords: antioxidant, elderberry, food, health, nutrition, phenolic compound

➢ *ORAL PRESENTATION*

Effects of Apis Totalis and Raw Honey on Wound Healing

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Abstract

Wounds and wound healing appear to be a major global health problem for various health and other professions [1]. Wounds should be treated as soon as possible to avoid undesirable effects and to eliminate any fatal danger. Wound healing is an active and dynamic process that begins in the first moments of injury [3,4]. Although, normal wounds tend to heal within 2 to 4 weeks, diabetic or contaminated from bacteria wounds present slow healing lasting weeks and they are difficult to manage[5]. This impaired healing in diabetes is resulted from a complex pathophysiology due to vascular, neuropathic, immune, and biochemical elements[6,7]

Apis totale, which is little known for its use in the treatment of human diseases, is obtained from the body of workers and drones and contains biologically active substances brought from the outside and produced by bees throughout their life. Apis totale is a complex, natural, complex of biologically active substances and the extract contains the nervous and endocrine systems of bees [8,9,10,11].

Honey has been reported to have great nutritional and prophylactic medical value due to its pronounced antimicrobial activity and potential wound healing activity [11]. The amino acids, vitamins and minerals contained in honey are believed to be responsible for its therapeutic and medical effects [12]. These substances and some enzymatic activities lose their activity either completely or partially in the processing of honey. Since raw honey is the first step product obtained before the processing processes of honey, it is superior to commercial honey in terms of some bioefficacy properties [13,14].

The aim of this study was to investigate the effects of raw honey and apis totalis wound healing properties on alloxan induced diabetic mice. Topical administration of raw honey and apis totalis were used to assess the role and function of raw honey and apis totalis in wound healing through wound contraction and histological analysis.

Keywords: Apis totalis, raw honey, apitherapy, wound healing

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➢ *ORAL PRESENTATION*

Miyosmin'nin camsı karbon elektrot ile elektrokimyasal özelliklerinin incelenmesi ve miktar tayini

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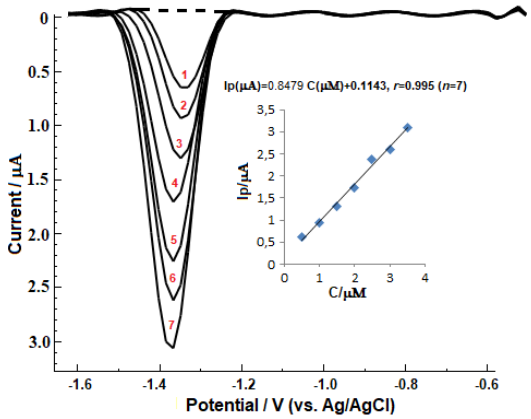
Özet

Tütün bitkisi olarak bilinen nikotin türleri (Solanaceae) dünyada en yaygın kullanılan farmasötik bitkilerdir[1]. Miyosmin, çeşitli doğal Nikotin ve ticari tütün çeşitlerinde (Nicotiana tabacum) bulunan yapısal olarak ilişkili küçük alkaloidler olan nornikotin, anabasin, anatabin grubundadır[2]. Nikotin cinsinin tüm üyeleri, tütün bitkileri ve ürünlerinin yaygın insan kullanımından sorumlu olan nikotin üretir[3]. Alkaloid miyosmin sadece tütün ürünlerinde değil aynı zamanda çeşitli yiyeceklerde de mevcuttur. Miyosmin, insan hücrelerinde genotoksiktir ve karsinojenik potansiyele sahip reaktif ara maddeler vermek üzere kolayca nitrozlanır ve peroksidasyona uğrar[4]. Miyosminin prevalansı nedeniyle, risk değerlendirmesi için metabolizması ve aktivasyonu üzerine araştırmalara ihtiyaç vardır[5]. Bu çalışmada, tütün alkaloidlerinden Miyosmin tayininde camsı karbon elektrot uygulaması sunulmuştur. Miyosmin´in elektrokimyasal indirgenmesi farklı destek ve geniş bir pH aralığında, CV (dönüşümlü voltametri) ve Diferansiyel Puls Voltametri (DPV) teknikleri ile araştırıldı.



Şekil 1. Miyosmin'nin GCE kullanılarak BR pH 12.0 tampon ortamında elde edilen DP voltamogramları. Kesik çizgili, destek elektrolit ve Pik akımı-derişim kalibrasyon eğrisi.

Miyosmin´in Britton Robinson, (BR, pH 12.0) tampon çözeltisinde camsı karbon elektrot (GCE) kullanılarak analitik sinyal duyarlılığı önemli ölçüde artmıştır. DPV tekniği kullanılarak, BR (pH 12.0) ortamında Miyosmin, yaklaşık -1.38 V (Ag/AgCl vs.) gerilimde iyi belirlenmiş voltametrik bir yanıt vermiştir. DPV tekniği ile GC elektrot üzerinde, analitik sinyal, BR (pH 12.0) tampon çözeltisinde 0.5-3.5 µM derişim aralığında doğrusal bir bağıntı göstermiştir(Şekil 1). Önerilen yöntem ticari tütün numunelerine başarıyla uygulanmıştır.

Anahtar Kelimeler: Voltametri, Camsı Karbon Elektrot, Miyosmin, Tütün

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➢ *ORAL PRESENTATION*

Effects of ellagic acid against methotrexate induced testicular toxicity in rats

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Abstract

Methotrexate (MTX) is a drug used for the treatment of various types of cancer and rheumatological diseases. Different side effects occur during the MTX treatment and one of them is testicular damage. Testicular damage is caused by increased free radicals. Ellagic acid (EA) is an antioxidant, free radical scavenger and anti-cancer agent and found in raspberries, grapes and hazelnuts. The aim of this study was to investigate the effects of EA against MTX-induced testicular damage in rats.
In this study 24 Sprague Dawley rats were randomly divided into four groups. Control group was given saline (0.6 ml) by gavage for 5 days. MTX group was administered (20 mg/kg) MTX intraperitoneally (i.p.) on the first day of the experiment. The EA group was given EA (25 mg/kg) by gavage for 5 days. The MTX + EA group was administered a single dose of MTX (20 mg/kg) i.p. on the first day of the experiment and EA (25 mg/kg) was administered by gavage for 5 days including the first day. At the end of the experiment, the rats were sacrificed by exsanguination and their testicles were taken for histopathological and flow cytometric analysis.
Histopathologically, testicular tissues were normal in Control and EA groups. In MTX group, seminiferous tubule epithelium was damaged. There was a slight increase in the number of immature cells in the seminiferous tubule lumen. Atrophy was observed in spermatogoniums and in the interstitial area Leydig cells atropy was observed. In MTX + EA group, the damage decreased compared to MTX group. In flow cytometry analysis Apopitotic index percentage was %13 in MTX group and decreased to % 4.7 in MTX + EA group. As a result histopathological and flow cytometric analysis showed that usage of MTX increased testicular damage and EA ameliorated this damage.

Keywords: Ellagic acid, Toxicity, Flow cytometry, Methotrexate, Testis.

Acknowledgment: This study was supported by the Karadeniz Technical University Scientific Research Project Coordination Unit (Project No: TYL-2018-7478)

➢ *ORAL PRESENTATION*

Leptin and Colorectal Cancer: Signaling and Pathogenesis

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Abstract

Adipose tissue is characterized by high expression of various cytokines and these cytokines are a known risk factor for many cancers such as colon, breast, endometrium and prostate. One of these cytokines, leptin is produced mainly by adipose tissue and cancer cells and mediates its action through the leptin receptor (LEPR), which is widely expressed in various tissues, including the colon mucosa. In previous studies in human and xenograft athymic mice with colorectal cancer, we found that LEP and LEPR ObRb expression was higher in tumor tissues than in normal tissues. This study demonstrated that leptin stimulates the phosphorylation and activation of STAT3 in a JAK2 dependent manner. Activated STAT3 may have played a role in proliferation, apoptosis inhibition, and cellular transformation. In another study, Ob-R expression was found to be higher in metastatic colon cancer patient tissues compared to those with local colorectal cancer. The results of these study demonstrated the role of leptin in the progression of colon cancer to metastatic disease without weight loss and that Ob-R may be a prognostic biomarker in CRC.
In this review, current understanding of leptin and its receptor's roles in the pathogenesis of colonogenic cancer will be presented.

Keywords: Leptin, ObRb, colorectal cancer, JAK2/STAT3

➢ *ORAL PRESENTATION*

Effects of L-Theanine against methotrexate induced testicular toxicity in rats

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Abstract

Methotrexate (MTX) is a folic acid antagonist. MTX inhibits dihydrofolate reductase enzyme and cell division. One of the major side effect of MTX treatment is testicular toxicity. L-Theanin (L-THE) is an amino acid and it is usually found in green tea and mushrooms. It has antioxidant and antiinflammatory effects. The purpose of our study was to investigate the effects of L-THE against MTX induced testicular damage in rats. 24 Sprague Dawley rats were randomly divided into four groups. Control group was given saline (0.6 ml) intraperitoneally (i.p.) for 5 days. MTX group was administered (20 mg/kg) MTX on the first day of the experiment. The L-THE group was given L-THE (200 mg/kg) i.p. for 5 days. The MTX + L-THE group was given a single dose of MTX (20 mg/kg) on the first day of the experiment and L-THE (200 mg/kg) i.p. for 5 days. At the end of the experiment, the rats were sacrificed by exsanguination and right testicles were taken for histopathological and flow cytometric analysis. Histopathologically, testicular tissues were normal in Control and L-THE groups. In MTX group, Johnson score levels were lower compared to control group. Johnson score was higher in MTX + L-THE group compared to MTX group. In MTX group Seminiferous tubule epithelium was disrupted. There was an increase of immature cells in the seminiferous tubule lumen. Atrophy was observed in spermatogoniums. Leydig cell atropy was observed in the interstitial area. In MTX + L-THE group, the toxicity was decreased compared to MTX group. In flow cytometry analysis Apopitotic index percentage was %12.4 in MTX group and decreased to % 3.6 in MTX + L-THE group. As a result with our histopathological and flow cytometric findings, L-Theanin has beneficial effects on methotrexate induced testicular toxicity.

Keywords: L-Theanin, Toxicity, Flow cytometry, Methotrexate, Testis.

Acknowledgment: This study was supported by the Karadeniz Technical University Scientific Research Project Coordination Unit (Project No: TYL-2018-7723)

➢ *ORAL PRESENTATION*

Katyonik sürfaktan madde varlığında bizmut film elektrot kullanarak desloratadinin elektrokimyasal olarak indirgenmesi: Lineer taramalı sıyırma voltametrisi ile ilaç ve idrar numunelerine uygulanması

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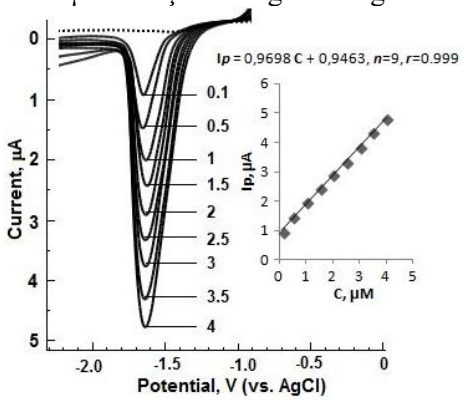
Özet

Histamin, mide salgısının güçlü bir uyarıcısı olan biyolojik bir amin olup etkili bir nörotransmitterdir[1]. Literatürde biyolojik sıvı ve ilaç şekillerinde desloratadin (DESL) analizi için önerilen elektrokimyasal yöntemlerin temel alındığı teknikleri kullanıldığı birkaç çalışma vardır[2-6]. Bu çalışmada, ikinci nesil antihistaminikler grubundan olan DESL'nin elektrokimyasal özellikleri, Bizmut Film eleketrot (BİFE) kullanılarak sulu ve sulu/yüzey aktif madde çözeltilerinde gerçekleştirildi. Bu bileşik dönüşümlü voltametri ile yaklaşık -1.65 V da tersinmez ve adsorpsiyon kontrollü bir indirgenme piki göstermiştir. Katyonik yüzey aktif madde (setiltrimetilamonyum bromür, CTAB) ilavesinin desloratadinin indirgeme akım sinyalini arttırdığı, anyonik (sodyum dodesilsülfat, SDS) ve iyonik olmayan (Tween 80) yüzey aktif maddelerinin ise ters etki sergilediği bulundu. Linear taramalı adsorptif sıyırma voltametrisi kullanılarak akım, 5 mM CTAB içeren Britton-Robinson tamponunda, pH 8.0'de 0.1 ile 4 µM derişim aralığında doğrusal bir bağıntı göstermiştir(Şekil 1).



Şekil 1. 0.1- 4 µM derişim aralığında BR tampon(pH 8.0 / 5 mM CTAB içeren) ortamında lineer taramalı sıyırma voltametri eğrileri

Bu çalışmada geliştirilen yöntem, farmasötik preparatlarda ve idrar örneklerinde herhangi bir ayırma işlemi yapılmadan DESL analizi için başarılı bir şekilde uygulanmıştır.

Anahtar Kelimeler: Desloratadin, Voltammetri, BİFE, İlaç, İdrar

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➢ *ORAL PRESENTATION*

Novel Applications of the Biopolymer Based Green Synthesis: Iron(III) Oxide Nanoparticles

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Abstract

The awareness of environmental problems and sustainability concepts around the world led to the development of green methods in chemistry and biochemistry. One of the main approaches in the environmentally-friendly methods is to use less toxic chemicals in synthesis methods. Biopolymer based methods include alginate decomposition, which was previously described for the synthesis of some nanoparticles such as cerium oxide and yttrium oxide. In this study, to the best of our knowledge, the synthesis of iron oxide nanoparticles using alginate biopolymer as a precursor, is reported for the first time, where iron(III) chloride and sodium alginate (2% solution) were used as only chemicals in the experiment. In addition to experimental study, a general view on green techniques in chemistry & biochemistry will also be delivered in the presentation.

Keywords: Green synthesis, Biopolymer, Alginate, Nanoparticle, Iron(III) oxide

➢ *ORAL PRESENTATION*

Spirulina platensis'in gıda patojenleri üzerindeki etkilerinin araştırılması

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Özet

Mikroalgler, protein, yağ asitleri, karbonhidratlar, mineraller, pigmentler, vitaminler, steroller, antioksidanlar ve polifenoller gibi değerli metabolitleri sentezleyebilen zengin biyoaktif bileşen kaynakları arasındadır. Bu değerli metobolitler sayesinde günümüzde mikroalgler gıda, kozmetik, ilaç, tarım gibi birçok endüstriyel alanda kullanım potansiyeline sahiptir. Günümüzde mikroalglerin gıdalarda katkı maddesi olarak kullanımına yönelik birçok çalışma yapılmaktadır. Farklı türlerden elde edilen mikroalgal ekstraktların, gıdalarda bozulmaya neden olan bakteri, küf ve mayalara karşı antimikrobiyal etkiye sahip olduğu düşünülmektedir. Bu etkilere göre bu çalışmadaki amacımız; *Spirulina platensis* ekstraktlarının gıda kaynaklı enfeksiyon ve intoksikasyon üzerine etkilerini disk difüzyon yönteminden yararlanarak incelemektir. *Spirulina platensis* 25 ± 2°C'de 25 günlük bir kültüvasyonun ardından sabit faza ulaşmadan hasat edilmiştir. Hasat sonrası dondurarak kurutulan biyokütle çözücü olarak "hekzan"ın kullanıldığı farklı "biyokütle/çözücü" oranlarında muamele edilmiştir. Elde edilen ekstraktlar farklı konsantrasyonlarda hazırlanarak "*Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Vibrio parahaemolyticus, Salmonella* sp. ve *Escherichia coli*:O157" patojenlerinin inhibisyon bölgelerinin belirlenmesi amacıyla "disk difüzyon testi" uygulanmıştır.En iyi sonuçların elde edildiği parametreler olan, 1/1 biyokütle solvent oranı ve 62 mg/mL ekstrakt konsantrasyonu ile yapılan inhibisyon testi sonuçlarında; *S.aureus* 15,5 mm, *B.cereus* 17,2 mm, *L.monocytogenes* 21,4 mm, *V.parahaemolyticus* 15,5 mm, *Salmonella* sp. 16,8 mm ve *E.coli* O157 için 8,5 mm çaplarında zonlar elde edilmiştir.

Anahtar Kelimeler: Antimikrobiyal aktivite, inhibisyon, zon, ekstrakt, *Spirulina platensis*.

➢ *ORAL PRESENTATION*

Kızılötesi Optik Biyosensör ile Numune Ön Hazırlama ve Etiketleme Gerekmeksizin Yapay Semen İçerisinde Folikül Stimülan Hormon Tespiti

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Özet

Semen analizi, infertil erkek değerlendirmesinde kullanılan yöntemlerin başında yer almaktadır. Ejakülatın (semen) kalitesi ve hormonal değerlendirmeler sonucu elde edilen veriler, infertil bireylerin teşhisinde önem taşımaktadır. Semen analizinden elde edilen veriler erkek bireylerin doğurganlığı hakkında bilgi sağlarken bazı sınırlamaları da beraberinde getirmektedir. Bu sınırlamaların başında gelen en önemli husus, semenin rutin değerlendirilmesi yapılırken deneyimli uzman kişiler tarafından özel donanımlı laboratuvarlar da yapılması gerekliliğidir. İnfertil erkek değerlendirmesindeki önemli basamaklarından bir diğeri de hormonal değerlendirmedir. Hormonal değerlendirmede kullanılan serum örneği invaziv numune toplamayı gerektirmektedir. Hormonal değerlendirmede folikül uyarıcı hormon (FSH), lüteinleştirici hormon(LH) ve testosteron(T) spermatogenezin belirteci olarak değerlendirilmektedir. Semen analizine alternatif olarak spektroskopik methodlar da kullanılmaktadır. Ekipman ve kullanılan sarf malzemenin ucuz olmasının yanı sıra az miktarda numune ile herhangi bir teknisyen tarafından hızlı değerlendirme yapılabilir. Biyospektroskopik analiz, infrared ışık ve madde arasında gerçekleşen fiziksel bir fenomen olan moleküler titreşimin sonucudur. Bu olgunun altında yatan absorbans mekanizması, numunenin kimyasal özgüllüğü hakkında spesifik bilgi verir ve biyobelirtecin tanımlanması için uygun ortam sağlar. İnfertilite teşhisinin pratikleşmesi için ön hazırlama, etiketleme olmadan, hızlı ve non-invaziv alınan numuneden ölçüm yapabilen sensörlere ihtiyaç duyulmaktadır. Bu çalışmada; optik biyosensör yardımıyla semen içerisinde biyobelirteç olarak belirlediğimiz FSH tespiti yapılmıştır.FSH hormonu, glikoprotein hormon grubuna ait olması sebebiyle 1500-1800 cm^{-1} aralığında yoğunlaşan amid-I ve amid-II absorbans piklerini baskın şeklide yansıtmaktadır. Tasarlanan sistemde emitter (verici) 1-20µm aralığında yayılım yaparken receiver (alıcı) bu dalga boyunun 3-20µm aralığını karşılayabilmektedir. Seminal sıvı içerisinde infertil bireylerde FSH oranı ortalama 0.65 mIU/ml iken fertil bireylerde 0.20 mIU/ml ‘dir. Birimsel çevrim için serum FSH değerlerinin ng/ml cinsinden karşılıkları bulunarak oransal hesaplamaya gidilmiştir. Bunun sonucunda, infertil ve fertil bireyler için belirlenen FSH değerleri standart ekleme yöntemi(infertil bireyler(120ng/ml-100ng/ml-80ng/ml-60ng/ml-40ng/ml)fertil bireyler(30ng/ml-20ng/ml-10ng/ml) kullanılarak belirlenmiştir. Elde edilen sonuçları 15 kat çapraz doğrulama ile destek vektör makinası(SVM) kullanılarak sınıflandırılmıştır. Sınıflandırma doğruluğu, %87.2 olarak tespit edilmiştir. Bu sonuçlar infertil bireylerin belirlenmesinde kullanılacak yöntemin umut vaat ettiğini göstermektedir.

Anahtar Kelimeler: Erkek İnfertilitesi, Semen Analizi, FSH, Optik Sensör, Moleküler Absorbans

➢ *ORAL PRESENTATION*

Biyojenik gümüş nanoparçacık içeren polisülfon nanofiberlerin nikel metalini mikrobiyal korozyondan koruma etkinliğinin belirlenmesi

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Özet

Korozyon; metal ve alaşımların, çevrenin etkileriyle kimyasal ve elektrokimyasal değişme ya da fiziksel çözünme sonucu aşınmasıdır. Korozyona uğramış metal veya alaşımın kullanım ömrü azalır ve bu durum ciddi ekonomik kayıplara neden olmaktadır. 2016 yılında raporlara göre Dünya çapında korozyonun ekonomik kaybının yıllık 2,5 trilyon dolar olduğu tahmin edilmektedir. Korozyonun bir çeşidi mikrobiyal korozyonda, mikroorganizmaların metabolik aktiviteleri sonucu doğrudan ve dolaylı olarak metalin bozulmasıdır. Bu çalışmada, nikel yüzeyler elektrospin tekniği ile üretilen ve biyojenik gümüş nanoparçacık (AgNP) içeren polisülfon nanofiber (PSU-Nf) ile kaplanmış ve kaplamanın yapay deniz suyu (ASW) ve bakteri varlığında ASW ortamında nikeli korozyondan koruma etkinliği araştırılmıştır. Kaplama karakterizasyonu taramalı elektron mikroskobu (SEM), temas açıcı analizi ve upright mikroskop ile araştırılmıştır. Buna ek olarak korozyon oranı kütle kaybı analizi ile belirlenmiş ve korozyon ölçümlerinde potansiyodinamik polarizasyon tekniği kullanılmıştır. SEM analiz sonuçlarına bakıldığında, nanofiberlerin homojen ve boncuksuz bir yapı gösterdiği ve kaplama kalınlığının yaklaşık 100 µm olduğu bulunmuştur. Sadece nikel yüzeyi 78,3° temas açısı değeri ile hidrofilik özellik gösterirken PSU-Nf kaplama ile bu oran 170,4° ve nanofibere AgNP entegrasyonu ile açı 166,7° artmıştır. Bu durumda yüzey hidrofobik özellik göstermiştir. Kütle kaybı değerlerine göre sadece nikel için 0,139 mm y^{-1} iken PSU-Nf ve AgNP-PSU-Nf kaplama ile kütle kaybı azalmıştır (0,104 ve 0,07 mm y^{-1}). Ayrıca, ASW ortamında sadece nikel yüzeyin korozyon akım yoğunluğu 170,6 µA cm^{-2} iken PSU-Nf ve AgNP-PSU-Nf kaplama ile I_{kor} değeri 12,1 µA cm^{-2} ve 2,94 µA cm^{-2} azalmıştır. Bu durumda PSU-Nf kaplamanın korozyondan koruduğunu göstermektedir. ASW ortamına bakteri inoküle edildiğinde ise I_{kor} 15 µA cm^{-2} iken PSU-Nf ve AgNp içeren Nf ile kaplama sonrası akım yoğunluğu azalarak (I_{kor}: 9,06 µA cm^{-2} ve 8,1 µA cm^{-2}) nikeli mikrobiyal korozyondan da korumuştur. Sonuç olarak elektrospin ile nikel yüzeye kaplama yapılan PSU-Nf'lerin abiyotik ve biyotik ortamlarda etkili antikorozif materyaller olarak kullanılabilecekleri kanıtlanmıştır. Çalışma TÜBİTAK 218M508 numaralı proje ile desteklenmiştir.

Anahtar Kelimeler: Elektrospin, Kütle kaybı, TAFEL

➢ *ORAL PRESENTATION*

The effect of dexamethasone on CYP3A is dose-dependent but not on P-gp*

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Abstract

Dexamethasone, which is a synthetic glucocorticoid with anti-inflammatory and immunosuppressant effects, is used a wide range of dose for different indications. It modulates both P-gp and CYP3A4, which plays an important role in drug-drug/drug-food interactions.
We investigated the effect of different doses of dexamethasone on the expressions and functions of P-gp and CYP3A using P-gp and CYP3A substrates. A total of 54 mice were divided into 9 groups in the study. The first group was considered as the control group. Animals in groups 2, 3, 4 and 5 received low dose dexamethasone (5 mg/kg, IP), high dose dexamethasone (50 mg/kg, IP), fexofenadine (40 mg/kg, PO) and midazolam (20 mg/kg, IP), respectively. Animals in groups 6 and 7 were administered fexofenadine (40 mg/kg, PO) and midazolam (20 mg/kg, IP), 24 hours after low dose dexamethasone (5 mg/kg, IP) administration, respectively. Animals in groups 8 and 9 received fexofenadine (40 mg/kg, PO) and midazolam (20 mg/kg, IP), 24 hours after high dose dexamethasone (50 mg/kg, IP) administration, respectively. The mdr1a/b and CYP3A11/13 expressions in the liver and small intestine were determined by RT-PCR. The plasma concentrations of drugs were determined using HPLC-UV.
It can be indicated that while the effect of dexamethasone on P-gp expression is not dose-dependent, its effect on CYP3A11 is dose-dependent. Midazolam, CYP3A substrate, causes to induction of P-gp in the liver but not in the small intestine, and the effect of dexamethasone on CYP3A11 expression in the intestines was reduced by fexofenadine and midazolam.

Keywords: P-glycoprotein, CYP3A4, dexamethasone, dose, mice

*This study was supported by Selcuk University Scientific Research Projects (18401108).

➢ *ORAL PRESENTATION*

Comparison of Physio-Biochemical Responses of Common Vetch (*Vicia sativa* L.) Seedling Organs to Salinity

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Abstract

Shoots and roots are autotrophic and heterotrophic organs of plants with different physiological and biochemical functions under stress conditions. The metabolites involved in tolerance enhancement differed between roots and shoots. In this study, the physio-biochemical changes occurring in shoot and root organs under salt stress, and the level of these changes were investigated. However, these changes in shoot and root organs were compared. For this aim, seeds of common vetch were sown and subjected to 14 days of salt stress in basal MS medium containing 100 mM NaCl. In shoot and root tissue, physio-biochemical parameters such as antioxidant enzymes activities (GR, APX, SOD, and CAT), malondialdehyde (MDA) content and proline accumulation were determined. Results of the study indicated that the activities of antioxidant enzymes (SOD, CAT (except in shoot), GR, and APX), MDA, and proline accumulation enhanced by salt stress in both organs. On the other hand, morphological parameters decreased in both tissues. It seemed that antioxidant enzyme activities more active in root tissues. However, proline accumulation was found higher in shoot tissues than root tissue, while MDA content was higher in root tissue than shoot tissue. The present investigation provides essential information for the antioxidant components of the shoot and root organs of vetch seedlings under salt stress.

Keywords: Salt (NaCl) stress, *in vitro* culture, antioxidant enzymes, proline, malondialdehyde (MDA)
.

➢ *ORAL PRESENTATION*

Methylation dynamics of axolotl transcriptome during regeneration

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Abstract

Functional restoration of the amputated or damaged tissue, organ or appendage is defined as regeneration. Regenerative capacity varies significantly within the animal kingdom and *Ambystoma mexicanum*, also known as Axolotl, is one of the exceptional vertebrates with extra ordinary regenerative potential. Axolotl can regenerate all of its internal organs, including heart, central nervous system, including brain, and extremities following the amputation. Hence, this model organism attracts the attention of stem cell researchers to utilize this animal in stem cell and regenerative medicine studies. Till now, several reference genomic, transcriptomic and proteomic datasets of axolotl have been generated. More recently, microbiome and metabolome profile during axolotl limb regeneration have been studied. Accumulation of datasets on limb regeneration makes remarkable contribution to expand the mechanisms underlying regeneration. However, the RNA modifications of axolotl have not been investigated yet and lack of this essential dataset limits the fully utilization of axolotl as a fruitful model. During the last couple of years, it has been postulated that RNA modifications, particularly RNA methylation, is one of the key regulatory steps to modulate the gene expression. In this study, we interrogated the dynamics of RNA modifications during axolotl limb regeneration. Using bisulfite sequencing and antibody-based technologies we mapped the methylation pattern of axolotl RNAs. 718 mRNAs were found significantly modified (hyper-methylated or hypo-methylated) for regeneration timepoints compared to 0 dpa (day post amputation). GO and KEGG enrichment of identified mRNAs highlighted the modulation of regeneration related pathways such as cell cycle, migration and immune system. Overall, this work would contribute to the concept of epitranscriptome during regeneration and close the gap between regulatory mechanisms and successful regeneration. This work was financially supported by TUBİTAK (project number: 217S296)

Keywords: Epitranscriptome, Axolotl, Regeneration, RNA modifications

➢ *ORAL PRESENTATION*

Yeşil kimya yöntemiyle oluşturulan PLGA-bakır hibrit nanopartiküllere yüklenen epigallokateşinin meme kanseri üzerindeki in vitro ve in vivo anti-tümör etkilerinin incelenmesi

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Özet

Fitokimyasal bileşikler genellikle antioksidan bileşiklerdir, ancak Fenton reaksiyonu adı verilen serbest demir ve bakır varlığında pro-oksidan etkiye (oksidasyonun uyarılması) sahip olabilirler. Böylece hücrenin oksidatif stres seviyesindeki artış, DNA hasarı yoluyla kanser tedavisinde kullanılabilir. Özellikle kanser hücrelerinde sağlıklı hücrelere kıyasla daha yüksek bakır konsantrasyonları, bu hücrelerdeki doğal pro-oksidanların etkinliğini artırır. Bu nedenle fitokimyasal bileşiklerin bakır ile birlikte hedef dokuya nano ilaç verme sistemleri kullanılarak taşınması, sağlıklı dokulara zarar vermeden sadece kanser dokusu üzerindeki etkilerinin artmasını sağlayabilir. Bu çalışma ile bakır taşıyan poli (d, l-laktit-ko-glikolid) (PLGA) nano partikülleri (son boyut 150 nm) kullanılarak, hedef kanser dokusuna doğal bir antioksidan Epigallokateşin (EGC) taşınması amaçlamıştır.
Bakır, yeşil kimya yöntemiyle yeşil çay ekstresi kullanılarak indirgenmiş, 40 nm boyutunda bakır nano partikülleri (NP) oluşturulmuştur. Bu NP'ler daha sonra EGC ile birlikte PLGA misellerine yüklenmiştir. Epigallokateşin antioksidan bir fitokimyasal olarak kullanılırken, bakır Nps varlığında bir pro-oksidan görevi göreceği varsayılmıştır. Partiküllerin sitotoksisitesinin artışı meme kanseri hücreleri (4T-1) üzerinde test edilerek, genotoksik, apoptotik ve sitotoksik etkiler serbest EGC ile karşılaştırılmıştır. Bakırın varlığı, serbest EGC ve EGC yüklü PLGA NP'ye kıyasla EGC'nin sitotoksisitesini önemli ölçüde artırdığı gösterilmiştir.

Anahtar Kelimeler: Doğal Antioksidanlar, Hedefli Kanser Tedavisi, Bakır, Fenton Reaksiyonu, Pro-oksidan Etki, Kombin Terapi ,Yeşil Kimya

➢ *ORAL PRESENTATION*

Electrospun nanofibers and applications

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Abstract

Nanotechnology has significantly influenced advances in materials science. This technology is of great interest due to its use in many different science and engineering disciplines such as medicine, electronics, materials science and polymer engineering. With the development of nanoscience and technology, interest in nano metric materials with delicate structures such as nanofibers has increased. Nanofibers, especially due to their high surface area and porosity, find applications in several areas [1-2]. Several methods such as electrospinning, melt or solution blowing, phase separation, self-assembly, and template synthesis have been used for producing nanofibers [3]. Electrospinning is a progressive method which produces fibers ranging from the submicron level to several nanometers in diameter and it is one of the most advanced simple and versatile methods in producing high performance nanofibers [4-5]. So, it is the most suitable technique used to produce nanofibers from different materials (eg polymers, metals) with different morphologies and functionalities. The electrospun fibers show excellent advantages such as complex porous structure, simplicity in processing, high surface area-to-volume ratio and so on. The electrospinning technique has become widespread for its ability to create high-performance fibers in numerous areas of research, including sensors, supercapacitors, biomedical applications, filtration, food package, drug delivery, and tissue engineering [6].

Keywords: Electrospinning, polymer, electrospun applications, nanofibers, nanomaterials.

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➢ *ORAL PRESENTATION*

Investigation of Biochemical Kit Interaction of Metclopramide, Ranitidine, Hyosin-N-Methyl Bromide, Dexketoprofen Parenteral Forms

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Abstract

Objective: Concomitant use of parenteral drugs, depending on the intensity of emergency room conditions, causes polypharmacy and impairment of bioavailability. The importance of avoiding polypharmacy is recognized by many clinicians and allied health professionals. However, these drugs can sometimes interfere with kit reagents during analysis. Depending on this, measurement ancestors may be overlooked. Such situations have the potential to cause malpractice. The aim of this study is to experimentally demonstrate the effect of parenteral forms of four different drugs commonly used in the emergency room on routine biochemical test results
Methods: Added to control material containing Metoclopramide, Ranitidine, Hyosin-N-Methyl Bromide, Dexketoprofen. Four separate mixtures were prepared by taking 20µL of each drug solution and 180µL of the control solution. Urea, creatinine, ALT, AST, GGT, ALP, CRP, CK-MB mass, troponin-I tests were performed from the prepared mixture in the biochemistry autoanalyzer. The same work was repeated by adding 20uL of distilled water. Deviation amounts calculated with bias%.
Results: There was a deviation of -19.75% in LDH due to metoclopramide and -9.07% in CK-MB mass. Ranitidine caused -9.17% deviation in AST, -6.02% in ALT. The deviation due to Hyosin-N-Methyl Bromide remained below 5%. Dexketoprofen created -9.6% deviation in ALT and -14% in CK-MB mass. The deviation rates in other tests were minimal.
Conclusion: The deviation amounts of biochemical tests performed after the infusion of frequently used drugs in emergency services may differ according to the kit and method used. In particular, neagtive interference in the result of CK-MB mass may miss acute cardiac syndrome. It is recommended that sampling before drug infusion to avoid such errors.

Keywords: Metoklopramid, Ranitidin, Hyosin-N-Metil Bromur, Deksketoprofen, False Measurement

➢ *ORAL PRESENTATION*

Postnatal dönem domuz idrar kesesinde zonula okludens-1 ve uroplakin III ekspresyonu

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Özet

Alt üriner sistemin lümene bakan yüzeyindeki epitele tranzisyonel epitel (üroepitelyum) denir. Bu epitel örtüsü bazal, intermediyer ve süperfisiyal hücreleri içeren üç hücre katmanından oluşmaktadır. Süperfisiyal hücrelere şekilleri nedeniyle şemsiye hücreleri de denilmektedir. İdrar kesesi boş olduğu zaman şemsiye şeklinde olan bu hücreler, kese dolduğu zaman yassı şekle dönüşürler. Şemsiye hücrelerinin apikal membranları asimetrik ünit membran yapısından oluşur ve bu membranda üroepitelyumun belirleyicileri olan üroplakin (UP) olarak adlandırılan, UP1a, UP1b, UP2 ve UP3 olmak üzere dört tip membran içi yerleşimli protein bulunur. Komşu epitel hücreleri arasında tight-junction adı verilen hücreler arası bağlantılar bulunmaktadır. Tight junctionlar maddelerin lumenden epitel altı dokuya hücreler arasından geçişini engeller. Bu çalışma ile hücreler arasındaki zonula occludens-1 (ZO-1) ve epitel hücre yüzeyindeki uroplakin III (UP III) proteinlerinin postnatal dönem dağılımları ve yerleşim yerlerinin immunohistokimyasal olarak incelenmesi amaçlanmıştır. Çalışma materyali olarak 14 tane sağlıklı Yorkshire ırkı domuz kullanıldı. Domuzlar 7 ve 30 günlük olmak üzere iki yaş grubuna ayrıldı. İdrar kesesi doku örnekleri 24 saat 10% tamponlu formaldehit solüsyonunda tespit edildikten sonra rutin histolojik doku takibi prosedürlerinden geçirilerek parafinde bloklandı. Hazırlanan idrar kesesi bloklarından 5µm kalınlığında kesitler alındı. İmmunohistokimyasal boyamada rabbit poliklonal anti-zonula occludens-1 ve mouse monoklonal anti-uroplakin III primer antikorları kullanıldı. İmmunohistokimyasal boyama streptavidin biotin kompleks metodu ile yapılarak ZO-1 ve UP III ekspresyonları incelendi. Tranzisyonel epitelinin lumene bakan süperfisyal katmanı oluşturan hücrelerinin apikal membranlarının UP-III ile immun pozitif boyandığı görüldü. Uroplakin III ekspresyonunda iki grup arasında belirgin bir immum boyanma faklılığı gözlenmedi. ZO-1 proteinleri tranzisyonel epitelin tüm katmanlarında boyanmakla birlikte daha çok süperfisiyal katmanda görüldü. Zonula okludens-1 ekspresyonunda 21 günlük idrar kesesinde çok az da olsa daha soluk immunreaksiyon belirlendi. Sonuç olarak, UP III ekspresyonunda belirgin bir fark gözlenmedi. ZO-1 ekspresyonunda 7 günlük domuz idrar kesesi tranzisyonel epitelinde yoğunluğunun fazla olması bazal, intermediyer ve süperfisiyal hücre katmanlarının postnatal dönemde epitel katmanının oluşumuna devam ettiği kanısına varmamızı sağladı.

Anahtar Kelimeler: Domuz, idrar kesesi, zonula okludens-1, uroplakin III

➢ *ORAL PRESENTATION*

Deep learning applications in ornithology

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Abstract

The conservation of biodiversity is the one the most important challenges in the twenty-first century and according to the assessment of IUCN, more than 32.000 species are threatened with extinction. Birds are also declining in recent years due to habitat loss, invasive species, overexploitation, pollution and climate change associated with global warming. Both spatial and temporal trends of biological diversity are needed to conserve and manage species in a particular environment. This means that occurrence, distribution, movement trend and density data need to be collected for each species for conservation. However, observing biodiversity can be time-consuming, expensive, logistically difficult, and needs experienced manpower. Deep learning-based computer vision and image recognition applications, which is rapidly developing in recent years, provide us with alternative solutions to overcome these problems. Deep learning is a machine learning method which allows us to train artificial intelligence to predict outputs with a given data set using computer algorithms and large data sets can be analysed using deep learning applications. These applications are used to individual detection, species identification, bird counting and regional mapping using inferred data from photographs taken by unmanned aerial vehicle, satellite-derived images, on-ground photography and/or photo-trapping. Furthermore, using deep learning applications humans can be assisted in bird monitoring activities and participation of citizen scientists can be increased.

Keywords: Deep learning, artificial intelligence, computer vision, image recognition, ornithology, ecology.

➢ *ORAL PRESENTATION*

Polikaprolakton ve ipek fibroini esaslı nanofibere immobilize edilen laktaz enziminin aktivitesi

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Özet

Günlük diyetimizin önemli bir kısmını oluşturan karbonhidratlar ile ilgili olarak en sık rastlanılan sindirim bozukluklarından biri laktoz intoleransıdır. Laktoz intolerasında sütte bulunan laktoz sindirilemediği için çeşitli gastrointestinal semptomlara neden olmaktadır. Bu nedenle laktoz intoleransı olan kişiler ya laktoz içermeyen ürünler tüketerek ya da dışarıdan laktaz enzimi kullanarak bu sorunu ortadan kaldırmaya çalışmaktadırlar. Kullanılan laktaz enzimin kararlılığındaki sapmalar nedeniyle çoğu zaman laktoz sindirimi beklenilen şekilde olmamaktadır. Enzimler uygun bir destek materyaline bağlandığı zaman kararlılıklarını koruyarak tekrar kullanılabildikleri için, farklı destek materyalleri kullanılarak enzim kullanımını en aza indirmek, enzimin etkinliğini artırmak ve daha iyi bağlanmasını sağlamak ile ilgili çalışmalar devam etmektedir.
Çalışmamızda polikaprolakton ve ipek fibroini esaslı nanofibere immobilize edilen laktaz enzimi aracılığı ile sütte bulunan laktozun hidroliz olması ve sütün biyokimyasal yapısı değişmeden laktozsuz süt elde edilmesi amaçlanmıştır. Polikaprolakton ve ipek fibroini esaslı nanofiber elektro-dokuma yöntemi kullanılarak elde edilmiştir. Destek materyali olarak kullanılan bu nanofibere laktaz enzimi immobilize edilerek biyoaktif hale getirilmiştir. Biyoaktif nanofiber ve süt örnekleri 1 gece +4^{o}C'ta inkübe edildikten sonra laktaz enziminin laktozu hidroliz edip etmediği sütte glukoz tayini yapılarak takip edilmiştir. İmmobilize laktaz ve serbest laktaz enzimi aktiviteleri orto-nitrofenil-β-galaktozid kullanılarak tayin edilmiştir. İnkübasyon sonrasında sütün besinsel içeriği de incelenerek, immobilize laktazın sütün besinsel içeriği üzerine olan etkisi araştırılmıştır. Serbest ve immobilize laktazın optimum pH ve sıcaklık aralıkları incelendiğinde, serbest laktaz 50^{0}C ve pH 6.5'te en iyi aktiviteyi gösterirken, immobilize laktazın ise 37^{0}C ve pH 6.5 en iyi aktiviteyi göstermiştir. Süt örneklerinin immobilize laktaz ile muamelesinden sonra yağ ve protein içeriğinde ve pH değerinde istatiksel olarak anlamlı bir değişiklik oluşmamıştır. Sonuç olarak; laktaz enzimi immobilize edilerek biyoaktif hale getirilen polikaprolakton ve ipek fibroini esaslı nanofiberin sütteki laktozun hidrolizini sağladığı tespit edilmiştir.

Anahtar Kelimeler: polikaprolakton, ipek fibroini, laktaz immobilizasyonu, nanofiber

➢ *ORAL PRESENTATION*

Ankara Eğitim Araştırma Hastanesi'nde antiepileptik ilaç düzeylerinin retrospektif olarak değerlendirilmesi

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Özet

Amaç: Çoğunlukla yaşamın erken yaşlarında başlayan ve uzun yıllar ilaç tedavisine gereksinim duyulan epilepsi hastalarının yarısı ilk antiepileptik ilaca yanıt vermemekte, üçte biri ise birden fazla antiepileptik ilaç tedavisine ihtiyaç duymaktadır. Bu çalışmada, hastanemizde son 3 yılda yapılan ilk kuşak antiepileptik ilaçlar arasında yer alan Fenitoin, Fenobarbital ve Karbamazepin düzeyleri retrospektif olarak araştırılmıştır.
Yöntem: Çalışmaya Ankara Eğitim ve Araştırma Hastanesi Tıbbi Biyokimya Laboratuvarı'nda terapötik ilaç düzeyi izlemi (TİDİ) amacı ile 2018 Ocak ve 2020 Haziran arasındaki zaman diliminde ölçülen Fenitoin (n=167), Fenobarbital (n=212) ve Karbamazepin (n=1565) örnekleri alınmıştır. Örnekler HPLC yöntemi ile ölçülmüştür. Yaş, cinsiyet, tanı ve ilaç düzeylerinin gönderildiği poliklinik açısından karşılaştırılmıştır. İstatistiksel analizler GraphPad Prism 5.0 programı kullanılarak yapılmıştır. Poliklinik, cinsiyet, tanı ile ilaç düzeylerinin karşılaştırmalarında ki-kare testi, yaş ile ilaç düzeylerinin karşılaştırmalarında ise Spearman korelasyon testi kullanılmıştır. $p<0.05$ istatistiksel olarak anlamlı kabul edilmiştir.
Bulgular: Antiepileptik düzeyleri terapötik aralık açısından karşılaştırıldığında Fenitoin kullananların %14,9'unun, Fenobarbital kullananların %71,7'sinin, Karbamazepin kullananların ise %80,9'unun önerilen terapötik düzeylerde ölçüldüğü görülmüştür. Tanı açısından değerlendirildiğinde epilepsi hastaları arasında diğer hastalara göre Fenitoin ve Karbamazepin düzeyleri terapötik değerlerin altında ölçülenlerin daha fazla olduğu (sırasıyla $p<0.05$, $p< 0.0001$), Fenobarbital düzeylerinin ise fark göstermediği bulunmuştur ($p>0.05$). İlaç düzeylerinin gönderildiği poliklinik açısından karşılaştırıldığında ise Nöroloji polikliniğinden gönderilen hastalarda diğer polikliniklerden gönderilen hastalara göre Fenitoin düzeyleri terapötik değerlerin altında ölçülenlerin daha fazla olduğu ($p< 0.001$), Karbamazepin düzeylerinin ise fark göstermediği bulunmuştur ($p>0.05$). Yaş, cinsiyet açısından ise antiepileptik düzeylerinin fark göstermediği görülmüştür.
Sonuçlar: TİDİ yeterli etkinlik sağlamak ve toksisiteden korunmak için uygulanan etkili bir yöntemdir. Hastalardan analiz öncesi kullanmakta olduğu ilaçlar hakkında bilgi alınması ve kan örneğinin doğru zamanda alınmasının sağlanması, analiz sonrasında sonuçların yorumlanmasında önem arz etmektedir. İlaç düzeylerinin subterapötik düzeyde seyretmesi müdahale edilmesi gereken ciddi bir konudur. Hastanemizde, poliklinik hekimlerinin bilgilendirilmesini içeren klinikler arası işbirliğinin daha fazla olduğu yeni bir TİDİ sürecinin başlatılması gerekmektedir.

Anahtar Kelimeler: fenitoin, fenobarbital, karbamazepin, TİDİ

➢ *ORAL PRESENTATION*

Amlodipine release from polymeric nanomaterial for hypertension treatment

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Abstract

The major objective of our study was to investigate synthesized a new polymeric nanomaterials for controlled oral delivery of Amlodipine (AML). Amlodipine is a calcium channel blocker commonly used in hypertension treatment, chronic stable angina pectoris, and Prinzmetal's variant angina. For this purpose, poly(hydroxyethyl methacrylate-methacryloyl amido phenylalanine)-based polymeric nanomaterials (HEMPA) was synthesized using mini emulsion polymerization technique. The synthesized p(HEMPA) nanomaterial was characterized by different techniques such as Fourier Transform İnfrared Spectroscopy (FTIR), Zeta-Size Analysis, Scanning Electron Microscopy-Energy Dispersive Spectroscopy. Some of results are listed below; The average particle diameter of HEMPA was about 113 nm. Adsorption studies were carried out after characterization studies to examine the effects of pH, time, initial concentration, and temperature. Under optimum conditions, the maximum adsorption value (Qmax) of p(HEMPA) nanopolymer was found 145.76 mg/g for Amlodipine. In vitro controlled drug release studies of Amlodipine, which is bound to the nanopolymer at the optimum conditions, was studied by the dialysis method in the simulated gastrointestinal systems pH values of 1.2, 6.8 and 7.4. In conclusion, AML-p(HEMPA) nanopolymers may be reduce the dosing frequency, increase the bioavailability and deliver the drug complex as extended release with minimal side effects.

Keywords: nanomaterials, oral administration, amlodipine, hypertension

➢ *ORAL PRESENTATION*

Kuzey Kıbrısta Yetiştirilen Bezelye (*Pisum sativum* L.) Yerel Sebze Genotiplerin Tohum Morfolojisinin İncelenmesi

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Özet

Bu araştırma, Kuzey Kıbrıs'ta *Leguminosae* familyasına giren Bezelye (*Pisum sativum* L.) genotiplerinin tohum morfolojisinin incelenmesi amacıyla yürütülmüştür. Araştırma toplama ve karakterizasyon olmak üzere iki aşamalı olarak yürütülmüştür. Çalışmanın birinci aşamasında bezelye yerel genotiplerin toplanması hedeflenmiş ve bu hedef doğrultusunda toplam 94 merkeze ulaşılmış ve bu ulaşılan alanlardan toplam 7 örnek elde edilmiştir. Araştırmanın ikinci yılında ise toplanan bu genetik materyal tohumlarda morfolojik karakterizasyon çalışmaları UPOV ve IPGR kriterlerine göre gerçekleştirilmiştir. Araştırma sonucunda bezelye bitkilerine ait tohumlarda yapılan gözlemlerde olgunlaşmamış tohumda yeşil renk yoğunluğu bütün örnekler ile kontrol bitkisinde koyu olarak tespit edilmiştir. Örneklerin tamamında ve kontrol çeşidinde tohum şekli elips olarak belirlenmiştir. Tohum tanelerinde yapılan nişasta durumları mikroskopta yapılan incelemede tamamında bileşik nişasta oldukları tespit edilmiştir. Bileşik nişasta içeren tohumlarda kırışıklık yoğunluğu bakımından bütün genotipler ile kontrol bitkisinde güçlü olarak belirlenmiştir. Tohumda kotiledon rengi bütün örneklerde ve kontrol bitkisinde yeşil olarak tespit edilmiştir. Tohumdaki hilyum rengi örneklerin tümünde tohumla aynı renk olarak tespit edilmiştir. Bezelye bitkilerinin tohum ağırlıkları bakımından yapılan ölçümlerde 5 genotip ile kontrol bitkisi orta ağırlıkta, 1 tanesi de ağır olarak tespit edilmiştir.

Anahtar Kelimeler: Kuzey Kıbrıs, Yerel genotipler, Tohum morfolojisi

➢ *ORAL PRESENTATION*

Bazı Mikotoksinlerin Detoksifikasyonu ve Biyodegradasyonunda Yeni Yaklaşımlar: Antagonistik Mayalar

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Özet

Mikotoksinler, küfler tarafından üretilen toksik ikincil metabolitlerdir ve hem insanlarda hem de hayvanlarda sağlık sorunlarına ve ölüme neden olabilmektedirler. Doğada 100 'ün üzerinde küf türü tarafından üretilen 300 'e yakın metabolitin toksik aktiviteye sahip olduğu ve dünyada üretilen tarım ürünlerinin yaklaşık %25'inin bu metabolitlerle kontamine olduğu bilinmektedir. Mikotoksinler, küflerin normal gelişimini tamamladıktan sonra sentezlenmeye başlamakta ve küfün büyümesi için biyolojik önemleri bulunmamaktadır. Mikotoksinler, yemler ve gıdalarda yaygın kullanımları, insanlarda ve hayvanlarda neden olabileceği ciddi sağlık sorunları nedeniyle halk sağlığını tehdit eden en önemli toksik maddeler arasında bulunmaktadır. Gıdaların mikotoksin oluşumundan korunması ile ilgili çalışmalar genellikle küf gelişiminin önlenmesi üzerine yoğunlaşmıştır. Ancak son yıllarda, küflerin gelişiminin engellenmesinden çok mikotoksinlerin farklı yöntemlerle detoksifikasyonu ile ilgili araştırmalar dikkat çekmektedir. Mayaların çeşitli üretim proseslerinde uygulanması, belirli küflerin toksin üretimi üzerinde doğrudan inhibe edici bir etkiye sahip olmaktadır. Bunun yanı sıra birçok maya türü mikotoksini bağlayabilmekte ve böylece ürünleri etkili bir şekilde detoksifiye edebilmektedir. Ayrıca birkaç maya suşu da mikotoksinleri daha az toksik ve hatta toksik olmayan maddelere indirgeyebilmektedir. Bazı mayalar da mikotoksinlere direkt etki edebilmelerinin yanı sıra çok farklı tipte ve miktarlarda enzim üretebilme yeteneklerinden dolayı belirli mikotoksinlere karşı etkili olabilmektedir. Ancak bu durum biyoteknolojik açıdan her zaman bir avantaj sağlamamaktadır. Mayaların birçoğu insanlar açısından zararsız kabul edilmekle birlikte bazı detoksifikasyon yeteneğine sahip mayaların fungal hastalıklara neden olduğu bilinmektedir. Bu nedenle detoksifikasyon ya da biyodegradasyon için kullanılacak maya suşunun seçiminde oldukça dikkatli olunmalıdır. Çeşitli gıda kaynaklarından izole edilebilen *Debaryomyces hansenii, Saccharomyces cerevisiae, Metschnikowia pulcherrima, Kazachstania exigua* gibi maya türlerine ait suşların, detoksifikasyon ve biyodegradasyon işlemlerinde kullanılabilir olduğu birçok çalışma ile ortaya koyulmuştur. Suş seçiminde ürün çeşidi, tüketim durumu, mevcut mikotoksin tipi ya da tipleri, üründeki küf miktarı, ürünün muhafaza şartları, ürünün pH' sı v.b. parametreler önemli rol oynamaktadır. Bu derlemede maya suşu/mikotoksin ilişkileri, ilgili suşların detoksifikasyon/biyodegradasyon özelliklerinin geliştirilme potansiyelleri konuları incelenmiştir.

Anahtar Kelimeler: Mikotoksin, detoksifikasyon, biyodegradasyon, antagonistik maya, *Debaryomyces hansenii, Saccharomyces cerevisiae.*

➢ *ORAL PRESENTATION*

Molecularly-imprinted silica nanoparticles for selective quantification of ganciclovir in serum samples

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Abstract

Ganciclovir is a type of anti-viral drug which is used for treatment of Hepatitis B infected patients. In this case, molecularly imprinted silica nanoparticles was synthesized via surface-initiated reversible addition fragmentation chain transfer polymerization to selective and sensitive quantification of ganciclovir in human serum samples. The imprinted silica nanoparticles was detail characterized by combination of several surface analysis techniques and the results showedthere was a thin polymer layer on the silica nanoparticles. Moreover, the rebinding properties of ganciclovir on the imprinted sites were investigated. The imprinted nanoparticles showed high adsorption capacity, fast kinetics as well as high selectivity. Moreover, the imprinted nanoparticles were used as selective adsorbent for selective extraction and determination of ganciclovir from serum samples. The results indicated that the proposed method had high recovery rates with lower standard deviations. As a result, the proposed method can be useful for determination of ganciclovir in clinical applications.

Keywords: Molecularly imprinted polymers, silica nanoparticles, ganciclovir.

➢ *ORAL PRESENTATION*

Investigation of the cytotoxic effects of three different food additives using MTT assay

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Abstract

Food additives are substances that are added to foods in stages such as production, processing, storage or packaging. It is allowed to be used for purposes such as ensuring food safety, increasing taste, and extending shelf life however their impact on human health is controversial. These substances can be harmful to public health when they are used in foods out of regulation and that are toxicologically dangerous when consumed in random amounts. Monosodium glutamate (MSG), monopotassium glutamate (MPG) and magnesium diglutamate (MDG), the salts of glutamic acid, are food additives used as flavor enhancers. The aim of this study was to evaluate the cytotoxic effects of MSG, MPG and MDG using MTT (3-(4,5-dimethylthiazol-2-YL)-2,5-diphenyltetrazolium bromide) assay in human breast cancer (MCF-7) and normal breast epithelium (MCF-12A) cell lines. For this purpose, cells were grown to confluence at 37°C under 5% CO_2 in flasks with Dulbecco's Modified Eagle's Medium (DMEM) (MCF-12A) and RPMI-1640 (Roswell Park Memorial Institute) (MCF-7) including 10% fetal bovine serum. Different concentrations of MSG (437.5-27.34 mM), MPG (218.75-13.67 mM) and MDG (109,3-6.83 mM) treated with cells for 24 hours. Cell viability was measured using the following formula: Viability (%) = mean experimental (optical density) OD value /mean control OD value) x100%. The results showed that all three food additives have the ability to induce cytotoxicity in the cell lines used, depending on the concentration. According to the results, IC50 values of MSG, MPG and MDG were measured as 227.17, 84.64 and 80,27 mM for MCF-7 and 156.53, 197.24 and 14,05 mM for MCF-12A respectively. The results showed that MSG, MPG and MDG exhibited cytotoxic effect in MCF-7 and MCF-12A cell lines. However there is a need for further research with different cell lines.

Keywords: MSG, MPG, MDG, MTT assay, Cytotoxicity, Cell line.

Acknowledgement: This study was supported by Amasya University Research Fund under the project number FMB-BAP 19-0421.

➢ *ORAL PRESENTATION*

The evaluaiton of cell death and cell viability of YKG1 glioblastoma cell line treated with Panax ginseng before cisplatin.

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Abstract

The evaluaiton of cell death and cell viability of YKG1 glioblastoma cell line treated with Panax ginseng before cisplatin. Commercially purchased YKG1 glioblastoma cell line were incubated in DMEM F12 media until being confluence in %5CO2 incubator. Cells separated four groups. The first was control, group 2; 50 mg / ml Panax ginseng for 48 hours, group 3; 50 mg / ml Panax ginseng for 48 hours and then cisplatin treatment, and group 4 only cisplatin treatment. Cell viability was assessed by MTT analysis. Immunocytochemically, apoptatic cell markers bax / bcl 2 and caspas 3, and the MMPs from invasion markers were used for primary antibodies and the results were evaluated statistically. In addition, the cells were evaluated by performing PCR analysis using BAX, BCL and CASPASE 3 primers. According to immunocytochemical results, the staining intensity of apoptatic markers increased significantly in group 3 and 4. In addition, according to the results of the immunocytochemical evaluation markers, the staining intensity for groups 2 and 3 was decreased. According to the results of MTT, which is the determination of cell viability, the number of cells in groups 3 and 4 decreased significantly. When the PCR results were analyzed, it was determined that there were changes in the gene expression levels of apoptatic markers and that these changes were significant in the 3rd and 4th groups. It was thought that panax application before cisplatin treatment in human testicular tumor cells may have sensitized cells to cisplatin and the application maybe effective in cancer cells in terms of viability and cell death.

Keywords: YKG1 glioblastoma cell line, cisplatin, panax ginseng, bax/bcl, caspase 3.

➢ *ORAL PRESENTATION*

Investigation on Dynamic Mechanical Properties of Dextran and Polymethacrylamide Blends

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Abstract

In recent years, the development of new polymeric materials prepared by blending natural and synthetic polymers with better mechanical properties and biocompatibility than their single components have become more interesting because of the application especially in biomedical field. The biocompatibility, compatibility and phase separation investigations of dextran and its synthetic polymer blends are of great interest as intramolecular and intermolecular interactions allow the obtain novel polymeric materials especially in biomedical applications. Intermolecular interactions and compatibility of two components in polymer blends can be investigated by different techniques. In order to determine the dynamic mechanical properties of polymeric blends, DMA (Dynamic Mechanical Analysis) is an effective technique. DMA is also useful technique for explaining various types of transitions, especially the T_g values of blends and their individual components, as well as enable accurate and rapid determination of viscoelastic behavior and compatibility at a certain constant frequency over a specific temperature range. The objective of this study is to prepare, investigate and analyse dynamic mechanical properties, type of transitions, intra- and intermolecular interactions and compatibility/phase separation-structure-property relationships of the blends of Dextran (DEX) and Polymethacrylamide (PMAM). DEX/PMAM blends were prepared at different compositions by solvent casting method. Thermal transitions and α-relaxations were observed results from DMA measurements with increasing temperature. The temperature was increased at a heating rate of 3 °C/min over a temperature range of 30 °C to 250 °C at a constant frequency (ω=1 Hz). It was found that temperature dependence of dynamic mechanical properties and curves exhibit typical behaviours and strongly depended on the intra- and intermolecular interactions due to the hydrogen bonding in these blend systems.

Keywords: Polymer blend, DMA, Dextran, Polymethacrylamide

➢ *ORAL PRESENTATION*

Manyetize suyun ketende (*Linum usitatissimum* L.) *in vitro* tohum çimlenmesi ve sürgün rejenerasyonu üzerine etkisi

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Özet

Bu çalışmada, ketende çimlenme ve rejenerasyon ortamlarının hazırlanmasında kullanılan saf suyun manyetize edilmesi yoluyla *in vitro* tohum çimlenmesi ve sürgün rejenerasyonunun artırılması amaçlanmıştır. Farklı sürelerle (0-kontrol, 2, 4 ve 6 saat) 150 mT manyetik alana maruz bırakılan saf su hem *in vitro* tohum çimlendirme ve hem de rejenerasyon ortamlarının hazırlanmasında kullanılmıştır. Keten 'Madaras' çeşidine ait tohumlar, 10°C'lik sıcaklığa sahip %40'lık ticari çamaşır suyu içerisinde 12 dk. çalkalandıktan sonra, aynı sıcaklığa sahip steril saf su ile 3 kez 2'şer dakika durulanarak steril edildikten sonra manyetize su ile hazırlanan ortamda 2 hafta boyunca çimlendirilerek steril fideler elde edilmiştir. Bu steril fidelerden izole edilen hipokotil eksplantları, yine manyetize su ile hazırlanan rejenerasyon ortamına aktarılarak 4 hafta boyunca kültüre alınmıştır. Üzerinde rejenere olan sürgünleri taşıyan köklü eksplantlar, sıcaklık ve nemi kotrollü iklim odasında içerisinde torf bulunduran magenta kaplarına aktarılmış, üzerlerine ince şeffaf naylon poşet geçirilerek, nem oranının yüksek tutulması sağlanmıştır. Böylece, henüz dış ortam şartlarına alışmamış bitkiciklerin aniden su kaybederek ölmesi engellenmiştir. Saksı üzerindeki naylon poşete belirli aralıklarla (2-3 gün) makas yardımıyla delikler açılarak nem oranı yavaş yavaş azaltılmış ve en sonunda da saksı üzerindeki naylon poşet tamamen kaldırılmıştır. Bu şekilde bitkiciklere iklimlendirme uygulanmış ve dış ortam şartlarına alıştırılmıştır. Araştırma sonunda, manyetik alana maruz bırakılmayan su (kontrol) kullanılarak hazırlanan çimlendirme ve rejenerasyon ortamlarından en düşük sonuçların alındığı görülmüştür. Çimlendirme ve rejenerasyonda en yüksek sonuçlar 6 saat manyetik alana tabi tutulan saf su ile hazırlanan ortamdan elde edilmiştir. Bu araştırma, dünyada *in vitro* bitki kültüründe bir ilk olma niteliği taşımaktadır.

Anahtar Kelimeler: Keten, manyetize su, *in vitro*, çimlenme, rejenerasyon

➢ *ORAL PRESENTATION*

Yüksek fruktozlu mısır şurubunun MTT testi ile sitotoksik ve kromozomal anormallik testi ile genotoksik etkilerinin incelenmesi

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Özet

Yüksek fruktozlu mısır şurubu (YFMŞ), gazlı ve gazsız içeceklerde, unlu mamullerde, şekerlemelerde, konserve meyvelerde, reçellerde ve süt ürünlerinde gıda tatlandırıcısı olarak yaygın şekilde kullanılmaktadır. Bu araştırmada, YFMŞ'nun insan hepatoselüler karsinoma HepG2 hücre hattında 3-(4,5-dimetiltiyazol-2-il)-2,5-difenil tetrazolyum bromür (MTT) testi ile potansiyel sitotoksik etkisi incelenmiştir. Genotoksik etkisi ise, insan periferal kan lenfositlerinde kromozomal anormallik (KA) testi kullanılarak değerlendirilmiştir. Hücreler, YFMŞ'nun çeşitli konsantrasyonları (%5; %7,5; %10; %15; %20 ve %30) ile 24 ve 48 saat muamele edilmiştir. Ayrıca bir pozitif kontrol (Mitomisin C) ve bir de negatif kontrol (distile su) kullanılmıştır. Elde edilen verilere göre,YFMŞ'nun HepG2 hücreleri ile 24 ve 48 saat muamelesi sonucunda özellikle çalışılan yüksek konsantrasyonların (%7,5 - %30) hücre canlılığını doza bağlı olarak anlamlı oranda düşürdüğü belirlenmiştir. Kromozomal anormallik testinde ise her iki muamele süresinde de YFMŞ'nun yüksek konsantrasyonlarının (%15, %20) anormal hücre frekansını ve hücre başına düşen anormallik oranını kontrole göre istatistiksel olarak anlamlı düzeyde ve konsantrasyona bağlı şekilde artırdığı belirlenmiştir. Ayrıca en yüksek konsantrasyonun (%30) toksik etki gösterdiği gözlenmiştir. Buna göre, YFMŞ'nun özellikle çalışılan yüksek konsantrasyonlarının (%15-%30), HepG2 hücrelerinde sitotoksik etki sergilediği ve insan periferal lenfositlerinde ise KA frekansını artırarak genotoksik etkiye neden olduğu belirlenmiştir.

Anahtar Kelimeler: Yüksek fruktozlu mısır şurubu (YFMŞ), gıda tatlandırıcısı, MTT testi, kromozomal anormallik (KA) testi.

Teşekkür: Bu çalışma Gazi Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından 64/2020-01 nolu proje ile desteklenmektedir.

➢ *ORAL PRESENTATION*

Development of SSR markers targeting fatty acid synthesis genes for molecular breeding in soybean (*Glycine max* (L.) Merrill)

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Abstract

Soybean is major oil crop with more than 350 million tons production in the World. Turkish soybean production does not meet domestic needs. Thus, development of soybean cultivars have high yield and quality traits is necessary using modern breeding methods. Oil quality is important trait in soybean breeding. Soybean contains five types of fatty acids. The mail aim of soybean breeding is development cultivars have linolenic and high oleic acid contents due to health benefits of oleic acid. Thus, molecular breeding tools need to be developed for more efficient soybean breeding. Although QTLs control fatty acid content were mapped in the genome, these QTLs is insufficient for soybean breeding. Although transcriptomic methods identified a total of 124 genes associated with faith acid synthesis, none of markers were developed targeting the genes. In the present study, SSR markers targeting the fatty acid synthesis genes were developed. All the gene (209.3 kb in size) were screened for SSR. As result, 50 SSR were identified in 37 genes. Dinucleotide SSRs were the most prevalent (72%) followed by trinucleotide repeats (26%). AG/CT (18%) and TC/GA (18%) were the most frequent SSR motifs. A total of 40 SSR primers were developed for PCR. Also, physical map of the genes revealed five fatty acid gene clusters at chromosome 3, 5, 11, 13 and 20. This is the first study developed fatty acid specific SSR markers in soybean. These markers can be used in QTL and association mapping studies for fatty acid content in soybean.

Keywords: Marker development, fatty acid synthesis genes, genome mapping.

➢ *ORAL PRESENTATION*

Evaluation of Metimazole Induced Pancreatitis Formation Mechanisms in *In Vitro* Conditions

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Abstract

Methimazole is one of the first-choice antithyroid agent used especially in the treatment of hyperthyroidism and Graves' disease. In addition to the known adverse effects of methimazole, such as nausea, vomiting, and urticaria, different case reports reported pancreatitis as adverse effect of this active substances. Data on the risk of drug-induced acute pancreatitis are mostly based on case reports and informations obtained from case-control, animal and in silico studies and detailed in vitro studies are needed to elucidate the mechanisms of formation of pancreatitis risk. It is foreseen that in vitro studies to clarify the mechanisms of pancreatitis induced by drugs will be very useful in terms of eliminating the deficiency in this field. In this study the aim was to define the toxic effect mechanisms underlying metimazole induced pancreatitis. In this study, cell viability determined by MTT test in human pancreas/duct (PANC-1 / CRL-1469) cell line. Total Oxidant Status and Total Antioxidant Status analysis were performed for oxidative stress evaluation and endoplasmic reticulum stress biomarkers Grp78, Hsp90, DDTI3, ERN1, Caspase 12 were evaluated with Elisa tests with 200, 280 and 400 mM metimazole concentrations. According to our results, Total Oxidant Status, ERN1 and caspase-12 significantly increased in 400 mM concentration group compared to control. It is thought that the formation of reactive oxygen species may increase the risk of methimazole-induced pancreatitis via endoplasmic reticulum stress pathway induction. The results obtained are intended to contribute to the literature and provide a basis for the planning of further new researches on the subject.

Keywords: Methimazole, Acute Pancreatitis, Drug induced pancreatitis, Toxicity, Reactive oxygen species

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➢ *ORAL PRESENTATION*

Some useful strategies in metagenomic studies on various materials in the aquatic environment

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Abstract

Microbiological culture is a method that has been used for diagnostic purposes for decades and is almost the same age as modern microbiology. However, the scientific studies conducted today have revealed that a very small part of the bacteria found in nature can be cultivated under laboratory conditions, therefore, culture-based techniques can provide limited information about the diversity of existing microbiota in such studies. Starting with the first phylogenetic studies based on molecular biology more than forty years ago, and thanks to the invention of PCR and the great development in automated DNA sequencing technologies, the phenotypic-based classification has been completely replaced by molecular approach classification today. In this study, the experiences we have gained in our laboratory that can be helpful in conducting successful metagenomic studies on many different biotic and abiotic materials found in aquatic environments, including those commonly found in aquaculture, were shared. While doing this, attention was paid to discuss all the steps involved in such a study including sample collection, DNA extraction, amplification and next-generation sequencing (NGS).

Keywords: metagenomics, microbial diversity, aquatic environment, aquaculture

➢ *ORAL PRESENTATION*

Effects of usnic acid on oncomiR expressions, oxidative stress and cell proliferation of Caco-2 colon cancer cells

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Abstract

Usnic acid (UA), a secondary metabolite, is extracted primarily from some types of lichens. UA exhibits anti-microbial, anti-cancer, antioxidant and anti-inflammatory activities *in vitro* and *in vivo*. It is known that UA shows antiproliferative effects in different tumor cell lines, including lymphoma, glioblastoma, pancreatic, cervical and hepatocellular carcinoma. The aim of the study is to investigate the effects of UA on cell proliferation, oxidative stress and possible action mechanism via oncomiR expression changes. The effect of UA on cell proliferation was determined by XTT Cell Viability Assay. Expression profiles of oncomiRs elevated in colon cancer such as *miR-21, miR-155* and *miR-146a* were determined by real-time PCR. Total oxidant, antioxidant capacity of UA and oxidative stress index were determined by TAS/TOS Assay. IC_{50} dose of UA in Caco-2 human colon cancer cells was detected as 50 μM at the 24th hour. It was observed that UA significantly downregulates miR-21, miR-155 and miR-146a expression in Caco-2 cells. UA may have a role via decreasing oncomiR expressions and inducing oxidative stress in Caco-2 cells. Therefore, UA can be a good lichen secondary metabolite for further anti-cancer studies.

Keywords: Usnic acid, OncomiRs, Caco-2, colon cancer

➢ *ORAL PRESENTATION*

Coenzyme Q10 effect on cisplatin-induced oxidative optic nerve injury in rats

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Abstract

In this study, it was aimed to investigate the effect of coenzyme Q10 (CoQ10) on cisplatin-induced oxidative optic nerve damage in rats biochemically and histopathologically. 30 male Wistar albino rats were divided into 3 groups randomly: untreated control (C group), only 2,5 mg/kg cisplatin daily administrated group for 2 weeks (CP group), 2,5 mg/kg cisplatin + 20 mg/kg orally CoQ10 daily administrated group for 2 weeks (CoQC group). At the end of experimental period, blood samples obtained before sacrification for the biochemical examination of serum malondialdehyde (MDA), total glutathione (tGSH), total oxidant system (TOS), total antioxidant systemic (TAS) levels and after eyes were removed for examined histopathology. As a result of our study, severe histopathological damage was detected in the optic tissue of the cisplatin group as destruction, vacuolization, edema with serum malondialdehyde (MDA) and total oxidant system (TOS) levels were high and total glutathione (tGSH) and total antioxidant systemic (TAS) levels were low. However, it was observed that the histopathological damage associated with cisplatin was decreased in the optic nerve tissue of the CoQ10 group, which inhibited the increase in blood serum MDA / TOS levels and decrease in tGSH / TAS levels. The biochemical and histopathological results of our study were compatible with each other, so we concluded that the damage to the rat optic nerve tissue caused by cisplatin may be reversible with coenzyme.

Keywords: Cisplatin, coenzym Q10, optic nerve toxicity, oxidative damage

➢ *ORAL PRESENTATION*

Effect of pycnogenol on ethanol-related oxidative optic nerve injury: An experimental study

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Abstract

We aimed to determine the protective effects of Pycnogenol on ethanol induced optic nerve toxicity in an experimental model. 30 male Wistar albino rats were divided into 3 groups randomly: untreated healthy control (HC group), only ethanol daily administrated group for 6 weeks (EtOH group), ethanol + 40 mg/kg orally Pycnogenol daily admisinstrated group for 6 weeks (PEtOH group). To the rats in HC and EtOH groups, the same volume (0.5 ml) of distilled water as solvent was applied in the same manner. To the rats in PEtOH and EtOH groups, one hour after application of Pycnogenol or distilled water, 32% ethanol with a dose of 5 g/kg was administered via oral gavage. At the end of expiemental period, blood samples obtained before sacrification for biochemical examination of serum malondialdehyde (MDA) and total glutathione (tGSH) levels and after eyes were removed for examined histopathology. Histopathological evaluations in the EtOH group showed significant destruction in optic nerve tissue with marked edema, degeneration, vacuolization. However, it was observed that MDA values increased and tGSH values decreased in EtOH group. In the PEtOH group, MDA values decreased and GSH values increased. Again, degenerative changes were considerably reduced in this group. In the light of biochemical markers and histopathological evaluations, it was observed that ethanol exposure caused a significant degeneration in the optic nerve tissue. It was found that Pycnogenol administration significantly reduced the destructive effects seen histopathologically. Biochemical results also coincided with other findings. It was concluded that ethanol-induced toxicity can be prevented to a large extent by pycnogenol administration.

Keywords: Ethanol, Pynogenol, optic nerve toxicity, oxidative damage

➢ *ORAL PRESENTATION*

Lilium candidum L.'nin Kriyoprezervasyonu için Biyoteknolojik Yöntemlerin Oluşturulması

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Özet

Lilium candidum L. önemli bir tıbbi ve süs bitkisidir. Liliaceae familyasına ait olan bitki soğanlı bir bitki olup, mide iltihabı, şişkinlik gibi birçok hastalığın tedavisi için halk tarafından kullanılabilmektedir. Ancak, son yıllarda doğadan direkt sökülerek ve değişen iklim şartlarına bağlı olarak, *Lilium candidum* L. bitkisi nesli tükenme tehlikesi altında olan türler arasına girmektedir. Bu çalışmada, *Lilium candidum* L. bitkisinin gövde bulbillerinden elde edilen kalluslar kullanılarak kriyoprezervasyon işlemi gerçekleştirilmiştir. Kriyoprezervasyon, hücrelerin metabolizmasını minumum düzeye düşürerek ultra soğuk ortamda (-196°C) uzun süreli muhafaza işlemidir. Kriyoprezervasyon yapılırken hücrelerin bütünlüğünü koruma amaçlı belli kriyoprotektanlar kullanılmaktadır. Çalışmada, kriyoprotektanlardan biri olan PVS-2 (Plant Vitrificitaion Solutiun-2) kullanılmasıyla hücrelerin kristalleşmesi engellenerek amorf bir yapıda hücrelerin elde edilmesi hedeflenmiştir. PVS-2 canlı hücreler için toksik bir madde olabileceğinden farklı uygulama sürelerinde canlılığa zarar verebilmektedir. Bu nedenle, PVS-2 uygulama süresine (60, 75, 90, 105 ve 120 dakika) bağlı denemeler kurularak *Lilium candidum* L. bitkisi için en uygun PVS-2 süresi belirlenmiştir. Kriyo sonrası besin ortamında (aktif kömür içeren 1 mg/L BA ilaveli Olive Medium (OM) besin ortamı) çoğaltılmasına devam ettirilen kallusların çaplarına bağlı olarak yapılan istatistiki analizler sonucunda, 90 dakika PVS-2 uygulamasının bitki hücrelerinin gelişimi için en uygun süre olduğu belirlenmiştir.

Anahtar Kelimeler: *Lilium candidum* L., kriyoprezervasyon, kallus, PVS-2

[+]Bu çalışma, TÜBİTAK tarafından desteklenmektedir (Proje no: TOVAG-217O009 nolu proje).

➢ *ORAL PRESENTATION*

The evaluation of the effects of *Helichrysum plicatum* subsp. *pseudoplicatum* plant extract containing chlorogenic acid to the wound healing on human dermal fibroblast cell line

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Abstract

Helichrysum species (Altınotu) are rich in phenolic compounds used in traditional medicine for wound healing. The main component in their flowers is chlorogenic acid. It is aimed to determine chlorogenic acid in methanol extract of the flowers of *H. plicatum* subsp. *pseudoplicatum* by HPLC method and to investigate the proliferative, oxidative stress, and wound healing effects of the plant extract on human dermal cell line *in vitro* on cell viability in this study.

The flowers of *H. plicatum* subsp. *pseudoplicatum* were collected in Erzurum Turkey (altitude 1950 m), dried, pulverized, and extracted with methanol. For the determination of chlorogenic acid in the obtained plant extract, the experimental conditions in the HPLC method were optimized by the central composite design method. Chromatographic separation was performed at 330 nm wavelength by using a mobile phase of acetonitrile and water containing orthophosphoric acid with a reversed-phase column. The proliferative effect, oxidative stress activities, and wound healing effects on human dermal cells were evaluated by XTT test, TAS-TOS commercial kits, and the scratch experiment by taking microscopic images of the cells at 0, 12, 18, and 24 hours, respectively. The HPLC method for determining chlorogenic acid exhibit linearity in the concentration range of 0.1-100 µg/mL. The proposed method was precision with 3.32% RSD values and accurate with ± 4.05 RE values. According to the developed method, the chlorogenic acid amount in plant extract was determined as 9.77 ±1.818 (µg/mg extract). According to the XTT test results, the plant extract has a proliferative effect at 0.5-10 µg/mL concentrations. It was observed that TAS levels significantly increased in plant extract at the dose ranges 1-10 µg/mL. 1-5 µg/mL plant extract started to increase cell migration at the 12th hour and significantly closed the wound area at the 24th hour. It has the strongest effect on both cell viability, antioxidant effect, and wound healing was found to be in this dose range.

It was concluded that chlorogenic acid is an important natural product found in the flowers of *H. plicatum subsp. pseudoplicatum* plant and the plant extract accelerates wound healing by increasing cell migration in low doses.

Keywords: *H. plicatum* subsp. *pseudoplicatum*, HPLC, chlorogenic acid, wound healing

➢ *ORAL PRESENTATION*

Gıdalarda Akrilamid Oluşumu ve İnsan Sağlığına Etkileri

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Özet

Gıdalardaki düzeyi üretim yöntemine göre değişmekle beraber akrilamid bisküvi, kraker, ekmek, kahvaltılık tahıl ürünleri, cips ve patates kızartması gibi ürünlerde sıklıkla bulunan bir bileşendir. Akrilamidin temel oluşum mekanizması, pişirme ve kızartma gibi yüksek sıcaklıklara maruz kalması sonucunda başta asparajin olmak üzere aminoasitler ve bazı indirgen karbonhidratların Maillard reaksiyonuna katılmasıdır. Mevcut araştırmalar sonucunda akrilamidin vücudun birçok farklı bölgelerinde toksik etkilere neden olduğu ortaya konmuştur. Merkezi sinir sistemindeki, üreme hücrelerindeki ve hamilelerde plasenta gelişimindeki olumsuz etkileri akrilamidin toksisite belirtilerinden bazılarıdır. Ayrıca fetal gelişimde de kardiyovasküler sistem, beyin gibi çeşitli yapıların gelişmesinde toksik etkilere sebep olmuştur. Diğer bir taraftan oksidatif strese sebep olan akrilamid DNA hasarına ve apoptoza neden olabilmektedir. Akrilamid içeren gıdaların yaygın tüketimi söz konusu olduğundan dolayı akrilamidin gıdalardaki oluşum koşulları ve miktarının bilinmesi önem arz etmektedir. Ayrıca tüketimi nedeni ile akrilamidin absorbsiyon mekanizması, metabolik yolları ve atımı üzerine araştırmaların yapılması akrilamidin sağlık üzerine endişelerine ışık tutmaktadır. Bu çalışmada, gıdalarda akrilamid oluşum mekanizmalarına, metabolik yollarına ve akrilamidin insan sağlığı üzerindeki olumsuz etkilerine değinilmiştir.

Anahtar Kelimeler: Akrilamid, Maillard reaksiyonu, sağlık, akrilamid oluşumu

➢ *ORAL PRESENTATION*

Çiftlik hayvanlarında genom çapında ilişkilendirme çalışmaların rolü

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Özet

Şüphesiz çiftlik hayvanları, hayvansal üretime bağımlı olan ülkelerin ekonomisine doğrudan etkisi vardır. Önemli besin değerleri nedeniyle hayvansal ürünlere gün gittikçe talepler artmaktadır. Çoğu ekonomik özellikler, kompleks bir genetik sisteme sahiptir. Önemli ekonomik özelliklerin, kantitatif özellik lokuslarıyla (QTL), bu kompleks genetik sistemi tanımlamak mümkün hale gelmiştir. Ekonomik açıdan önemli özelliklerden sorumlu olan kantitatif özellik lokuslarının (QTL) haritalanması önemli sonuçlar elde etmesine rağmen, QTL haritalama çalışmalarında kullanılan genetik belirteçlerin düşük verimliliği nedeniyle, kompleks özelliklerdeki tüm genetik varyasyonlar tanımlanmamıştır. QTL'nin dezavantajlarını gideremek için, Son zamanlarda, genom çapında ilişkilendirme çalışmaları (GWAS) yöntemi geliştirilmiştir. Çok sayıda tek nükleotid polimorfizmi (SNP) kullanmasında dolayı, GWAS yöntemi hayvan yetiştiriciliğinde büyük ilerleme kaydetmiştir.

Anahtar Kelimeler: GWAS, QTL, SNP, Çiftlik hayvanları.

➢ *ORAL PRESENTATION*

Deep Eutectic Solvents (DES) and Their Applications in Extraction Processes

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Abstract

Chemicals used in the industrial production process play an essential role in people's daily life. On the other hand, the use of chemicals has negative consequences on human health and the environment. Recently, many studies have examined the effects of chemicals on living things. These studies have shown that chemicals known as Volatile Organic Compounds (VOCs) constitute one of the most dangerous groups considering world consumption. Many chemicals under the solvent class in this group have a significant negative impact on the world's ecological balance [1-3].
Studies are carried out in the chemical industry to ensure that production can be carried out in more environmentally friendly ways. These studies focus mostly on the discovery of new, environmentally friendly solvents. In the early 2000s, inexpensive, easy-to-prepare, and environmentally friendly new solvents, named as "deep eutectic solvent" (DES) in the literature, were discovered as promising alternatives in this field. DESs are eutectic mixtures prepared from Lewis or Bronsted acids and bases containing various anionic and/or cationic species [4].
In our study, the use of deep eutectic mixtures as environmentally friendly solvents was investigated. In this context, the definition, classification, the thermodynamic examination of DESs, and their usage areas subject to recent research will be discussed. In addition, some data from our experimental work on ongoing extraction applications using deep eutectic solvents will be shared.

Keywords: Green solvent, deep eutectic solvent, extraction

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➢ *ORAL PRESENTATION*

Determination of biotin with affinity based potentiometric sensor

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Abstract

Biotin (Vitamin B7) acts as the prosthetic group of four carboxylase enzymes (propionyl-CoA carboxylase, 3-methylchrotonyl-CoA carboxylase, pyruvate carboxylase and cytosolic acetyl-CoA carboxylase) that play a central role in protein, carbohydrate and fat metabolism in humans, and thus plays an important role in gluconeogenesis, fatty acid synthesis and catabolism of branched chain amino acids. Although biotin is continuously recycled by the effect of biotinidase, biotin and biotinidase enzyme deficiency is rarely seen in humans. Biotinidase deficiency disrupts the biotin cycle in the organism and causes a disease with clinical findings such as metabolic acidosis, neurological symptoms and skin findings like dermatitis, conjunctivitis and alopecia. Streptavidin is widely used in bioanalytical applications since its interaction with biotin is one of the most powerful non-covalent interactions. In this study, nanoenzyme synthesis was carried out by providing photosensitive conjugation and cross-linking with the ANADOLUCA method, where ruthenium-based amino acid monomer chelates were used as crosslinkers. Potentiometric sensor was prepared by using dibutyl phthalate and graphite after conjugating enzyme nanoparticle and streptavidin with covalent conjugation. The effect of pH, response time, lifetime, selectivity, reusability, reproducibility, accuracy, and stability of the sensors were analyzed. The limit of detection of the prepared potentiometric sensor was 0.3 10^{-15} M, with the linear dynamic range from 10^{-15} M to 10^{-7} M. The response time for the prepared sensor was 7 min and the operational lifetime was 3 months.

Keywords: Biotin, potentiometry, biosensor, affinity

➢ *ORAL PRESENTATION*

ABS-oxaliplatin combination enhances anti-cancer efficacy in HT29 human colorectal tumor cell line

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Abstract

Colon cancer is a malignant type of cancer with a high prevalence and is one of the primary causes of cancer-related deaths. Oxaliplatin plays a significant role in the treatment of cancer, but the application of oxaliplatin is restricted due to its toxic side effects and drug resistance in clinical practice. Therefore, there is an urgent need for new strategies that can synergize with oxaliplatin for confronting colon cancer. Ankaferd Blood Stopper (ABS) is a standardized herbal extract mixture prepared from Thymus vulgaris, Glycyrrhiza glabra, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica*, and it is known as a hemostatic agent, possesses antitumor properties in many cancer cell lines. This study evaluated the therapeutic potential of ABS, alone and in combination with oxaliplatin in *in vitro* human colon cancer model. HT29 cells were exposed to oxaliplatin (0-100 µg/ml), ABS (0-90 µL) or oxaliplatin plus ABS for 24 h. The cell viability was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The combination index was calculated according to Chou's model using the CalcuSyn program. Gene expression of apoptosis markers such as bax, bcl-2, cytochrome-c, apaf-1, caspase-3 and p53 was determined by Real-Time PCR. Results prove that ABS alone or in combination with oxaliplatin has a strong effect on cell proliferation and vitality in a dose-dependent manner. ABS and oxaliplatin combination has increased the expression of cytochrome-c, apaf-1, caspase-3 and bax genes which induce apoptosis and decreased the bcl-2, p53 genes in colon cancer cells. These data indicated that ABS-oxaliplatin combinatory treatment promotes apoptosis and enhances anti-cancer efficacy in colon cancer cells.

Keywords: HT29, colon cancer, chemotherapy

➢ *ORAL PRESENTATION*

Diyabetik Ratlarda Fesleğen (*Ocimum Sanctum*) Uygulamasının Böbrek Dokusunda Tümör Nekrozis Faktör-α ve İnterlokin 1 β Salınımı Üzerine Etkileri

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Özet

Bu çalışmada, fesleğen (*Ocimum Sanctum*) uygulamasının, streptozotocin (STZ) ile deneysel diyabet oluşturulan ratların böbrek dokusunda tümör nekrozis faktör alfa (TNF-α) ve interlökin 1β (IL-1β) üzerine meydana getirdiği değişikleri incelemek amaçlanmıştır. Çalışmamızda her bir grupta 8 adet *Spraque Dawley* cinsi erkek rat olmak üzere toplam 40 adet sıçan kullanıldı. Ratlar, diyabet, diyabet+*Ocimum sanctum, Ocimum sanctum*, kontrol ve sham olmak üzere 5 gruba ayrıldı. Kontrol grubuna herhangi bir uygulama yapılmadı, sham grubuna intraperitonel (i.p.) olarak 50 mg/kg sodium sitrate uygulandı. Diyabet ve diyabet+*Ocimum sanctum* gruplarına i.p 50 mg/kg STZ enjeksiyonu yapılarak diyabet oluşturuldu. Daha sonra *Ocimum sanctum* ve diyabet+*Ocimum sanctum* gruplarına *Ocimum sanctum* ekstraktı 200 mg/kg olacak şekilde oral gavaj yolu ile 14 gün boyunca verildi. Histopatolojik incelemeler için dokulara hematoxylin & eosin ve üçlü boyamalar yapıldı. TNF-α ve IL-1β'in böbrek dokusundaki immunohistokimyasal lokalizasyonu streptavidin-biotin peroxidaz yöntemi ile belirlendi. Bütün gruplardaki ratların böbrek dokusunda14. günlerde spesifik olarak TNF-α ve IL-1β immunoreaktivitesi görüldü. Diyabet ve diyabet+*Ocimum sanctum* grubundaki ratların tubulus proksimalis ve tubulus distalis hücrelerinde 14. günlerde güçlü immunoreaktivite tespit edilmesine karşın, *Ocimum sanctum*, sham ve kontrol grubundaki ratlarda zayıf immunoreaktivite tespit edildi. Diyabet grubunun böbrek korteksinde güçlü, diyabet+*Ocimum sanctum* grubunda ise böbrek korteksinde orta düzeyde IL-1β immunoreaktivitesi tespit edildi. Diyabet grubundaki ratların tubulus proksimalis, tubulus distalis, makula densa ve henle kulpundaki hücrelerinde 14. günlerde güçlü immunoreaktivite tespit edilmesine karşın, diyabet+*Ocimum sanctum* grubunda orta düzeyde, *Ocimum sanctum*, sham ve kontrol grubundaki ratlarda ise zayıf düzeyde immunoreaktivite tespit edildi.

Anahtar Kelimeler: Diyabet, *Ocimum sanctum*, TNF-α, IL-1β, Böbrek,

➢ *ORAL PRESENTATION*

In vitro biological activities of *Colchicum speciosum* Steven corm extract assessment

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Abstract

This research's basic purpose was to investigate the antimicrobial and antioxidant activities and total phenolic and flavonoid contents *Colchicum speciosum* corm ethanol extract. The crude extract was collected from Tokatlı Canyon in Karabük province in Turkey during 2016-2017. Antimicrobial activities of ethanol extract were investigated by the disk diffusion method according to the CLSI procedures against eight bacteria (3 non-filamentous Gram-positive, three filamentous Gram-positive, 2 Gram-negative bacteria) and two yeast strains. 70% ethanol and ciprofloxacin were used as control to evaluate the antioxidant activity two different methods (DPPH radical scavenging activity assay and ferric reducing antioxidant power analysis) were followed. The total phenolic content was determined according to the Folin-Ciocalteu method. The differences between the means of the inhibition zones were tested with a Tukey HSD test with one-way variance analysis. Mean diameters of inhibition zones were found in the range of 12.80±0.18 mm (*Candida albicans*) to 28.57±0.47 mm (*Nocardia cyriacigeorgica*). *N. cyriacigeorgica*, *Proteus vulgaris* (27.55±0.19) and *Micrococcus luteus* (24.06±0.18) were the most sensitive microorganisms to the extract. The total antioxidant activity value of the extract was calculated at 7.28 mg AAE/g. The IC_{50} value was calculated at 27.455 mg/mL for DPPH free radical scavenging activity. The total phenolic content of the extract was determined as 0.483 mg GAE/g. The FRAP value indicated that the reducing power of 1 gram of sample was equivalent to 3.75 µmol of Trolox. The flavonoid content of the extract was calculated as 6.48 mgQE/g. We conclude that the analyzed ethanol extract of *C. speciosum* corm was pointed out that antioxidant and antimicrobial activity level could be considered useful.

Keywords: antibacterial activity, antifungal activity, pathogenic actinomycetes

➢ *ORAL PRESENTATION*

Proliferatif Konsantrasyondaki Timokinonun NRK-52E Hücre Hattında TP53 Gen Ekspresyon Düzeyine Etkisi

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Özet

Nigella sativa (çörek otu)'nın önemli içeriklerinden biri olan ve bazı ilaçların üretiminde kullanılan timokinonun (TQ) geleneksel tedavide birçok hastalık için kullanılmaktadır. TP53 geni, tümör proteini p53 adı verilen bir proteini yapmak için kodlanmıştır. Bu protein bir tümör baskılayıcı olarak işlev görür; hücrelerin büyümesini ve çoğalmasını kontrol altında tutar. Bu çalışmanın amacı sağlıklı hücrelerde TQ'nun TP53 genin ekspresiyon seviyesine etkisini araştırmaktı. Bu çalışmada sağlıklı bir hücre hattı olan, rat böbrek epitelyal hücre serisi (NRK-52E) kullanıldı. Hücre serisine proliferatif dozda etki gösteren 10 µM TQ'u uygulandı. Uygulama sonrası TP53 genin ekspresyon düzeyi gerçek zamanlı kantitatif polimeraz zincir reaksiyonu (RT-qPCR) yöntemiyle tespit edildi. RT-qPCR sonuçlarına göre TQ'nun NRK-52E hücrelerinde TP53 genin ekspresyonunu azalttığı tespit edilmiştir. Bu sonuçlar TQ'nun bir tümör baskılayıcı olarak görev gören TP53 genini NRK-52E hücre serisinde downregüle etiği sonucuna varılmıştır.

Anahtar Kelimeler: Timokinon, Ekspresiyon, TP53

➢ *ORAL PRESENTATION*

Beyaz Peynirde Bozma Etmeni Mayaların DNA İzolasyon Yöntemleri

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Özet

Süt ve süt ürünleri özellikle yüksek protein, vitamin (B_2, B_6, B_{12} ve A vitamini), mineral maddeler (yüksek oranda kalsiyumun yanında, potasyum, fosfor, magnezyum, iyot ve çinko) bakımından oldukça zengin gıdalardır. Bu nedenle, insanların sağlıklı beslenebilmesi için tüketilen besinler arasında süt ve süt ürünlerinin mutlaka yer alması gerekir. İnsan beslenmesinde bu kadar önemli olmasına karşın, süt çok çabuk bozulan bir gıda maddesi olması nedeniyle, sütün raf ömrünü arttırmak için çeşitli yöntemler geliştirilmiştir. Bu uygulamalardan birisi de çabuk bozulan sütün besin değeri yüksek ve uzun süre bozulmadan saklanabilen bir gıda ürünü olan peynirin yapılmasıdır.

Uygun çevre koşulları buldukları takdirde, birçok mikroorganizma peynirde de bozulma yapabilir. Bu mikroorganizmalar arasında mayalar önemli bir yer tutmaktadır. Özellikle beyaz peynirlerde gelişen mayalar sağlık açısından önemli bir sorun oluşturmasa bile, peynirde görünüş ve yapı gibi bazı duyusal özellikleri bozduğu için tüketici tarafından kabul edilemez kusurların oluşmasına neden olabilmektedir.

Mayalar uygun ortamlarda yalancı misel oluştursalar da tek hücreli, büyük (5-8µ m çapında), oval, uzun, eliptik veya yuvarlak hücre şekilleri ile ve bölünme esnasında tomurcuk oluşturmaları nedeniyle bakteri ve küflerden ayrılırlar. Geniş pH, şeker ve alkol sınırları içerisinde gelişebilirler. Krem renginden pembe kırmızıya kadar değişen renkte pigmentler oluşturabilirler.

Teknolojinin gelişmesiyle birlikte mayaların tanımlama yöntemlerinde çeşitlilikler görülmektedir. Bunlar klasik, test kiti ve moleküler tekniklerdir. Bu analizlerin doğruluğunda saf kültür kullanımı çok önemlidir. Moleküler tekniklerde saf kültür kullanımın yanında DNA izolasyon yöntemlerinin seçimi ve kullanılması ayrı bir önem taşımaktadır. Çünkü moleküler tekniklerin başarısı DNA izolasyonunun etkinliği, DNA molekülünün saf olması ve artık kimyasal maddelerin bulunmamasına bağlıdır. Bu yöntemler çeşitlilik göstermektedir. Bunlar klasik yöntemler (enzim kullanılması, enzim kullanılmaması, fiziksel hallerin kullanası vb.), izolasyon kiti (genel ve mayaya özgü) ve aletler (Bioneer ExiPrep™ 16 Plus vb.) yardımıyla yapılmaktadır. Bu çalışmada DNA izolasyon yöntem prosedürlerine, aralarındaki faklara, klasik ve aletle yapılan DNA izolasyon değerlerinin karşılaştırılmasına değinilecektir.

Anahtar Kelime: DNA izolasyonu, Peynir, Moleküler Analizler

➢ *ORAL PRESENTATION*

TGF-β1 Induces ADAMTS8 (A Disintegrin and Metalloproteinase with Thrombospondin Motif 8) Expression in MG-63 Cells

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Abstract

The transforming growth factor-β (TGF-β) family members are secreted cytokines and represented three isoforms in mammals (TGF-β1, -β2 and, -β3). TGF-β1 is the most abundant isoform at the protein level in bone. TGF-β1 can regulate bone remodeling affecting osteoblast and osteoclast differentiation and activation. TGF-β1 precursors are synthesized by osteoblasts, deposited in the bone matrix, and activated by acids and matrix metalloproteinases secreted from osteoclasts. TGF-βs have known as pleiotropic cytokines because they can able to act as both tumor suppressors and tumor promoters, depending on the cancer type and tumor development stage. In contrast with the dual effects of TGF-βs on cancer progression, TGF-βs mainly have a pro-tumoral effect in osteosarcoma. ADAMTSs are secreted extracellular matrix proteases that can cleave a wide range of substrates in the extracellular matrix and can modify the tumor microenvironment. As a member of ADAMTS family, ADAMTS8 is a potential tumor suppressor gene having proapoptotic and antiangiogenic properties and is epigenetically silenced in common cancer cells. Expression pattern and the regulatory factors of the ADAMTS8 gene in osteosarcoma hasn't been known yet. In the present study, the effect of the TGF-β1 on ADAMTS8 mRNA expression level was evaluated in osteoblast-like MG-63 cells. ADAMTS8 mRNA level was determined after 500U/ml TGF-β1 stimulation by qRT-PCR based strategy. It was determined that TGF-β1 induced ADAMTS8 mRNA expression in MG-63 cells.

Keywords: ADAMTS8, TGF-β1, Osteosarcoma, MG-63

➢ *ORAL PRESENTATION*

Determination of colo 320 extracellular vesicles via gold electrode and calibration curve study for high concentration sample

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Abstract

Exosomes are sub-micron-sized lipid transporters released from cells by exocytosis. Studies to examine the intercellular communication process in normal and pathological cells have greatly increased the interest in exosomes. The most important reason for this is that factors known to be formed by cancer cells are released by exosomes [1]. In addition, the exosome level is high in blood samples of cancer patients (such as prostate, lung, stomach) [2]. For this reason, detection and determination of exosomes in body fluids is of vital importance in determining their prognostic, diagnostic and therapeutic values. In this study, we designed a biosensor system based on anti-CD63 is used to capture and determine the exosomes. Four different calibration graphs were created for the determination of Colo 320 cell lines with signal points obtained from five different concentrations.

Keywords: extracellular vesicles, exosomes, biosensor.

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➢ *ORAL PRESENTATION*

Association of VEGF +936 C/T Polymorphism with VEGF Expression and Glioma Risk In Turkish Population

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Abstract

Gliomas are the most prevalent malignant primary tumors of CNS. Due to the limited treatment options, it is very important to detect genes that cause gliomas to show high angiogenic activity and genetic susceptibility to glioma. VEGF is one of the most significant genes related cancer pathogenesis and angiogenesis. Although there is a study in Chinese population related with VEGF +936C/T polymorphism and glioma risk, in Turkish populations there is no study. The objective of current study was to evaluate the effect of VEGF +936C/T polymorphism on susceptibility to glioma in Turkish population and relationship between VEGF gene expression and glioma angiogenesis.

In the present study, 96 glioma patients and 104 healthy controls, matched for age (49.09±1.33, 48.60±1.34, respectively), were included. VEGF +936C/T polymorphism were evaluated with PCR-RFLP and relative VEGF expressions were measured by RT-PCR. The data were evaluated with SPSS version 22.0.

The CC, CT and TT genotypes of VEGF +936C/T polymorphism were determined 68, 26, 2 in glioma patients and 103, 1, 0 in controls, respectively. Although there was no significant relationship between the VEGF +936C/T polymorphism and glioma susceptibility in terms of genotype and allele distribution ($p>0.05$), the mean relative VEGF expression was found 9.27 times higher in gliomas compared to healthy controls ($p<0.01$).

In conclusion, the findings obtained from our study suggest that high relative VEGF expression in glioma patients may contribute to increased angiogenic activity. There was no difference in genotype and allele distribution in VEGF +936C/T polymorphism in terms of glioma risk.

Keywords: Glioma, Polymorphism, VEGF.

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➢ *ORAL PRESENTATION*

In vitro diyabetik koşullarda protirelinin fibroblast yara iyileşmesine etkisi

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Özet

Yara iyileşmesinin diyabette görülen hiperglisemiye bağlı olarak bozulduğunu gösteren birçok çalışma vardır. *In vitro* koşullarda da yüksek glukozun keratinosit ve fibroblast migrasyonunu inhibe ettiği gösterilmiştir (1). Protirelin bir tirotropin salgılattırıcı faktör (TRF) analoğudur. TRF ve analoğunun (taltirelin) yara iyileşmesini düzeltici özelliği gösterilmiştir (2). Bizim çalışmamızın amacı ise in vitro ortamda diyabetik koşulların taklit edildiği yüksek glukoz inkübasyonunda protirelinin yara iyileşmesine etkisinin araştırılmasıdır.

Hücre kültüründe L929 fibroblast hücre serisi kullanılmıştır. Hücreler 5 mM (kontrol grubu) ve 25 mM (yüksek glukoz grubu) glukoz içeren vasatta 48 saat inkübe edilmiştir. Protirelin grubuna ise 25 mM glukoz ve 100 nM protirelin uygulanmıştır. Deney koşullarının hücre canlılığına etkisinin incelenmesi için XTT, yara iyileşmesinin değerlendirilmesi için de migrasyon testi (scratch migration assay) yapılmıştır.

XTT testinde 48 saat sonra hücre canlılığı, kontrol grubuna göre (% 100) yüksek glukoz ve protrelin grubunda sırasıyla % 97.69 ve % 97.33 olarak bulunmuştur. Yüksek glukoz inkübasyonu 24 saat sonra fibroblastların migrasyonunu kontrol grubuna kıyasla % 8.39 ± 1.004 ($p<0.001$) azaltmıştır. Protirelin inkübasyonu ise hücre migrasyonunu 24 saat sonra yüksek glukoz grubuna kıyasla % 8.66 ± 2.304 ($p<0.01$) azaltmıştır. Protirelin inkübasyonu hücre migrasyonunu yara oluşumundan 48 saat sonra yüksek glukoz grubuna kıyasla % 5.596 ± 2.018 ($p<0.05$) azaltmıştır.

Çalışmamızda literatürle uyumlu olarak yüksek glukoz inkübasyonu fibroblast migrasyonunu azaltmıştır. Protirelin, yüksek glukoz düzeyinin azalttığı fibroblast migrasyonu üzerinde iyileştirici bir etki göstermemiştir. Protirelinin yara iyileşmesi üzerine etkilerinin aydınlatılması için daha fazla çalışma yapılması yararlı olacaktır.

Anahtar Kelimeler: Diyabet, Yüksek Glukoz, Fibroblast, Protirelin, Yara İyileşmesi

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➢ *ORAL PRESENTATION*

Identification of *Aspergillus niger* and *Aspergillus flavus* on Soybean Seed

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Abstract

Aspergillus species are widely distributed fungi worldwide and significantly affect soybean seed production. This study was conducted to detect and identify *Aspergillus* species on soybean seeds. From this purpose, we randomly chose soybean seeds showing different shape and color in the experimental field of Akdeniz University, Antalya, Turkey in 2017. In order to isolate the causal fungi, soybean seeds firstly surface sterilized with the 10% NaOCI for two min and then rinsed with sterile water. After drying on paper towel, five seeds were placed on each Petri plates containing PDA and incubated for seven days. After incubation period, two different fungal colonies were observed in culture. Based on the culture and colony morphologies, the fungi were identified as *Aspergillus niger* and *Aspergillus flavus*. Additionally, total genomic DNA of the isolates was extracted and the internal transcribed spacer (ITS) region of ribosomal DNA was amplified with ITS1 and ITS4 primers. Amplicons of 550 bp in size were successfully amplified with conventional PCR.

Keywords: *Aspergillus niger, Aspergillus flavus,* Soybean, Seed, ITS region

➢ *ORAL PRESENTATION*

Determination and Molecular Characterization of Bacteria in Mix of Sweet Sorghum and Soybean Silage

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Absract

Bacteria in silage are one of the main factors affecting the silage quality. This study was conducted to detect and identify bacteria in mix of sweet sorghum and soybean silage. Each silage samples were measured 30 g and mixed with 270 ml of sterilized water in a clean bench. Prepared each mix were placed in eppendorf tubes (2 ml) and then were serially diluted from 10^{-1} to 10^{-5}. Bacteria were taken into nutrient agar (NA) incubated at 24 °C for 48 h under an absence of free oxygen conditions. Genomic DNA was extracted for the molecular analysis of the microbial agents. The 16S rDNA Fd1 (CAGAGTTTGATCCTGGCTCAG) and Rd1 (AAGGAGGTGATCCAGCC) primers were used to amplify for the bacteria isolates in PCR. Conventional PCR was done in a thermal cycler BIORAD T100™ (Biorad, Hercules, CA). The amplified PCR products were sequenced with sanger sequencing method. The sequenced products were edited using Chromas software. According to the sequence analysis, *Enterococcus spp., Pseudomonas spp., Ralstonia spp. and Bacillus spp.* were identified.

Keywords: Bacteria, Sweet sorghum silage, Soybean silage, PCR, Sequence,

➢ *ORAL PRESENTATION*

Erken postnatal dönemde melamin maruziyeti hepatotoksisiteye neden olur

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Özet

Amaç: Sıçanlarda süt kesim döneminden itibaren, mama ve süt ürünlerinin protein içeriklerinde yalancı yüksek pozitiflik sağlamak için kullanılan melaminin karaciğer dokusu üzerindeki etkilerinin akım sitometri, elektron mikroskobu ve histopatolojik yöntemlerle incelenmesi amaçlanmıştır.
Materyal-metot: Çalışma Giresun Üniversitesi Deney Hayvanları Yerel Etik Kurul'dan alınan 2020/23 nolu etik izin ile Giresun Üniversitesi Deney Hayvanları Araştırma laboratuvarında gerçekleştirildi. Onsekiz adet dişi wistar albino sıçan 3 gruba ayrıldı. Postnatal 21.günden itibaren 21 gün süreyle; birinci gruba oral gavajla 0.1ml serum fizyolojik (SF) verildi ve bu grup kontrol grubu olarak kabul edildi. İkinci gruba 50 mg melamin 0.1ml SF ile çözülerek oral gavajla verildi. Üçüncü gruba ise 75mg melamin 0.1 ml SF ile çözülerek oral gavajla verildi. Sıçanlar günlük olarak tartıldı ve doz ayarlaması yapıldı. Postnatal 45.günde, tüm sıçanlar anestezi altında sakrifiye edildi ve karaciğer dokuları alınarak 3 parçaya ayrıldı. Karaciğer dokuları sırasısyla nötral formalin ve %2,5 gluteraldehit içerisine konuldu. Hematoksilen&eosin (H&E), masson trikrom, periyodik asit schiff (PAS) ile boyanan kesitlerde ve geçirimli elektron mikroskobu (TEM) ile histopatolojik analizler yapıldı. Akım sitometri yöntemiyle Annexin V pozitifliği ile apoptozis değerlendirildi.
Bulgular: 50 mg ve 75 mg melamin gruplarında kontrol grubuna göre apoptoziste anlamlı derecede artış görüldü. Histopatolojik değerlendirmede, özellikle 75 mg melamin uygulanan grupta hepatosit kordonlarının ışınsal tarzda görünümünde bozulmalar, hepatositlerde vakuol, portal alan ve parankim dokusu içerisinde inflamatuar hücre infiltrasyonu, PAS reaksiyonu sonucuna göre glikojen içerikte azalma görüldü. 50 mg melamin grubunda 75 mg grubuna göre daha az olmakla birlikte benzer histopatolojik bulgular gözlenirken, 3.zondaki hepatositlerde PAS (+) glikojen içerikte azalma gözlendi. TEM sonuçlarına göre 75 mg grubunda daha fazla olmak üzere her iki melamin uygulanan grupta hepatosit çekirdeklerinde anormal kromatin dağılımı, mitokondrilerin kristalarında kayıplar, sitoplazmada geniş alanlarda organel kayıpları görüldü.
Sonuç: Süt kesim döneminden itibaren doz artışı ile birlikte melamin maruziyeti karaciğer hasarına neden olmaktadır.

Anahtar kelimeler: Karaciğer, Melamin, Apoptozis, Akım sitometri, Elektron mikroskobu, Süt kesim dönemi

➢ *ORAL PRESENTATION*

Collagen extraction and purification based on human tissues

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Abstract

Collagen is the most abundant protein in the human body, corresponding to approximately 25% of the total protein weight. It is mostly found in bone, cartilage, skin and tendons. Thanks to its high applicability in the fields such as drug delivery systems, cell culture, cosmetic surgery, dermal injections, bone grafting and reconstructive surgery makes it a highly preferred biomaterial. In addition, since collagen resources used in today's industry are obtained from non-human sources, it causes negativities such as rejection potential, inflammation and various infectious diseases. This need for collagen has required collagen extraction methods which are autologous, fast, easy, and most importantly human-sourced.
It has been predicted that collagen extraction can be performed in a shorter time with higher efficiency by developing traditional methods such as dissolving collagen in neutral saline solutions, acid solutions, and acid solutions with additional enzymes. With agitating the highly accessible dermal sample in strong acids such as sodium acetate and sodium citrate, is expected to make the method we will use faster than traditional methods. In addition, filtering with a centrifugal filter device provides a higher efficiency for collagen extraction.
SDS PAGE (Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis) and Collagen Content Test Kit are used to determine the purity rate of the collagen source obtained. According to the literature research, it is predicted that the human-derived collagen can be used as an alternative to the commercial collagen sources with high efficiency.
In this study, we will talk about the extraction steps and the purification methods using waste human-resource of highly biocompatible, autologous, soluble collagens.

Keywords: Collagen, Collagen Extraction, Collagen Purification, SDS PAGE

➢ *ORAL PRESENTATION*

Paraoksonaz enziminin saflaştırılması ve alaklor herbisidine karşı inhibisyon etkisinin araştırılması

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Özet

İnsan serum paraoksonaz (PON1, EC3.1.8.1) HDL'ye bağlı olan, aktif bölgesinde kalsiyum içeren, organofosfat ajanlarını ve sinir gazlarını hidroliz eden, LDL'nin oksidasyonu ile lipit peroksitlerin oluşumuna ve bakteri endotoksinlerine karşı koruyucu etkisi olan önemli bir karaciğer enzimidir. Paraoksonaz enzimi, kan serumu, karaciğer, ince bağırsak ve böbrekler olmak üzere organizmada yaygın olarak bulunmaktadır. Enzim kan serumunda serbest olmayıp HDL (yüksek yoğunluktaki lipoprotein)'ye bağlıdır. Tarım ilacı olarak kullanılarn pestisitlerin ve herbisitlerin aşırı ve yanlış uygulamalarından en fazla etkilenenler zirai ilaçlama yapan kişilerdir. Yapılan araştırmalar zirai ilaçlama yapan kişilerin kanlarında ilaç kalıntılarının saptandığına ve bu kişilerin kanlarındaki enzimlerin ve organlarının olumsuz etkilendiğine dikkat çekmektedir. Çalışmamızda kullanılan insan serum paraoksonaz enzimini daha önce grubumuz tarafından sentezlenen Sepharose 4B-L-tirozin-1-naftilamin hidrofobik etkileşim kromatografi jeli ile saflaştırılmıştır. Enzimin saflık kontrolü SDS-PAGE ile yapılmıştır. Alaklor çeşitli konsantrasyonlarda hazırlanıp saf enzim üzerindeki inhibisyon etkisi belirlenmiş ve IC_{50} değeri hesaplanmıştır.

Anahtar Kelimeler: Paraoksonaz, Saflaştırma, İnhibisyon, Herbisit, Hidrofobik etkileşim kromatografisi

➢ *ORAL PRESENTATION*

Türkiye'de yetişen *Scabiosa* türlerinin fitokimyasal açıdan incelenmesi

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Özet

Caprifoliaceae familyasının bir üyesi olan *Scabiosa* L. cinsi dünyada 80 türden oluşmakta olup, Türkiye'de ise 12 tanesi endemik olmak (*Scabiosa balianii, Scabiosa hololeuca, Scabiosa lycia, Scabiosa kurdica* vd.) üzere 32 tür ile temsil edilmektedir (Göktürk, 2012). *Scabiosa* cinsi Akdeniz bölgesi başta olmak üzere hemen hemen Türkiye'nin tüm bölgelerinde yetişmektedir (Davis 1970).

Türkiye florası zengin bitki çeşitliliği ile birçok tıbbi bitkinin yetişmesine imkân sağlamakta ve buna bağlı olarak geleneksel tıp ve aramoterapide bitkiler büyük önem arz etmektedir. *Scabiosa* türleri de geleneksel tıpta geniş bir kullanım alanı bulmaktadır. *S. atropurpurea* akne ve kızamık tedavisinde (Bonet vd, 1999), *S. columbaria* yüksek tansiyon ve rahim rahatsızlıklarının tedavisinde, karaciğer koruyucu olarak (Köse vd, 2015; Rigat vd, 2007), *S. succisa* ülser, bronşit, bronşiyal pnömoni, grip ve astım tedavisinde (Pinto vd, 2018), *Scabiosa sulfurea* antiseptik (Altundağ vd, 2011, S. rotata bağırsak düzenleyici (Küpeli vd, 2008).

Doğal bileşikler, tedavi edici ve tedaviyi destekleyici ürünlerin en önemli kaynağını oluşturmaktadır. Aktiviteleri belirlensin ya da belirlenmesin doğal kaynaklı bileşiklerin yapılarının aydınlatılması sentetik kimyacılar ve biyokimyacılar için yeni açılımlar sağlamaya devam etmektedir. *Scabiosa* türleri üzerine yapılan aktivite çalışmaları incelendiğinde; *S. atropurpurea* hepatoprotektif, serbest radikal temizleyici, antioksidan ve antihiperglisemik aktivite (Elhawary vd, 2011), *S. sicula* antidiyabetik aktivite (Kılınç vd, 2020), *S. rotata* antifungal, antiinflamatuar ve antinosiseptif aktivite göstermiştir (Panayır vd, 1996; Kupeli vd, 2008). Literatürde *Scabiosa* türü ile yapılan izolasyon ve karakterizasyon çalışmalarına bakıldığında, iridoid, fenolik ve terpenoid yapıda bileşikler izole edilmiştir (Polat vd, 2010; Kılınç 2020).

Günümüzde gıda, kozmetik, sağlık gibi pek çok alanda kullanılan bitkiler genellikle göstermiş oldukları biyolojik aktiviteler ve içerdikleri sekonder metabolitler açısından değerlendirilirler (Kaltalıoğlu vd, 2019). Bu araştırma çalışmasında; Türkiye'de yer alan Scabiosa türlerinin fitokimyasal olarak incelenmesi yapılmış olup bu bitkilerinin potansiyel kullanım alanlarına katkı ve ülkemiz florasında yetişen bitkilerin fitokimyasal analizlerini içeren veri bankasına da katkı sağlanması amaçlanmıştır.

Anahtar Kelimeler: Caprifoliaceae, *Scabiosa*, sekonder metabolit, doğal bileşik kimyası

➢ *ORAL PRESENTATION*

Evaluation of ecological quality of Lakes İznik, Manyas and Eber by using Water Framework Directive with macroinvertebrates

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Abstract

Analyses of water quality parameters and also biological monitoring programs (including water organisms) are used for planned of aquatic systems and protected of water quality. Biological monitoring is informative about quality of a water resource long-term. Biological quality elements (fish, macrophyte, plankton, diatom, macroinvertebrate) in the direction of Water Framework Directive beside physiochemical and bacteriological parameters listed in Surface Water Quality Management Regulation are used in determination of aquatic systems' ecological situation in Turkey.

The aim of this study was that ecological quality of Lakes İznik, Manyas and Eber (located different geographical region of our country) were be evaluated with using Water Framework Directive based on macroinvertebrates and presented composition, diversity and abundance of macroinvertebrate according to geographical differences. Macrozoobenthic invertebrates were collected by going to three lakes in 2018 and 2019 years and at the same time, analyzed temperature, pH, dissolved oxygen and biological oxygen demand, total coliform and total fecal coliform listed in Surface Water Quality Management Regulation. Macrozoobenthic invertebrates which is methods of Water Framework Directive were evaluated with biological indices (Biological Monitoring Working Party (BMWP), Avarage Score Per Taxon (ASPT), Simpson Diversity Index, Margalef Diversity Index and dominancy). In consequence of this research, 30 taxa in Lake İznik, 24 taxa in Lake Manyas and 15 taxa in Lake Eber were identified.

While dominant taxa in Lakes Eber and Manyas were determined meso and polisaprob Oligochaeta and Chironomidae taxa, Chironomidae, Ephemeroptera and Gastropoda taxa were dominant groups in Lake İznik. It were determined increased temperature listed in physicochemical parameters and decreased dissolved oxygen and presence of fecal coliform in Lakes Manyas and Eber. In Lake İznik, levels of dissolved oxygen were determined as class II and III. Presence of fecal coliform is acceptable as sign of inflow sewage.

Key words: BMWP, ASPT, biomonitoring

Acknowledgement: This study was supported by ESOGU-BAP (Project number: 201819D15).

➢ *ORAL PRESENTATION*

Bioactive Components and Antioxidant Activity Relationship of Some Lamiaceae and *Verbascum* Species

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Abstract

The aim of this study is to determine the bioactive contents of four different species (*Phlomis armeniaca, Thymus syriacus, Stachys pumila, Stachys rupestris*) belonging to the Lamiaceae family and different four species (*Verbascum pestalozzae, Verbascum detersile, Verbascum speciosum, Verbascum bellum)* belonging to the Verbascum family by chromatographic methods (HPLC-UV) and to compare the antioxidant activities of methanolic extracts. 0.5 g of dried plants were extracted in an ultrasonic bath with methanol for 3 hours and methanol was evaporated at 40 °C in an rotary evaporator. The residue was dissolved in 5 mL distilled water, extracted with ethyl acetate and diethyl ether, and the extracts were combined and the solvents were evaporated. The residue was dissolved with appropriate amounts of methanol for HPLC analysis. Analyzes were performed with Shimadzu using a UV detector at 280 and 315 nm wavelengths, (150 mm x 4.6 mm i.d., 5µm particle; Agilent) C18 reverse phase column, 50 µL injection volume, 1 mL/min flow rate and column oven at 30 °C. The mobile phase were (A) %2 acetic acid in water and (B) 80/20 ACN/water. In order to determine antioxidant activities, activities of methanolic solutions were examined by DPPH method. In HPLC-UV method, it was concluded that *Verbascum pestallozzae* (207.49 µg/g) had the highest total phenolic component amount according to 18 standards among Verbascum species. As a result of the analysis made with DPPH method, it was concluded that *Stachys pumila* with the lowest IC_{50} (19.27) value among Lamiaceae plant species has the highest activity. The active nature of these extracts can be explained by its high phenolic content.

Keywords: Antioxidant activity, Bioactive component, DPPH, HPLC-UV, Total phenolic content

➢ *ORAL PRESENTATION*

Characterization of using molecular techniques of some species *Salvia* L. (Adaçayı) squamulate and ventricose with tube of flower petal, in the flora of Turkey

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Abstract

In this study, it is aimed to determine the genetic similarities or differences among nine *Salvia* species (*Salvia sclarea* L., *Salvia indica* L., *Salvia chrysophylla* Stapf, *Salvia vermifolia* Hedge & Hub.-Mor., *Salvia chionantha* Boiss., *Salvia cassia* Samuelss. ex Rech.f., *Salvia candidissima* Vahl, *Salvia microstegia* Boiss. & Bal., *Salvia argentea* L.), naturally grow in Turkey, having squamulate and ventricose corolla tube. Genetic similarities and differences among the studied species have been determined using the SRAP (Sequence Related Amplified Polymorphism) molecular marker. DNA's extracted from herbarium specimens using CTAB method. SRAP markers were used in PCR (Polymerase Chain Reaction) reaction, and the PCR fragments is separated in 3% agarose gel electrophoresis. PCR fragments were scored dominantly and analyzed using the DARwin5 program From the results obtained, phylogenetic trees showing the genetic differences of the studied species using the Dice coefficient were produced on the basis of UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and NJ (Neighbor Joining) algorithms. According to the preliminary results obtained in the study, 22 polymorphic bands are determined as a result of 2 different SRAP primer combinations. Phylogenetic trees shown that 9 species are divided into 3 different groups. With 71% of the most genetic differences, *S. argentea* and *S. sclarea* species, the least genetic difference is found between *S. microstegia* and *S. cassia* species with a rate of 14%. In this study, Sage (*Salvia* L.) species are characterized molecularly for the first time using SRAP markers.

Anahtar Kelimeler: Sage (*Salvia*), Genetic Characterization, SRAP-PCR

➢ *ORAL PRESENTATION*

Antioxidants, Free radicals, and Nutrition

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Abstract

In this review study, the damages of free radicals and the benefits of antioxidants and the importance of the antioxidant-rich diet were explained. Free radicals occur as a by-product of the digestion of sugars and fatty acids in the cell, mitochondria. The free radical is defined as a molecule or atom containing one or more unpaired single electrons. These unpaired electrons are generally highly reactive. Therefore, free radicals can disrupt the structure of proteins, nucleic acids and lipids, resulting in organ and tissue damage. Free radicals have these harmful effects on the basis of many diseases, especially cancer. The condition that the cell is constantly under attack by radicals is called oxidative stress. Antioxidants are molecules that can easily neutralize these radicals. The most important task of antioxidants is to fight free radicals that destroy tissues and cells. It strengthens the immune system. Antioxidants reduce the risk of cancer by preventing the formation of free radicals. It slows down the aging process throughout the body, including the skin, eyes, tissue, joints, heart, and brain. Antioxidants can be synthetic or naturally found in vegetables, fruits and spices. Colorful vegetables and fruits are stores of antioxidants. Regular consumption of healthy foods can increase antioxidant levels and develop a strong defense against oxidative stress damage. Some of the best antioxidant sources that can help you increase antioxidant levels in the blood are dark chocolate, walnut, raspberry, blueberry, artichoke, spinach, cabbage, beans. As a result, foods rich in antioxidants reduce the effects of free radicals. Therefore, it is important to consume more antioxidant-rich foods to protect our health.

Keywords: Antioxidants, health, healthy nutrition, nutrition and dietetics, proper nutrition.

➢ *ORAL PRESENTATION*

Evaluation of turmeric (*Curcuma longa* L.) as a functional food

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Abstract

Functional foods are foods that provide additional benefits on human physiology and metabolic functions as well as meeting the body's need for essential nutrients. Thus, food components are effective in preventing diseases and reaching a healthier life. In this review study, turmeric (*Curcuma longa* L.- Zingiberaceae family) was described as a functional nutrient. Turmeric has been consumed in Asian countries for thousands of years for food and treatment purposes. Turmeric is a yellow-flowered, perennial herbaceous plant. It grows in tropical and subtropical regions of Asia, especially in India, China, Indonesia, Jamaica, Peru and Pakistan. The plant contains omega-3 and omega-6 fatty acids. It contains calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium and zinc minerals. It also contains B group vitamins, except vitamin B_{12}, vitamins E, C and K and folate. The main component of the plant, also called turmeric, is "Curcumin". Curcumin, derived from the *C. longa*, is a low-weight, hydrophobic polyphenolic flavonoid. The plant used as a yellow-orange coloring agent contains polyphenolic components called "Curcuminoids". Curcumin acts as an effective antioxidant by removing superoxide radicals, hydrogen peroxide and nitric oxide. To date, hundreds of reports have been published showing the anti-cancer potential of curcumin. *In vivo* and *in vitro* studies, it has been determined that curcumin inhibits the growth of cancer cells in different organs such as blood, brain, breast and gastrointestinal system. Many studies indicate that curcumin has a synergistic effect with chemotherapy. Curcumin substance helps fat burning. For this reason, it is a very effective spice for digestion and digestive problems. Curcumin can help you fight a variety of viruses, including herpes and flu. Turmeric appears to be a valuable functional nutrient for human health with its curcumin and other components.

Keywords: Antioxidant, curcumin, healthy foods, turmeric, natural foods

➢ *ORAL PRESENTATION*

Serological investigation of west nile virus infection in feral horses in konya region

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Abstract

West Nile virus (WNV) is an Arboviral infection with encephalitis in humans, horses, birds, and various wild animals. It is a zoonotic cause of disease within the family *Flaviviridae*. In this study, it was aimed to investigate the West Nile virus infection serologically (serum neutralization) in feral horses in Konya. For this purpose, randomized sampling of blood serum samples from 36 horses from a farm with 250 feral horses in Konya was used. Samples were evaluated for the presence of antibodies by subjecting them to a microneutralization test. 7 of the samples (19.4%) were found to be seropositive and 29 were seronegative. These results obtained have shown the serological presence of WNV for the first time in feral horses in our country. Also, these data showed that the continued presence of WNV infection in Turkey.

Keywords: West Nile virus, Feral horses, Serum neutralization

➢ *ORAL PRESENTATION*

Investigation of clonal relationship, carbapenemases and colistin resistance in multi drug resistant *Acinetobacter baumannii* clinical isolates

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Abstract

Acinetobacter baumannii infections has become a major clinical concern in clinical settings due to the high rates of multidrug-resistant (MDR) phenotypes. Class D carbapenemases is the main reason for increasing resistance rates in *A. baumannii* isolates, which mainly includes acquired carbapenemases blaOXA-23, blaOXA-24 and blaOXA-58 as well as blaOXA-51, which is intrinsic to *A. baumannii*. Thus, it is crucial to determine beta-lactamases such as oxacillinases and metallo-betalactamases in order to control dissemination of these strains as a part of nosocomial infection control measures.

Herein, total of 28 MDR *A. baumannii* were studied in terms of colistin susceptibility, oxacillinase gene distributions, NDM-1 genotype, and insertion sequence ISAba1. The clonal relationship of the isolates was also determined by AP-PCR and genotypic differences were evaluated within the clusters. Antibiotic susceptibility profiles were determined by Kirby-Bauer disk diffusion susceptibility test for ampicillin/sulbactam, ceftazidime, imipenem, meropenem, gentamicin, ciprofloxacin, piperacillin-tazobactam, trimethoprim/sulfamethoxazole, amikacin, cefepime and netilmicin.

All of the isolates were MDRAB, while 6 of them also revealed colistin resistance (21.4%) by broth microdilution method. All isolates were harboring blaOXA-51 gene, as expected. blaOXA-23 was determined in 26 isolates (92.9%). A frequent insertion sequence ISAba1 was determined in 20 isolates (71.4%), while insertion of ISAba1 upstream of blaOXA-23 was determined in 18 isolates. None of the isolates were harboring mobile plasmid-mediated colistin resistance gene variants (mcr 1-5) and blaNDM. There were 3 distinct clusters according to AP-PCR patterns. Cluster 3 included 8 isolates, in which all isolates were blaOXA-51 and harboring ISAba1 located upstream of blaOXA-23. Isolation dates were also closer for these isolates indicating a possible clonal spread in intensive care unit.

According to present results, blaOXA-23 was mainly located in highly mobile genetic elements. The present results highlight the emergence of colistin resistance and oxacillinase-producing MDRAB in clinical setting.

Keywords: *Acinetobacter baumannii*, MDR, oxacillinases, colistin

➢ *ORAL PRESENTATION*

Antimicrobial activities of *Commelina communis* L. ethanolic extract

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Abstract

The present study aimed to determine the antimicrobial activity of *Commelina communis* L. ethanolic extract. For this purpose, the extract's antibacterial and antifungal activities were investigated by minimum inhibitory concentration and minimum bactericidal concentrations. To determine the applied doses for pathogen microorganisms, we used the peripheral lymphocyte culture technic. Mitotic index values were calculated for various concentrations of the extract. The dose, which killed 50% of the cells in culture, was chosen as the maximum dose. Half of the maximum dose was administered as a medium dose, and the quarter of the maximum dose was used as the minimum dose. 5, 2.5, and 1.25 mg/mL extract doses were chosen for further analyses. Four pathogenic actinomycetes (*Micrococcus luteus* NRRL B-1018T, *Nocardia abcessus* DSM 44432T, *N. cyriacigeorgica* DSM 44484T, and *Streptomyces murinus* ISP 5091T) and two yeast strain (*Candida albicans* ATCC®60192T and *Saccharomyces cerevisiae* ATCC®9763T) were selected for antimicrobial activity tests. 14% DMSO and Amikacin (250 mg/mL) were used as controls. According to the obtained results, the most sensitive strain was *N. abcessus* (MIC=1.25 mg/mL, MBC90=2.5 mg/mL), and the maximum dose for bactericidal effect was 5 mg/mL for *M. luteus* and *S. murinus*. On the other hand, the chosen doses of the extract were not effective by yeasts. We want to express our appreciation to the Ordu University Scientific Research Project Commission, which supported this study (AR-1829).

Keywords: MIC, MBC_{90}, Mitotic index

➢ *ORAL PRESENTATION*

Synthesis and characterization of ciprofloxacin imprinted polymer nanoparticles from renewable resources

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Abstract

In this study, novel molecularly imprinted polymer nanoparticles (MIPNPs) were synthesized from renewable resources. Maleinated castor oil (MACO) and lactic acid-maleic acid ester (LME), derived from renewable resources, were used as monomers in the synthesis. N,N′-methylenebis (acrylamide) (MBA) and TEMED/KPS (N,N,N,N-tetramethyl ethylene diamine/potassium persulfate were used as the cross-linker and the thermal initiator, respectively. Ciprofloxacin was used as the model drug molecule for the imprinting process. Polymerization was carried out at 50°C for 30, 60, and 90 minutes. Twelve different MIPs were synthesized, and the effects of the monomer types, initiator concentration, and duration of synthesis on the binding characteristics and particle sizes of MIPNPs were investigated. The produced polymeric materials were characterized by Fourier-transform infrared spectroscopy (FTIR) analysis. Furthermore, particle sizes of nanoparticles were measured using a Zetasizer particle size analyzer. Binding performances were investigated by UV-Vis spectroscopy. Finally, novel antibiotic imprinted polymer nanoparticles were obtained using renewable resources from plant oil-based materials. They can be used in many applications such as solid phase extraction, chromatography and drug delivery systems. The authors are grateful to TUBITAK (EURONANOMED III) for financial support under Project No: 217S071. The authors are thankful to Assoc. Prof. Dr. Serap Derman, Yildiz Technical University, Department of Bioengineering for particle size and zeta potential measurements.

Keywords: molecularly imprinted polymer nanoparticles, ciprofloxacin, renewable resources.

➢ *ORAL PRESENTATION*

Effect of Carbon Source on Mesoporous Carbon Materials

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Abstract

In recent years, ordered mesoporous carbon materials have attracted attention because of their remarkable physicochemical properties including tunable mesopore size, high surface area, large pore volume, high corrosion resistance, chemical inertness, and its easy production steps [1,2]. In the present study, the effects of different carbon sources in the preparation of mesoporous carbon materials by Hard Template method [4] were investigated. In this method, hydrothermally prepared KIT-6 was used as a hard template and mannose, sucrose, glycerol, and citric acid were used as carbon sources. Materials prepared with these carbons were named as C-mannose, C-sucrose, C-glycerol, and C-citric, respectively. Hard template method includes the impregnation of pores of mesoporous template with carbonaceous compound, polymerization of carbon source, carbonization in argon atmosphere at 900 °C, and silica removal using NaOH solution. Synthesized materials were characterized by X-ray diffraction (XRD), N_2 adsorption-desorption, Fourier-transform infrared (FT-IR), scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDS) analyses. The diffraction peak at $2\theta=1.1^{o}$ corresponding to (211) Bragg reflection of the 3D cubic body-centered mesoporous structure was observed in the XRD patterns of C-mannose and C-sucrose samples. Structural C=C bonds and surface oxygen functional groups were evaluated by FT-IR analysis. While a mono-disperse structure was observed in the pore size distribution of the C-mannose, C-sucrose, and C-glycerol samples, a low-intensity peak was obtained with the C-citric sample. N_2 adsorption-desorption analysis results showed that C-mannose and C-sucrose materials had higher pore volume (about 0.9 cm^3/g) and surface area values (>1000 m^2/g) compared to other samples. Morphological structures of the samples were determined by SEM analysis and the small amount of Si (less than 0.4 wt.%) remaining in the structure was confirmed by EDS analysis. Results showed that mesoporous carbon samples prepared using mannose and sucrose precursors can be used as efficient adsorbents for dye removal from industrial waste water.

Keywords: Hard Template, KIT-6, mesoporous carbon, dye removal

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➢ *ORAL PRESENTATION*

Determination of Ischemia Modified Album (Ima) Level in Coronary Artery Disease

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Abstract

Objective: Cardiovascular diseases that develope depends on the atherosclerosis are a serious social health problem all over the World. In this study, it was aimed to determine ischemia modified albumin (IMA) levels in individuals with angiographically defined atherosclerotic heart disease and to investigate the relationship between IMA's and extent and degree of atherosclerosis.
Materials and Methods: This study was performed on 64 patients who were diagnosed as coronary angiography and applied angiography. As a result of angiography, 32 individuals without any occlusion of the coronary arteries were defined as the control group and 32 individuals with various degrees of obstruction the coronary artery (CAD) as patient group. Serum IMA level was measured by photometric method.
Results: There was no significant difference between the patient and control groups in terms of demographic data. In the patient group, serum IMA (respectively, 0.7±0.1 ABSU – 0.6±0.1 ABSU, p=0.002) levels were found to be significantly higher than the control group. In patient group, ıt was found that there is a positive correlation between age and Gensini score (r=0.431, p=0.014). In the control group; a negative correlation between IMA and total cholesterol levels (r= -0.401, p=0.025) was found. In the ROC curve analysis, for the diagnosis of CAD, the diagnostic sensitivity of IMA was 80% and specificity 71.0%.
Conclusion: Our findings show that IMA level increases in individuals with coronary artery disease, but that IMA level is not related to the extent and degree of atherosclerosis. However, our results show that IMA level can be used as a diagnostic variable for CAD. This study should be supported by studies involving larger patient groups.

Keywords: CAD, MPO, Prolidase, IMA, Gensini score.

➢ *ORAL PRESENTATION*

Kurşun kalem uçu elektrotların sensör olarak kullanımı

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Özet

Kurşun kalem ve kalem uçları karbon tabanlı malzemeler oldukları için elektriksel iletkenliğe sahiptir. Günümüzde elektrokimyasal sensörlerin çeşitlenmesi ve modifikasyon yöntemleriyle yeni özellikler kazandırılması oldukça yaygınlaşmıştır. Klasik elektrot materyallerine ucuz ve kolay erişilebilir bir alternatif oluşturması nedeniyle kurşun kalem türleri bu çalışmalar arasında artan kullanımlarıyla yer almaktadır. Kurşun kalem uçlarının modifikasyon işlemine başlamadan önce aynı elektriksel karakteristik özelliklere sahip olması hayati öneme sahiptir. Taban malzemenin farklı özellikler taşıması modifikasyon sonrasında elde edilmesi beklenen sensörün doğruluğu ve hassasiyeti üzerinde kaçınılmaz bir rol oynamaktadır. Bu çalışmada kalem ucu elektrotların çeşitli aktivasyon işlemlerine maruz bırakılarak ve doğrudan uygulanmasının getirdiği sonuçlar voltametrik ve elektrokimyasal empedans ölçümleri alınarak incelenmiştir. Elektrotların dönüşümlü voltamogramlara ve empedans spektrumu analizlerine verdiği yanıtların değişkenlik gösterdiği ve bu tekniklerin kalem uçlarının elektrot olarak kullanılabilir özelliklere sahip olup-olmadıkları konusunda kantitatif ayrım sağlayabildiği belirlenmiştir.

Anahtar Kelimeler: elektrokimyasal sensör, kurşun kalem, elektrot, pencil, PGE

➢ *ORAL PRESENTATION*

Comparison of Bioelectrochemical Systems and Conventional Aeration Systems for Wastewater Treatment

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Abstract

Conventional activated sludge systems can efficiently degrade organic pollutants, but generally energy-intensive and require high investment, maintenance, and operating costs due to the aeration and sludge treatment associated processes, so aeration can amount to 45-75% of wastewater treatment plant energy costs, while the treatment and disposal of sludge may count up to 60% of the total operation cost. To reduce the cost, other options could be used to make an energetically sustainable process. Bioelectrochemical systems (BESs) is one of the promising technologies in which organic matter is oxidized using electrochemically active bacteria under anaerobic conditions to convert chemical energy to electrical energy, and have potential applications in wastewater treatment. In this study, we compared BESs and conventional wastewater treatment systems in terms of COD removal and energy performances. We used lab-scale reactors to quantitatively audit the power generated or consumed during the operation of a 3-electrode electrochemical cell with flow-through electrodes, a 3-electrode electrochemical cell using a planar electrode of the same size, and an aeration tank with a pump air diffuser during treatment of raw domestic wastewater. The bioelectrochemical experiments were carried out under potentiostatic control using a three-electrode electrochemical arrangement similar to the microbial fuel cell. The results showed that the current generation using flow-through electrodes is faster than using flat electrodes. We also found that both systems can achieve almost the same COD removal rate, but the conventional system can treat it faster than BESs. Our results demonstrated that BESs can be a viable wastewater treatment technology.

Keywords: bioelectrochemical systems, wastewater treatment, COD

➢ *ORAL PRESENTATION*

Determination of apolipoprotein A1 and apolipoprotein B by using simple and robust uplc/ms based proteomics method

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Abstract

The aim of this study was to analyze Apolipoprotein A1 and Apolipoprotein B biomarker proteins by using UPLC/MS based proteomics approach without any protein depletion method in human plasma.
Proteins were digested with trypsin and peptides were eluted in C18 column with 150 minutes gradient. The MS/MS data of peptides were recorded in auto MS/MS mode. Identification and label-free quantification of peptides were performed by using Maxquant. 10, 20, 40 and 80 µg protein was loaded to get optimum loading capacity for identification and quantification APOA1 and APOB. Protein quantification was evaluated in terms of linearity and reproducibility.
Identified peptide numbers showed that APOA1 and APOB could be identified easily by loading 20, 40 and 80 µg protein loading. Protein intensities were calculated for each amount of protein. Linearity studies showed that protein intensities were strong correlation with amount of protein in proteomics metrics (R2 > 0.950). Intra-day experiments showed that proteins could be quantified more reliable with 80 µg protein loading (C.V= % 9,2 for APOA1 and 13,8 for APOB). Inter-day experiments showed high reproducibility with 80 µg (C.V< % 20).
Results showed that UPLC/MS based proteomics approach with label free quantification could be used to evaluate APOA1 and APOB levels in clinical and research samples.

Keywords: APOA1, APOB, UPLC/MS, Biomarkers, label free quantification, Proteomics

➢ *ORAL PRESENTATION*

Keban ve Karakaya Baraj Göllerinde Hayalet Av Araçlarının Su Altı Dalış Tekniği ile Tespit Edilmesi

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Özet

Hayalet balıkçılığı, insan kontrolü olmaksızın suda yaşayan organizmaların ölümüne neden olan ve işlev görmeye devam eden terk edilmiş av araçlarını ifade eder. Bu ağlara su ortamında sürekli balıklar takılarak ölmekte ve daha sonra tekrara başka balıklar takılmaktadır. Bu çalışma direk bu av araçlarını tespit etmek üzere yapılmamış olmasına rağmen, ne yazık ki her dalışta bunlar ile karşılaşılmıştır. İlk dalış 15 Ekim'de Keban Baraj Gölü Pertek Bölgesi'nde yapılmıştır. Yaklaşık 10 m derinlik ve 100 m'lik kıyı boyu dalışında satışı ve kullanılması yasak olan tırı vırı isimli hayalet ağlardan 2 tanesine rastlanılmıştır. İkinci dalış 15 Ekim'de Keban Baraj Gölü Çemişgezek Bölgesi'nde yapılmıştır. Bu bölgede ise yine bir tırı vırı ağına yakalanan biri canlı ve biri ölü iki balık görülmüş, canlı balık ağdan kurtarılmıştır. 3 Ekim 2020'de Karakaya Baraj Gölü Kömürhan Bölgesinde yapılan dalışta ise su altında balıkçıların unuttuğu germe ağa rastlanılmıştır. Bu ağda birçok çürümüş ve çürümeğe yüz tutmuş balığın olduğu tespit edilmiştir. Çalışmada gözlemlenen materyaller fotoğraf ve video formatında kaydedilmiştir. Yapılan sadece 3 dalışta tespit edilen bu ağlar durumun ne kadar kötü olduğunu göstermektedir. Bu sorunun giderilmesi için daha fazla çalışmanın yapılmasına ve bu av araçlarının su ortamı dışına çıkarılmasına ihtiyaç vardır.

Anahtar Kelimeler: Hayalet, Su Altı, Dalış, Keban, Karakaya

➢ *ORAL PRESENTATION*

The chemical composition and biological activities of *Epilobium hirsutum* L. essential oil

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Abstract

Epilobium (willowherb), one of the genus in Onagraceae family, encompasses annual or perennial herbaceous plants spread all over the world. It comprises of 200 taxa distributed across the world. It has been represented in Turkey by 29 species. It is a rich source of biologically active compounds varies with the season, climate, soil, period of collection, plant materials and extraction process. *Epilobium hirsutum L.*, which is known as 'Hasanhüseyin çiçeği' locally, cannot resist unusual weather conditions because it is a softly-hairy herb. It gives flowers in July and August. In this study it is aimed to determine the chemical composition and antioxidant activities of *E. hirsutum* essential oil. The plant was collected from Gaziantep region. Scientific identities of the plants were confirmed at Herbarium of Gaziantep University, Science and Arts Faculty, Department of Biology (GAUNHERB) and the voucher specimens were stored at GAUNHERB. The essential oil was extracted by hydrodistillation method using a Clevenger apparatus from dry leaves, flowers and stems of *E. hirsutum*. Chemical composition of essential oil was determined by Gas Chromatography-Mass Spectrometry **(**GC/MS). Antioxidant, anticholinesterase, tyrosinase and urease activities of essential oil were investigated. The antioxidant capacities of essential oil were analyzed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), *β*-carotene-linoleic acid and cupric ion reducing capacity (CUPRAC) assays. The antioxidant activities of essential oil in the ABTS cation radical scavenging assay and CUPRAC reduction power assay were higher than BHT and α-TOC, respectively. It was determined that the essential oil of *E. hirsutum* showed anticholinesterase (AChE; $IC_{50:}$ 67.08±0.44 µg/mL) and tyrosinase activities (BChE; IC_{50}: 54.33±0.07 µg/mL). Urease inhibition activity of essential oil was found better than the standard of the test.

Keywords: *Epilobium hirsutum*, Essential oil, Antioxidant, Anticholinesterase, Tyrosinase, Ureas

➢ *ORAL PRESENTATION*

İzosiyanat grubu içeren polimerik kaplamaların hazırlanması ve işlevselleştirilmesi

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Özet

İzosiyanat grubu, DNA ve proteinler gibi biyomoleküllerde mevcut bulunan ya da sonradan kolaylıkla yerleştirilebilen aminlerle ve tiyollerle oldukça hızlı ve efektif tepkime vermektedir. İzosiyanat grubu, fonksiyonel grup olarak yüzey kaplama çalışmalarında tek tabakalı ya da iki boyutlu kaplamalar olarak mevcuttur,[1,2] fakat üç boyutlu çalışmalara literatürde rastlanmamaktadır. Üç boyutlu yapıların sinyal duyarlılığı, spot morfolojisi ve yükleme kapasitesi bakımından ince tek tabakalı yapılara olan üstünlüğü gösterilmiştir.[3,4] Bu çalışmada, cam yüzeyler üzerinde izosiyanat grupları içeren üç boyutlu çapraz bağlı hidrofilik polimerler fotopolimerleşme metodu ile hazırlanmıştır. Bunun için, izosiyanat grubu barındıran ve polietilen glikol grupları içeren monomerler akrilat tabanlı çapraz bağlayıcı ve fotobaşlatıcı varlığında UV ışık altında bekletilmiştir. Elde edilen bu çapraz bağlı hidrofilik polimerlerin amin ve tiyol grupları içeren çeşitli moleküllerle işlevselleştirilebildiği gösterilmiştir. Daha sonra, cam yüzeyler üzerinde izosiyanat grupları olan çapraz bağlı filmler ve UV maske yardımıyla izosiyanat işlevsel grubu barındıran polimerik mikro dizilimler de elde edilmiş ve işlevselleştirilmiştir. Böylece, tiyol veya amin grubu içeren moleküllerin/biyomoleküllerin sabitlenme çalışmalarında kullanılabilecek yeni platformlar elde edilmiştir.

Anahtar Kelimeler: fonksiyonel polimerler, izosiyanat-amin, izosiyanat-tiyol

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➢ *ORAL PRESENTATION*

miR-626 inhibits mTOR activity of retinal pigment epithelial cells by targeting SLC7A5 and Inducing effect on age-related macular degeneration

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Abstract

Aim: Age-related macular degeneration (AMD) is a chronic disease progressive to central retina, a major health problem in aging populations, and a leading cause of visual loss. MicroRNAs circulating in the serum may be novel biomarkers for many diseases, may show key processes in disease mechanism and may support the development of new treatment methods. The purpose of this study is to investigate of mir-626 miRNA expression profile analysis and the role and pathways of miRNAs in AMD.
Material and method: The expression of Mir-626 in human retinal pigment epithelial cell line was examined using Rt-pcr and western blot, respectively. We knocked down mir-626 levels and overexpression by mir-626-siRNA transfection of human RPE cell lines, and using an MTT assay, we assessed the role of SLC7A5 on RPE cell proliferation.We additionally measured the expression of Bcl-2, Bax, caspase-3, VEGFA, Akt1, mTOR and MAPKs.
Result: Our results show that miR-626 cells are damaged by suppressing the SLC7A5 gene in RPE cells. Inhibition of SLC7A5, a predicted target for Mir-626 by short interfering RNA (siRNA), has the same effect on RPE cells. We also show for the first time that SLC7A5 is a direct target of miR-626.

Keywords: AMD, mir 626 ,mTOR, SLC7A5,

➢ *ORAL PRESENTATION*

Chemical and thermal properties with biological activities of *Achillea arabica* Kotschy essential oil

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Abstract

The genus *Achillea L.* belongs to the family Asteraceae, the tribe Anthemideae. Aerial parts of the members of the genus have been used as medicinal herbs in folk and alternative medicines or traditional medicines in most countries. The genus comprises of about 115 species worldwide. It has been represented in Turkey by 69 species including 33 endemics. One of these species *Achillea arabica* Kotschy *o*ccurs naturally in many parts of Turkey and the local names of 'hanzabel, sarı civanperçemi'. The essential oil isolated from aerial parts of *A. arabica* was extracted by hydrodistillation method using a Clevenger apparatus. To date, many investigations have been conducted into essential oil compositions of the genus *Achillea*. It was found that the differences in oil composition were affected by different environmental factors such as the climate conditions and the cultural sites. The aim of this research was to determine the chemical composition, thermal properties, antioxidant, anticholinesterase, tyrosinase and urease activities with cytotoxic effect of the essential oil of *A. arabica* collected from Gaziantep region. Plant vouchers are deposited at the Herbarium of the Gaziantep University, Department of Biology (GAUNHERB). The chemical composition of the extracted oils was examined by Gas Chromatography-Mass Spectrometry (GC-MS). Thermal behavior of essential oil was explored with 5, 10 and 15°C/min heating-cooling rates by monitoring peak temperature transitions by Differential Scanning Calorimetry (DSC). Three DSC parameters of exothermic and endothermic events, namely, peak temperature (Tpeak), enthalpy and temperature range were identified. In order to assess any possible toxic effects of the compounds, they were evaluated according to the MTT assay. The serial dilutions 12.5-1000 µg of the compounds were incubated with HTB-54 and BEAS2B cell lines. Their antioxidant capacities were analyzed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), *β*-carotene-linoleic acid and cupric ion reducing capacity (CUPRAC) assays. It was evaluated that antioxidant capacities of essential oil in the ABTS cation radical scavenging and CUPRAC reduction power assays were founded active than BHT and α-TOC, respectively. It was calculated that essential oil showed anticholinesterase activity (AChE; IC_{50}: 54.29±0.37 µg/mL) and tyrosinase activity (BChE; IC_{50}: 18.23±0.07 µg/mL). In urease inhibition activity, it was more excellent than the standard of the test.

Keywords: *Achillea arabica*, Chemical and Thermal Properties, Antioxidant, Anticholinesterase, Tyrosinase

➢ *ORAL PRESENTATION*

Fall Season Megabenthic Invertebrates of Western Black Sea Coast of Turkey

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Abstract

The aim of this study was to determine of mobile fauna and their distribution depending on the depth in the western Black Sea coast of Turkey. Benthic samples were collected by RV Karadeniz Araştırma of the Recep Tayyip Erdoğan University. Samplings were carried out in 7 different transects consists of 3 depths, (10m, 20 m, 30m) by using 2 m wide and 15 mm mesh size beam trol. Soyer's Frequency Index (%F), dominance (%D), diversity index (H′), evenness index (J') and Bray-Curtis similarity index were used to determine frequency of the species in each stations. The maps of the locations have been prepared using geographic information systems. A total of 1395 individuals and 24 species belonging to order Decapoda, Amphipoda, Mytilida, Arcida, Venerida, Littorinimorpha, Phlebobranchia have been identified. *Liocarcinus depurator* was determined to be most encountered species in the stations.

Keywords Black Sea, Megabenthic communities, Beam trol, Fall

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➢ *ORAL PRESENTATION*

Interactions of DNA with the vinca alkaloids and paclitaxel, which are anti-cancer drugs targeting microtubules

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Absract

Microtubules are an important part of the intracellular cytoskeleton structure and have unique polymerization dynamics that are critical for many cellular functions including cell division. Anti-microtubule drugs that interfere with microtubules are important chemotherapeutic agents for the treatment of various cancer. These drugs that block mitosis seem to work by a common mechanism, which is suppress the dynamic of microtubules, slow cells, induce apoptosis and subsequently kill tumor cells. Vinca alkaloids (vinblastine, vincristine and vinorelbine) and Taxanes (paclitaxel) are two different classes of anti-microtubule drugs that cause microtubule dysfunction and inhibit cancer cell proliferation.The main activity profile of vinca alkaloids and taxanes is due to their reversible binding interactions with tubulin protein. However, studies on DNA interactions of these anti-microtubule drugs are not sufficient. In this study was aimed to investigate that DNA binding activity of the vinca alkaloids (vinblastine, vincristine, vinorelbine) and paclitaxel. The interactions of the drugs with DNA were analyzed by agarose gel electrophoresis assay. Three types of DNA was used in each experiment, including 100bp marker DNA, pUC19 plasmid DNA (2686 bp) and pBR322 plasmid DNA (4361 bp). After the DNAs were incubated with different concentrations of the drugs under certain conditions, agarose gel electrophoresis was performed. DNA band distributions were analyzed with a gel analysis system so that the drugs-DNA interactions could be interpreted. The results showed that the Vinca alkaloids bind with DNA suggesting a possible similarity in their DNA binding motifs. The Vinca alkaloids possess features like several H-bond acceptor/donor atoms, planar ring systems, and a large aromatic skeleton that are essential for the DNA binding activity. As a result of paclitaxel-DNA interaction study, it was observed that there was no significant change in DNA band distributions. This may be because the chemical structure of paclitaxel is not suitable for binding to DNA.

Keywords*;* Vinca alkaloids, Paclitaxel, Anti-microtubule drugs, DNA binding, Cancer treatment

➢ *ORAL PRESENTATION*

Çiftlik hayvanlarında markör destekli seleksiyon (MAS) uygulaması

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Özet

Ekonomik öneme sahip çiftlik hayvanların genetik ıslah programlarında dikkate alınan özelliklerin çoğu kantitatif özelliklerdir. Hem çevresel faktörlerden etkilenerek hem de çok sayıda gen tarafından kontrol edilmektedir. Geleneksel yöntemler, fenotip bilgilerine dayanarak genetik olarak üstün ebeveynler seçilerek bir seleksiyon uygulamaktadır. Ancak bu yöntem sınırlı kalıp fazla kazanç elde edememiştir. Son yıllarda, moleküler markörlerin geliştirilmesi hayvancılık alanda büyük bir ilgi çekmiştir. Markör Destekli Seleksiyon (MAS) dolaylı bir seleksiyon yöntemidir. Özelliğin kendisini seçmeyip, özelliğe ilişkili veya yakın olan (morfolojik, biyokimyasal ve moleküler) markörleri seçme işlemidir. Aynı zamanda MAS, genetik markörler ile kantitatif özellik lokusları (QTL) arasındaki bağlantı ve ilişkinin mesafesine dayanır. QTL ile markörler arasındaki bağlantı tanımlandığı takdirde seleksiyon programında kullanılabilmektedir. Ölçülmesi zor, pahalı ve kalıtım derecesinin olan özellikler üzerinde MAS'ın etkisi daha etkili olabilmektedir.

Anahtar Kelimeler: MAS, QTL, Markörler.

➢ *ORAL PRESENTATION*

Design and Synthesis of New 1,3-indandione Derivatives

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Abstract

Indandione is a hydrocarbon classified as bicyclic aromatic β-diketone. It is successfully used as a substrate in organic synthesis and pharmaceutical sciences. According to literature studies, indandiones are known to have various biological properties such as anticancer, anticoagulation, anti-inflamatory, antimicrobial. In this studies, we focused our attention on the preparation of a new series of indandion substituted urea derivatives. Indandion substituted urea derivative was prepared with 2mmol indandion and 1mmol 4-nitrobenzaldehyde in the presence of Et_3N in DMF, and nitro group was reduced to amine derivative with $Na_2S.H_2O/S_8$. Finally, the compound was reacted with isocyanates and urea derivatives synthesized . Chemical structures of all compounds were defined by $^1H/^{13}C$ NMR and IR spectra.

Keywords: Indandione, synthesis, reduction, urea derivatives.

➢ *ORAL PRESENTATION*

Terapötik ultrasonun cisplatin-dirençli yumurtalık kanseri hücreleri üzerindeki etkisinin incelenmesi

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Özet

Cisplatin direnci, yumurtalık kanseri tedavisinde önemli bir sorundur. Cisplatine karşı direncin oluşmasında farklı yollar mevcuttur. İlacın hücre içine alımı (influx), ilacın hücre dışına atımı (eflux), ilaç detoksifikasyonu, DNA onarımı ve apoptoz arızası gibi yollarla ilaca karşı direnç gelişmektedir. Farklı direnç oluşum mekanizmalarının olması, belirli bir yolu hedefleyen bir stratejinin ilaca karşı verimli bir duyarlılaştırma üretemeyebileceğini göstermektedir. Bu yüzden ilaç direncine karşı daha etkili stratejilere ihtiyaç duyulmaktadır.
Ultrason, tıpta geniş bir uygulama alanına sahiptir ve hem tanı hem de tedavide yaygın olarak kullanılmaktadır. Biyolojik sistemlerin, düşük yoğunluklu ultrasonik dalgaya maruz bırakılması, biyolojik etkiler olarak bilinen mekanik ve termal etkilere sebep olur. Ultrasona maruz kalma, termal etkiler sonucunda ablasyona, mekanik etkiler sonucunda hücre membran geçirgenliğinin artmasına sebep olur. Bu özelliklerinden dolayı anti-kanser tedavide umut verici bir yöntem, kemoterapi ve düşük yoğunluklu ultrasonun kombine etkisinin kullanılması olarak görülmektedir. Ultrason enerjisi varlığında artan membran geçirgenliği sonucunda hücre içine daha çok ilaç alımının sağlanması cisplatine karşı oluşan dirence karşı etkili tedavi olacağının hipotezlemekteyiz.
Bu çalışmada, *in vitro* ortamda cisplatin duyarlı ve dirençli A2780 yumurtalık kanseri hücre hatlarında ilaç direncine karşı etkili tedavi geliştirmek için terapötik ultrason (TUS) + cisplatin tabanlı kombine tedavinin etkileri incelenmiştir. Sadece cisplatin, sadece TUS, cisplatin + TUS şeklinde tedavi grupları oluşturulmuştur. TUS için %10 duty cycle, 1 MHz, 1 W/cm^2, 1 dakika ve %50 duty cycle, 1 MHz, 1 W/cm^2, 3 dakika olmak üzere iki farklı parametre grupları kullanılmıştır. Hücre canlılık testi sonuçlarına göre her iki hücre hattında, cisplatin + TUS (%50 duty cycle, 1 MHz, 1 W/cm^2, 3 dakika) tedavi grubunun diğer tedavi gruplarına kıyasla istatistiksel olarak anlamlı ve etkili bir tedavi yöntemi olduğunu in vitro şartlarda %90'a kadar dirençli kanser hücrelerini yok ettiği gösterilmiştir. Elde edilen sonuçlar, cisplatin direncine karşı etkili tedavinin geliştirilmesinde umut vadetmektedir.

Anahtar Kelimeler: Yumurtalık kanseri, ultrason terapi, cisplatin direnci, ikili terapi

➢ *ORAL PRESENTATION*

Berrak, papiller ve kromofob renal hücreli karsinomda ve her alt tipin evrelerini ayırmada kullanılabilecek moleküler biyobelirteç araştırılması

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Özet

Renal tümörler diğer tümör tiplerine kıyasla toplam sağkalım açısından daha iyi bir sağkalım profili gösterse de bu tümörlerin histolojik, fenotipik ve genetik olarak heterojen olmaları önemli bir sorun teşkil etmektedir. Bu durum çeşitli alt tipler arasında bile prognoz ve tedavi oranlarının değişken olmasına yol açmaktadır. Günümüze kadar bu heterojeniteyi ayırt ederek renal tümör alt tiplerinin ayırımını daha kesin sınırlarla ortaya koyabilecek pek çok çalışma yapılmıştır. Ancak halen bu kanser tipinin alt gruplarını ayırmada kullanılan spesifik bir biyobelirteç bulunmamaktadır.

Mevcut çalışmamız renal tümörlerin üç alt grubu olan berrak hücreli (clear cell) renal kanser, papiller renal hücreli kanser, kromofob renal hücreli kanser alt tiplerini ve her alt tipin evrelerini ayırmada kullanılabilecek moleküler biyobelirteç araştırılması ile ilgilidir. Bu amaçla GEPIA veritabanından üç renal kanser alt tipinden en yüksek ifadeye sahip 25 gen incelenerek çakışan genler analize alınmış ve makale taramaları ile elde ettiğimiz genler araştırmaya dahil edilerek çoklu ifade analizleri ve patolojik tümör evreleme diyagramı analizleri gerçekleştirilmiştir.

Elde edilen analizler kromofob renal hücreli kanserin CA9 genini ifade etmemesi, TNFAIP6 genini ise az miktarda ifade etmesi ancak CD117 genini yüksek ifade etmesi ile diğer renal kanser alt türlerinden ayrılabileceğini öngörmektedir. CA9 geni ise berrak hücreli ve papiller renal hücreli kanserde ayırıcı ifadelere sahip olması dolayısıyla iki kanser türünü birbirinden ayırabilir görünmektedir. Mevcut genler patolojik tümör evreleme diyagramı analizine sokulduğunda ise; PAX2, CK7, KEAP1, CTSK, TFE3, CA9, APOC1, TNFAIP6 genlerinin her üç kanser alt tipini evrelere ayırmada oldukça anlamlı olduğunu göstermiştir. Özellikle CK7 geninin papiller renal hücreleri kanser evrelerini ayırmadaki anlamlılığı çok çarpıcıdır (F değeri:19, Pr(>F):$3.36x10^{-11}$). Ancak sonuçların ıslak laboratuvar çalışmalarıyla doğrulanması gerekmektedir.

Anahtar Kelimeler: Biyobelirteç, in-siliko analiz, renal kanser, tümör evreleme

➢ *ORAL PRESENTATION*

PLGA-Kurkumin Nano-formülasyonunun Kanser Hücrelerinde NF-kB Alt Birimleri Düzeyi Üzerindeki Etkisinin İncelenmesi

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Özet

Kanser kemoterapi ajanlarının sağlıklı hücreler üzerindeki toksik etkilerine ek olarak bu ilaçların özellikle klinik kullanımdaki yüksek maliyeti göz önüne alındığında prooksidan polifenoller ile etkinliklerinin artırılması önem taşımaktadır. Polimerik materyaller arasında en sık kullanılan polilaktik-ko- glikolik asit (PLGA), FDA tarafından onaylanan ve ilaç taşıma sistemlerinde yaygın olarak kullanılan bir biyomateryaldir. Yapılan birçok çalışma ile kurkuminin, kanser gelişimini önlemesi ile kanser tedavisinde güvenilirliliği ve etkinliği kanıtlanmıştır. NF-kB; inflamasyon, immun yanıt, proliferasyon ve apoptoz gibi hücre mekanizmalarından sorumlu birçok genin düzenlenmesinde rol alan önemli bir transkripsiyon faktörüdür. Stres nedeniyle ile reaktif oksijen türlerinin hücre içerisinde artması NF-kB transkripsiyon faktörünü etkilemektedir. NF-kB yapısının ve mekanizmasını aydınlatılması, hücresel stresin azaltılmasında ve bununla birlikte yeni bakış açıları ile stresin negatif etkilerinin ortadan kaldırılmasında önemli rol oynayacağı düşünülmektedir. Bu çalışma ile PLGA-Kurkumin nano-formülasyonun kanser hücrelerinde NF-kB alt birimleri düzeyi üzerindeki etkisi incelenmiştir.
PLGA-Kukumin nano-formülsyonunun, p65, RelB, c-Rel, p100/p52, p105/p50 olmak üzere 5 NF-kB alt birimlerinin oranları üzerindeki etkisi paklitaksel uygulanan MDA-MB-231 meme kanseri hücreleri üzerinde incelenmiştir. P65, PLGA-Kurkumin tarafından en çok baskılanan birim olmakla birlikte bu nanoformülasyon ile birlikte kanser hücrelerindeki inflamasyonun engellenmesi nanoformülasyonun güvenilirliğini arttırmaktadır.

Anahtar Kelimeler: NF-kB, nano-formülasyon, curcumin, PLGA.

➢ *ORAL PRESENTATION*

Effects of indoline on the intracellular LPS-induced inflammation pathway and interaction between actin cytoskeleton

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Abstract

Actin filaments and bundles are involved in many processes such as movement, chemotaxis, secretion and cell division, as well as structural functions in cells. Actin, which constitutes 5% of the total protein in eukaryotic cells and 20-25% in muscle tissue, is a network system that plays an active role in many signalling pathways within the cell. It is also known that the actin cytoskeleton is regulated by RhoGTPases and plays a role in cell movement, polarity and intracellular transport. In recent studies, it has been reported that RhoGTPases also participate in the formation of inflammation response. In this study, the effects of indoline on the inflammatory response through RhoA and pyrin inflammatory complexes indoline and actin cytoskeleton interactions were investigated in the inflammatory disease model. Altought, indoline is one of the frameworks of FDA approved drugs, there are not many studies on it. In recent years, it has been reported that compounds containing indoline have antibacterial activity, as well as resistance modifying agents and reduce cytokine release in LPS-induced inflammation. In this study, the effects of indoline treatment on pyrin inflamosome complex formation mechanisms and RhoA pathway in LPS-induced THP-1 cells were investigated by electrophoretic and western-blot methods. Besides, interaction of actin and indoline was realized by the thermal shift assay (TSA). As a result of our findings, the indoline-actin interaction and effects of indoline on inflammation pathway will contribute to elucidate the molecular mechanism of pyrin inflammasome formation in various autoimmune diseases.

Keywords: Actin, Indoline, RhoA, RhoGTPases, autoimmune disease.

➢ *ORAL PRESENTATION*

Chromosomal Analysis of Endemic Fish Species *Salmo macrostigma* (Teleostei: Salmonidae)

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Abstract

Fish cytogenetics is a field of study that allows the analysis of chromosomes of a fish species, the number of chromosomes, morphology and species determination and contributes to the clarification of the biology of living organisms. Fish chromosomes can be seen and defined well via a light microscope in the metaphase phase of mitosis. Salmo species has wide distribution and high economic value in Turkey as endemic trout species. There are four species belonging to the genus Salmo including *S. abanticus*, *S. caspius*, *S. labrax*, *S. macrostigma* species in Turkey. It is important to know the genetics, origin and phenotype differences of the species to interpret the ecology, physiology, behavior and systematics of salmonid fish correctly. Karyological studies have provided basic information on the number, size and morphology of chromosomes. The study of karyotype is also important in aquaculture in connection with the use of chromosome manipulation techniques, including induction of polyploidy, gynogenesis, androgenesis and inter or intra-species hybridization. For this purpose, endemic species *S. macrostigma* were captured and transported as alive from Cınarcık Creek, Evciler Village, Bayramic, Canakkale to Bartin University research laboratory inside well aerated aquarium. The individuals are kept for relaxation and adaptation until laboratory study. The air-drying technique was used to obtain the chromosomes from tissues of kidney, spleen, gill. Metaphase chromosomes were photographed via Leica DM3000 research light microscope. Result of the analysis, diploid chromosome number ranges from 70 to 80. The most common pattern of chromosome number was 80 and the number of diploid chromosomes was thus confirmed as 2n = 80. This study will provide contribution for chromosomal data of Anatolian salmon.

Keywords: Chromosome, Trout, Diploid number.

➢ *ORAL PRESENTATION*

The effect of the originally synthesized curcumin analogue (JWB1) on triple breast cancer

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Abstract

Curcumin is a yellow-colored natural pigment isolated from the roots of the Curcuma Longa plant. Synthetic and natural curcumin compounds are known to have various biological activities such as anti-inflammatory, anti-oxidant, anti-viral, anti-infection, anti-allergy and anti-HIV. Curcumin isolated from natural sources in very small amounts and the variation in structure is limited. In addition, due to low bioavailability, low cellular uptake, low solubility and lack of persistence, it is essential to develop synthetic analogues in order to benefit from curcumin. Breast cancer is the second most common type of cancer in the world. Mortality rate is increasing despite many standard treatments. In this case, researchers have turned to the development of more effective therapeutic agents. Approximately 70% of breast cancers have oestrogen positive ER (+) characteristics. Triple negative breast cancer cells; Oestrogen negative ER (-), progesterone negative PR (-), epidermal growth factor 2 HER2 / EGFR2 deficient cells are the most aggressive breast cancer cells and their prognosis is poor. In vitro cytotoxicity tests are measurement methods performed in cell culture in order to evaluate substances with drug characteristics. DNA methylation is the reaction of covalent attachment of a methyl group to the structure from the 5-carbon of Cytosine in a CpG dinucleotide. In this way, alteration of cell function occurs as a result of change in gene expression. Studies in cancer development and mechanism are examined at epigenetic level and it was observed that global DNA hypomethylation plays a role in the carcinogen process. Moreover, the relationship between breast cancer and global DNA methylation was also determined. In our study, we performed cytotoxicity and global methylation (determination of methylation %) analyzes in vitro. It was concluded that the benzoquinone curcumin analogue (JWB1) we distinctevely synthesied was effective on triple breast cancer MDA-MB-231 cells.

Keywords: Curcumin analogue (JWB1), triple breast cancer, global DNA methylation, cytotoxicity

➢ *ORAL PRESENTATION*

Effect of CuO nanoparticle on *Ceratophyllum demersum*

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Abstract

In recent years, nanoparticles with sizes below 100 nm have been used in numerous commercial applications. Therefore, the development of nanomaterials and their use in various industrial fields causes more pollution of the environment by nanoparticles. Aquatic macrophytes are an important part of aquatic ecosystems. *Ceratophyllum demersum* with a cosmopolitan distribution is a rootless submerged aquatic macrophyte. In this study, it was aimed to determine some effects of nano-CuO on *C. demersum*. The macrophytes used in the study were collected from the uncontaminated pond in Gaziantep province. Different concentrations of CuO (0-200 mg/L) were applied to the macrophytes after being acclimatized in controlled conditions. Some analyzes were made on macrophytes harvested at the end of the application. It was determined that protein contents of the macrophyte tissues were increased by CuO concentrations. The highest increase was found in 50 mg/L. In addition, under the effect of the applied CuO concentrations, contents of total phenolic compound and non-protein sulfhydryl groups of the tissues increased with the increasing CuO concentration. As a result, it was determined that the applied CuO concentrations caused some physiological changes in *C. demersum*.

Keywords: *Ceratophyllum demersum*, nano-CuO, physiological effect, Oxidative Stress.

➢ *ORAL PRESENTATION*

Türkiye'de yetiştirilen Öküzgözü ve Boğazkere (*Vitis vinifera* L.) üzüm varyetelerinin tohumlarının yağ asidi ve fenolik içeriklerinin değerlendirilmesi

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Özet

Vitis vinifera L. üzüm türü dünyada en yaygın olarak yetiştirilen ve ekonomik önemi olan bir meyvedir. Türkiye'nin iklim koşulları nedeniyle *Vitis vinifera* L. bakımından zengin bir kaynağa sahip olduğu bilinmektedir. Bizde bu çalışmada *Vitis vinifera* L.'nin Öküzgözü ve Boğazkere varyetelerinin fenolik bileşiklerini ve yağ asidi içeriklerini analiz etmeyi amaçladık.

Lipit ekstraktı içindeki yağ asitleri metil esterlerine dönüştürüldükten sonra gaz kromatografisi ile analiz edilmiştir. Flavanoid analizi için HPLC cihazı kullanıldı. Flavonoid türleri DAD dedektörü kullanılarak analiz edildi. Çalışmanın sonunda, çalışmada kullanılan iki varyetenin bazı doymuş ve doymamış yağ asidi içerikleri ve fenol bileşiklerinin içeriği elde edilmiştir. Yağ asidi içerikleri incelendiğinde, özellikle de doymamış yağ asitleri olmak üzere bütün yağ asidi miktarlarının Öküzgözünde Boğazkere varyetesini göre belirgin düzeyde fazla olduğu gözlenmiştir ($p<0,001$, $p<0,01$). Fenolik içerikler bakımından sonuçlar yağ asitlerinde olduğu gibi Öküzgözünde Boğazkere çeşidine göre yüksek miktarlarda tespit edildi ($p<0,001$, $p<0,01$).

Bitki ekstraktları içeriğindeki fenolik bileşiklerin düzeyi ile antioksidan etkinliği arasında güçlü bir ilişki bulunduğu bilinmektedir. Yine doymamış yağ asitlerinin fosfolipit yapısına katıldığı ve faydaları bilinmektedir. Bu etkilerin birbiriyle orantılı çıkması Öküzgözü çeşidinin antiosidan etkisinin daha yüksek olduğunu düşündürmüştür. Bu etkiyi sağlayan maddeler bitkiden doğru şekilde izole edilirse hem gıda hem de ilaç sektöründe faydalı bir şekilde kullanılabilecektir.

Keywords: *Vitis vinifera* L., fenolik, yağ asitleri.

➢ *ORAL PRESENTATION*

Bioinformatic Analysis of Biomarker Potentials of miRNA-Associated ceRNAs in Breast Cancer

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Abstract

Objectives: Breast cancer (BCa) is a heterogeneous disease, which is the most common malignancy in women. The incidence and mortality rates of BCa indicate that it is the leading cause of cancer-related deaths. In recent years studies have reported that competing endogenous RNAs (ceRNAs) mechanisms, which play critical roles in the different biological processes of breast cancer such as proliferation, migration, and apoptosis. The goal of the present study is to identify the potential predictive biomarker for BCa diagnosis via in silico analysis that takes BCa specific miRNAs, finds their combinatorial target genes (ceRNAs), selects ones containing Transcribed Ultra Conserved Region (T-UCR) among them.
Methods: 40 miRNAs miRWalk were obtained from the database and their combinatorial target genes (ceRNAs) were identified with ComiR. Genes containing T-UCR and showing potential ceRNA activity were extracted. Among BCa-associated ceRNAs including T-UCR, we determined genes with remarkable expression differences between BCa and normal breast tissue using the GEPIA database. Moreover, Spearman correlation test in GEPIA database was used for the statistical analysis of the association of CLK3 and NFAT5 genes pairs.
Results: The analysis conducted using GEPIA database indicated that CLK3 and NFAT5 genes were significantly more expressed in BCa than in normal breast tissue. And also, CLK3 and NFAT5 genes pairs were found to be correlated with BCa ($p<0.001$; $R=0.35$).
Conclusion: These genes may be considered as potential predictive biomarkers for discriminating BCa patients from healthy persons. Our preliminary results can supply a new perspective for *in vitro* and *in vivo* studies in the future.

Keywords: Breast cancer; ceRNA; T-UCR; miRNA; In silico analysis

➢ *ORAL PRESENTATION*

Coenurus cerebralisli koyunların beyin kist sıvısında mineral madde düzeylerinin araştırılması

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Özet

Coenurus cerebralis, koyun ve keçi yetiştiriciliğini etkileyen önemli parazitlerden biridir. Taenia multicepsin larvası olan coenurus cerebralisin son konağı karnivorlardır. Son konağın dışkısı ile bulaşan arakonaklarda merkezi sinir sistemine ulaşarak nörolojik bozukluklar yapmaktadır. Bu çalışma ile Coenurosisli koyunlarında beyin kist sıvısında bulunan bazı mineral maddelerin seviyesinin belirlenmesi amaçlandı. Çalışmanın hayvan materyalini Van Büyükşehir Belediye Mezbahasına kesim için getirilen koyunlar oluşturdu. Depresyon, başın bir tarafa eğilmesi, dairesel hareketlerle yürüme ve arka bacaklarda felç gibi belirtiler gösteren ve kesim sonrası beyinde kist varlığı tespit edilen beş koyun seçildi. Beyinde oluşan kist sıvısı alınarak bazı mineral maddelerin (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Zn,Na,Ca,Mg,K) miktarları tespit edildi. Makro element düzeyi için atomik absorbsiyon spektrofotometre mikro element düzeyleri tespiti için ise Typical Optima 7000 DV ICP-OES cihazı kullanıldı. Tespit edilen mineral madde düzeyleri literatür bilgisine sunuldu.

Anahtar Kelimeler: Coenurus cerebralis, mineral maddeler, kist sıvısı.

➢ *ORAL PRESENTATION*

Anaplasmosisli koyunlarda kisspeptin düzeylerinin araştırılması

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Özet

Anaplasma etkenleri zorunlu hücre içi, gram negatif ve kene kaynaklı riketsiyal parazitlerdir. Anaplazmoz, tropikal ve subtropikal bölgelerde canlı hayvan sağlığı ve üretimi için zararlar oluşturmaktadır. Azalan üretim, ölüm oranı ve etkilenen hayvanların iş verimliliğinin düşmesi nedeniyle büyük ekonomik kayıplara neden olmaktadır. Anaplasma enfeksiyonları genelde subkliniktir. Fakat şiddetli enfeksiyonlarda anemi, abort ve ölümler görülebilmektedir. Anaplasma sonuçlarından bir olabilen abortusların araştırılması için bu çalışmada Kispeptin düzeylerinin tespit edilmesi amaçlanmıştır. Kisspeptin(KISS1) son yıllarda keşfedilen puberta ve fertilite üzerinde önemli etkileri olan bir nöropeptiddir. Yapılan bu araştırmada; çalışma materyalini Van Büyükşehir Belediye mezbahasına getirilen 91 koyundan alınan kan örnekleri oluşturmuştur. Anaplasmosisin mikroskobik teşhisi için; koyunların kulak uçlarından alınan kanlardan yapılan sürme ince kan frotisi, giemsa boyama yapılarak x100 büyütmede eritrositler içinde 0.3 -0.1 mikron büyüklüğünde mavi- mor renge boyanmış formlar yönünden incelenmiştir ve serolojik teşhis için ise ticari kompetetif ELISA (c-ELISA) kiti (Anaplasma antibody test kit, c-ELISA, no: 282- 2VMRD-USA) kullanılmıştır. Pozitif bulunan 10 koyun çalışmanın deney gurubunu, negatif çıkan 10 koyun ise kontrol grubunu oluşturmuştur. Alınan kan örneklerinden elde edilen serumlarda ELİSA (Sheep Kisspeptin 1 ELISA Kit) kiti ile kispeptin düzeyleri belirlenmiştir. Çalışmanın deneysel şekilde planlanıp yaş gruplarına göre ayrılarak daha detaylı yapılmasının faydalı olacağı kanısına varılmıştır.

Anahtar Kelimeler: Anaplasma, kisspeptin, koyun.

➢ *ORAL PRESENTATION*

Regorafenib treatment induced Wnt/b-catenin pathway mediated stemness in HCC

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Abstract

Hepatocellular carcinoma (HCC) is the most common type of liver cancer and the third leading cause of cancer-related deaths worldwide. Sorafenib and recently lenvatinib is the only systemic therapy approved in the first-line by Food and Drug Administration (FDA). But this drug is related to poor therapeutic response and high rates of drug resistance after approximately 3 mounts. Regorafenib, an analog of sorafenib with improved target affinity and higher potency, is one of the second-line treatments in patients who failed sorafenib therapy. But its molecular mechanism still needs investigation to improve treatment efficiency in HCC. In our study, we show that regorafenib treatment activates Wnt/β-catenin signaling in HCC cell lines and induces enrichment of markers associated with hepatic stem/progenitor cells. Also, when cells were treated with regorafenib in combination with Wnt3a/R-Spo1, the induction of EpCAM+ cancer stem cell subpopulation was greatly enhanced. Finally, when regorafenib was used in combination with Wnt3a/R-Spo1, regorafenib-induced apoptosis of HuH7 cells was completely inhibited. Thus, for HCC tumors with Wnt/β-catenin activation, the inhibition of this pathway along with regorafenib administration might increase regorafenib-induced cell death and thus ameliorate treatment outcome.

Keywords: HCC, Regorafenib, Wnt-b-catenin pathway, Stemness,

➢ *ORAL PRESENTATION*

Eosinophilic Gastroenteritis And Accompanying Biochemical Changes

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Abstract

Objective: Eosinophilic gastrointestinal diseases (EGD) are characterized by eosinophilic infiltration and inflammation in the gastrointestinal system without any other disease that may cause eosinophilia (such as parasitic infection, drug reaction, inflammatory bowel disease). EGD is subdivided into eosinophilic esophagitis (EE), eosinophilic gastroenteritis (EGE), and eosinophilic colitis (EC). There are three main types: mucosal, muscular and serosal. Serum Ig E increase and peripheral osinophilia are generally observed in patients. The definitive diagnosis is made by biopsy. In this presentation, our patients diagnosed with EGD are evaluated.
Methods: Between 2010 and 2014, our patients with EGD who had various digestive problems and determined by endoscopic pathological examinations were investigated. These cases were evaluated retrospectively.
Results: The mean age of our 4 patients evaluated at the time of diagnosis was 41 (30-56 years). All of them were sensitive to dairy products. All patients had stomach pain and various digestive system symptoms. The duration of the complaints ranged from 1 to 10 years. Serum total Ig E level was normal in all patients. Three of our patients had no peripheral eosinophilia. No pathology was found in the ultrasonic examination of all patients. Upper and lower digestive system endoscopy and endoscopic biopsy examinations were performed in all patients. One of them was diagnosed with IE, 2 with EC, and 1 with eosinophilic rectitis. The patients were treated with food elimination and short-term steroid therapy.
Conclusion: Since EGE symptoms are not specific to the disease, they can be confused with other digestive system diseases. Blood eosinophilia and increased Ig E are common. However, the definitive diagnosis is made by biopsy. EGD should be considered and diagnostic steps should be taken by patients with digestive problems that increase after certain foods.

Keywords: Eosinophilic gastrointestinal diseases, eosinophilia, Ig E

➢ *ORAL PRESENTATION*

Osteosarkom Hücrelerinde Spektroskopik Apoptozis İndeksinin Belirlenmesi

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Özet

Apoptozisin belirlenmesi, hücre kültürü çalışmalarında kanser ilaçlarının değerlendirmesi için oldukça önemlidir. Apoptotik süreçleri belirlemeye yönelik kullanılan yöntemler, hücre kültürü ortamına fiziksel ve/veya kimyasal olarak müdahale edilmesini gerektirir. Ayrıca bu yöntemler fazladan maliyet ve hücre kayıplarına yol açmasının yanı sıra deneyin sadece bir zaman noktasında değerlendirilmesi ile sınırlıdır. Bu çalışmanın amacı osteosarkom hücrelerinde geri yansıma spektroskopisi tekniği kullanılarak yapılan ölçümlerle apoptozisin gerçek zamanlı belirlenebileceği yeni bir yöntem geliştirmek. Osteosarkom hücre kültürü ortamından geri yansıyan ışığın spektrumu ölçülerek, hücrelerdeki biyokimyasal değişikliklere bağlı sinyal elde edilebilir. Apoptozis ile bu sinyaldeki farklılıklar belirlenerek, apoptozisi gösteren spektroskopik bir indeks geliştirilebilir. Bu çalışmada osteosarkom (Saos-2) hücrelerinde apoptozisi indüklemek için beş farklı dozda (5-100 µM) doksorubisin (Dox) ile inkübasyon yapıldı. Geri yansıma ölçümleri için kullanılan deney düzeneği, spektrometre, tungsten-halojen ışık kaynağı ve fiber optik probtan oluşmaktadır. Kontrol ve beş farklı dozdaki Dox grubu numunelerinden 6 saat sonra geri yansıyan ışığın spektrumu 400-800 nm dalga boyu aralığında ölçüldü. Kontrol ve Dox spektrumlarındaki değişimler analiz edilerek yeni bir indeks olan "Spektroskopik Apoptozis İndeksi" tanımlandı. Spektroskopik Apoptozis İndeksi ölçülen spektrumlardaki 550 ve 800 nm deki ışık şiddeti oranları ile hesaplandı. Apoptotik süreci değerlendirebilmek için MTT yöntemi ile hücre canlılığı ölçüldü ve toluidin mavisi boyama yöntemi ile hücre morfolojisi görüntülendi. Sonuç olarak Kontrol ve Dox numunelerinden ölçülen geri yansıma spektrumunun analizinden apoptozisin meydana getirdiği sinyal farklılığı belirlendi. Buna bağlı olarak hesaplanan Spektroskopik Apoptozis İndeksinde gruplar arasında istatistiksel olarak anlamlı fark olduğu bulundu. MTT yöntemi ile hücre canlılığı ölçümünde 24 saat sonra tüm gruplarda canlılığın kontrole göre azaldığı bulundu. Toluidin mavisi yöntemi ile 6 saatte görüntülenen hücrelerde morfolojik olarak anlamlı bir fark görülmedi. Ancak hücresel bütünlük bozulmaya başladığı belirlendi. Spektroskopik Apoptozis İndeksi deneysel uygulamalarda hücre kültürü koşullarına müdahale etmeden apoptozisi gerçek zamanlı belirleyebilecek yeni bir yaklaşımdır. Bu yaklaşımın apoptozisin zamana bağlı izlenmesini sağlayan ve invaziv olmayan bir teknik olarak geliştirilme potansiyeli bulunmaktadır.

Anahtar Kelimeler: Geri Yansıma Spektroskopisi, Apoptozis, Osteosarkom.

➢ *ORAL PRESENTATION*

Germ cells in infertility treatment

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Abstract

Isolation of germ cells and their ability to reproduce under laboratory conditions can be effective in the diagnosis and treatment of many diseases. In the treatment of infertility, which is one of these diseases, it is possible to reach the stem cell sources from various places with the in vitro proliferation method, differentiate them into germ cells or directly isolate PGD or SSC. In approximately 5-20% of men who apply for infertility, live sperm is not found in the ejaculate sample. Studies conducted to induce spermatogenesis and obtain spermatozoa in patients with only early stage spermatogenesis (maturation pause) or only Sertoli support cells (Sertoli-cell only) have not been successful in the clinical field in histopathological evaluations. It is aimed to share the findings obtained based on the current literature.

Keywords: Infertility, stem cell, germ cell, spermatogenic stem cell

➢ *ORAL PRESENTATION*

In-Vitro Demonstration of the Effect of Quarterner Ammonium Content Antiseptic Mouthwash on Biochemical Tests

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Abstract

Objective: Antiseptic mouthwashes are chemical plaque control methods that have been shown to be effective in reducing gingivitis by preventing biofilm accumulation. The antiseptic solution can be absorbed through the mucosa and enter to the blood stream. Although physicians are aware of the systemic side-effects of active and auxiliary substances, their interferance properties to biochemical test results are not known enough. The aim of this study is to experimentally investigate the effect of frequently used oral antiseptic on routine biochemical test results.
Methods: A commercially available alcohol-free mouthwash containing the quaternary ammonium compound was used as an antiseptic. The ingredients of the antiseptic were aqua, glycerin, propylene glycol, sorbitol, poloxamer 407, cetylpyridinium chloride, potassium sorbate, sodium fluoride, sodium saccharine. The mixture was prepared by taking 20µL of the solution and 180µL of the biochemical control solution with the same content as blood. Glucose, urea, creatinine, sodium, potassium, chlorine, AST, ALT, GGT, ALP, direct bilirubin, total bilirubin, calcium, magnesium, phosphorus tests were studied with the prepared mixture in biochemistry autoanalyzer. The same experiment was repeated by adding 20µL of distilled water to the control solution. Deviation amounts were calculated with bias%.
Results: The detected deviation range was calculated between -2.78% and 10.82%. The highest deviation was in potassium with 10.82%. Sodium deviated 4.72% and ALT deviated 5.13% from the target value. The deviation rates in other tests were minimal.
Conclusion: Despite its low potassium content, the antiseptic caused a false high result in potassium. The interferant properties of the ingredients may have affected the ion selective electrode in the analyzer. Minimal deviations in other tests are not clinically significant. The deviation amounts of biochemical tests may vary depending on the content of the oral antiseptics used and their elimination time from the blood.

Keywords: Antiseptic, mouthwash, interferan, glucose, urea, creatinine, sodium, potassium

➢ *ORAL PRESENTATION*

Türkiye' de Yayılış Gösteren *Bolboschoenus* (Palla) Cinsi Taksonların Tohum Morfolojisi

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Özet

Cyperaceae familyasının taksonlarından olan, dünyada yaklaşık olarak 15 türe sahip olan *Bolboschoenus* (Palla) cinsi geniş bir yayılışa sahiptir. Türkiye florasında Kittan tarafından yazılan *Bolboschoenus maritimus* türü 2 varyete içermektedir. Daha sonra yapılan çalışmalar sonucunda ülkemizdeki *Bolboschoenus* (Palla)'un takson sayısı 3 tür olarak belirlenmiştir. Bu çalışma ile son zamanlarda yapılmış çalışmalarla ortaya çıkan taksonomik değişiklikleri kontrol etmek ve taksonların betimlemelerini genişletmek amaçlanmıştır. Nut (Meyve) morfolojisi çalışmalarında stereo ışık mikroskobu kullanılmıştır. Her özellik için 30 ölçüm yapılmıştır ve ölçümlerin ortalamaları hesaplanmıştır. Ayrıca daha detaylı incelemek için SEM'den yararlanılmıştır. Elektron mikroskobu çalışmaları, Erciyes Üniversitesi Teknoloji Araştırma ve Uygulama Merkezinde (TAUM) bulunan Leo 440 marka Bilgisayar Kontrollü Dijital SEM (Scanning Electron Microscope) kullanılarak yapılmıştır. *Bolboshoenus* (Palla) taksonlarında nutlar fındıksı,ters yumurtamsı biçimde, üstü düz, parlak uçta kısa gagalıdır. *B. maritimus* taksonunda yüzey süslemesi düz iken *B. glaucus* ve *B. laticarpus* türlerinde mikro perforattır. *B. maritimus* ve *B. laticarpus* türlerinde tohumlar enine kesitte üçgen şekilli, kahverengi renklidir. *B: glaucus* türünde ise tohum enine kesit alındığında üçgen şekilli, kahverengi ya da sarımsı kahverengi renklidir. Çalışılan türlerdeki tohumların en ve boyları *B. maritimus* 2,73(B) X 1,70 (E), *B. glaucus* 2,44(B) X 1,82 (E), *B. laticarpus* 2,57(B) X 1,78 (E) olarak ölçülmüştür.

Anahtar Kelimeler: Nut, Bolboschoenus, Türkiye, SEM

Bu çalışma 117Z036 numaralı TÜBİTAK projesi tarafından desteklenmiştir.

➢ *ORAL PRESENTATION*

Türkiye' de Yayılış Gösteren *Bolboschoenus* (Palla) Cinsi Taksonların Polen Morfolojisi

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Özet

Dünyada yaklaşık olarak 15 türe sahip olan *Bolboschoenus* (Palla) cinsi geniş bir yayılışa sahiptir. Cyperaceae familyasının taksonlarından olan Türkiye florasında Kittan tarafından yazılan *Bolboschoenus maritimus* türü 2 varyete içermektedir. Daha sonra yapılan çalışmalar sonucunda ülkemizdeki *Bolboschoenus* (Palla)'un takson sayısı 3 tür olarak belirlenmiştir. Bu çalışma ile Türkiye' de yayılış gösteren *Bolboschoenus* (Palla) taksonlarının palinolojik özellikleri hakkında bilgi verilerek karakterlerinin belirlenmesi amaçlanmıştır. Herbaryum materyali haline getirilen örneklerden Wodehouse metoduna göre polen preparatları hazırlanıp, bazik fuksin ile boyandıktan sonra preparatlar ışık mikroskobunda incelenmiştir. Her bir özellik için 30-50 ölçüm yapılmıştır ve bu ölçümlerin ortalamaları hesaplanmıştır. Ayrıca polenlerin daha ayrıntılı incelenmesi amacıyla taramalı elektron mikroskobu (SEM) ile çalışılmıştır. Elektron mikroskobu çalışmaları, Erciyes Üniversitesi Teknoloji Araştırma ve Uygulama Merkezinde bulunan Leo 440 marka Bilgisayar Kontrollü Dijital SEM (Scanning Electron Microscope) kullanılarak yapılmıştır. *Bolboshoenus* (Palla) taksonları 6 apertüre (1 distal ulkus + 5 yanal kolpus) sahip olup *B.maritimus* prolat-spheroidal şekillidir. Diğer *B. glaucus* ve *B. laticarpus* türleri subprolat şekillidir. *Bolboschoenus* (Palla) cinsi taksonlarının yüzey süslemesine bakıldığında türlerin tamamının mikroekinat-perforat yüzey süslemesine sahip olduğu görülmüştür. Çalışılan türlerin ölçümleri *B. glaucus* 42,66 (P) x 23,71 (E), *B. maritimus* 45,72(P) x 29,57 (E) ve *B.laticarpus* 42,72 (P) x 23,96 (E) olarak ölçülmüştür. Bu çalışma 117Z036 numaralı TÜBİTAK projesi ile desteklenmiştir.

Anahtar Kelimeler: Polen morfolojisi, Bolboschoenus, Türkiye, SEM

➢ *ORAL PRESENTATION*

Interaction between SCH-442416 and Telomeric G-quadruplex DNA: A possible Anticancer Mechanism

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Abstract

Human telomere consists of tandem repeats of guanines so it can form intramolecular G-quadruplex structure which was found to inhibit telomerase enzyme that responsible on more than 85% of cancer. The present work investigated the interactions of (SCH-442416), pyrazolo-triazolo-pyrimidines derivative, with telomeric G-quadruplex $AG_3(TTAGGG)_3$. We found that SCH has shown a good affinity towards G-quadruplex DNA with a binding constant of $1.05x10^6$ M^{-1} and a melting temperature shift ΔTm of 9.6 °C. Increasing SCH ratio with DNA up to 5 times has shown inverse effect to G-quadruplex DNA stability. The stoichiometric ratio between SCH and telomeric G-quadruplex (per strand) was 2:1. Results obtained from absorption, fluorescence and CD indicated that SCH interacted with G-quadruplex through intercalation binding mode. SCH has shown a good specificity to quadruplex over duplex DNA by 4.00 folds. These results indicated that stabilizing of telomeric G-quadruplex, consequently inhibiting of telomerase enzyme could be one of the possible anticancer mechanisms of SCH.

Keywords: Quadruplex, SCH, Cancer, Telomere, Telomerase.

➢ *ORAL PRESENTATION*

Isopoda Fauna of Some Brackish Waters in the Rize Province Coasts

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Abstract

The aim of this study was to determine of Isopoda fauna and their distribution in the eastern Black Sea coast of Rize. Sampling was done by free diving technique depending on the depth. Benthic samples were collected using a 30x30 cm quadrat with 250 micron mesh size. The first fixation was made in the field with 90% ethyl alcohol. The maps of the locations have been prepared using geographic information systems. As a result of the morphological diagnoses, 4 species belonging to the Isopoda orders were identified. In the sampling made throughout the coast, *Idotea balthica, Idotea metallica, Lekanesphaera monodi, Dynamene bicolor* were detected. *Idotea balthica* was determined to be most encountered species in the stations.

Keywords: Isopoda, coast, Eastern Black Sea, estuary

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➢ *ORAL PRESENTATION*

Comparison of Gene Expressions of Astrocyte and Glioblastoma Cells

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Abstract

Cancers are the most common cause of death in the society after cardiovascular system diseases. Glioblastome Multiforme (GBM) is the most common primary brain cancer of the central nervous system that progresses aggressively and results in death in a short time. It forms from astrocytes which support nerve cells. GBM, which is of neuroectoderm origin, is grade IV astrocytoma, the most dangerous group of the astrocytoma cancer family. Current treatment practices such as surgery, chemotherapy, radiotherapy, and immunotherapy only extend the survival of patients. In addition to the ineffectiveness of treatment options, significant differences are observed in patient survival and treatment response in GBM, which includes many mutation subgroups, both genetic and epigenetic. Astrocyte and GBM cell lines are frequently used for studies. In the study, the gene expressions of astrocyte (SVG p12 cell line) and GBM cells (U87 and LN229 cell lines) are examined by PCR primarily. BAX, BCL2, BIRC5, Akt, PUMA genes' expressions for cell apoptosis; CCND1, CCNE1, CDK4, CDK6 for cell cycle; CDKN1A, CDKN1B, GADD45A, TP53 for cell cycle senescence; PCNA, MKI67, DNMT1 for cell proliferation; ATM, BRCA1, BRCA2 for DNA damage response are checked. The genes' expressions related to cell apoptosis,decreased in U87 GBM cells compared to SVG p12 astrocyte cells. The genes' expressions related to cell cycle senescence and DNA damage response decreased in both U87 and LN229 GBM cells. According to the results, due to the expression difference in SVG p12 and U87 cell lines, a wound healing assay is performed to see the difference in migration. SVG p12 and U87 GBM cells are divided into 4 time points (0,3,6,24 hours) with 6 replicates at each time point. When U87 and SVG p12 cells are compared according to 0h, there is statistical significance in each time point: U87-SVG p12(3h/0h) p=0.0038; U87-SVG p12(6h/0h) p=0.0011; U87-SVG p12(24h/0h) p=0.0034.

Keywords: astrocyte, glioblastoma multiforme, wound healing assay

➢ *ORAL PRESENTATION*

Clinical Applications of Enzyme Inhibition

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Abstract

Enzymes are biological catalysts, mostly in the protein structure, that catalyse chemical reactions in living things. It is possible to interfere with any metabolic pathway by increasing or decreasing the activities of enzymes. Recently, scientists have recognized enzymes as the most attractive targets for small molecule drug intervention in human diseases. Enzymes are often the primary molecular targets of drug search efforts. Much of the information driving these medicinal chemistry efforts comes from in vitro evaluation of enzyme-inhibitor interactions. Therefore, it is important to correctly identify the type of inhibition. Enzyme inhibitors are low molecular weight chemical compounds that reduce or permanently (irreversibly) inhibit the enzyme's catalytic activity. Irreversible inhibitors that are covalently bound to the enzyme provide an alternative and complementary way to elucidate functional groups in the enzyme active region so they limit competition with high concentrations of endogenous ligands and achieve desired pharmacological effects even at lower drug concentrations/doses. Or, in cases where the cellular substrate concentration is high, enzyme inhibitors that work with non-competitive inhibition are ideal for disrupting enzyme targets in metabolic pathways because the accumulation of the substrate cannot prevent inhibition owing to the fact that it is not competing. In enzymes, the active site is more protected than the allosteric site, and a mutation in this region is a low probability to resist inhibition. Therefore, competitive inhibition with an inhibitor that is structurally similar to the substrate can often be preferred in the treatment of many diseases.
The purpose of this presentation is to give an overview of enzyme inhibition, application of enzyme inhibition in the clinic, and examples of drugs targeting enzymes.

Keywords: drug, competitive inhibition, enzyme, non-competitive inhibition, therapeutic target

➢ *ORAL PRESENTATION*

Electrostatic deposition with chitosan as a novel approach for stabilisation of pomegranate seed oil bodies

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Abstract

Oli bodies are plant organogels that stores energy for the germination of seeds. Therefore, it is rich in triglycerides and oil soluble bioactive compounds. During generally oil extraction methods, the structure is destroyed by solvents or mechanical energy to obtain bulk oil. However, aqueous extraction provided the extraction of the oil bodies in the natural structure which is a microcapsule formed by triglyceride core coated with protein and phospholipid layer. Because of this form, oil bodies have high chemical stability. On the other hand, there is physical stability is low and affected from environmental and processing conditions. In the present study, effectiveness of chitosan on the stabilisation of oil bodies were tested under different conditions. The results showed that optimum chitosan concentration was 0.25%. Addition of the chitosan to the oil body dispersion provide stability against pH (in the range of 2 to 7), ionic strength (up to 500 mM) and freeze-thaw cycle (up to 3 cycles). Moreover, both control and stabilised samples determined to be stable when exposed to the heat treatment. Overall, the study proved that chitosan is a good alternative to stabilise oil bodies under various conditions.

Keywords: pomegranate, oil bodies, phyical stability, chitosan

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➢ *ORAL PRESENTATION*

Controlled Drug Release of Dexketoprofen from Chitosan-Halloysite Composite Beads

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Abstract

Nowadays, biodegradable and biocompatible polymers have become great attention in many fields such as the environment, controlled drug release, biomedical, tissue engineering. The use of natural polymers and their semi-synthetic derivatives in drug delivery systems is of particular interest. Natural polymers are primarily preferred because of their cheapness, readily available, capable of a multitude of chemical modifications, and potentially degradable and biocompatible [1,2]. Chitosan, a natural polymer, has been widely used in pharmaceutical and biomedical areas because of its favorable biological properties such as safe, biocompatibility, biodegradability, low-toxicity, bacterio-static, fungi-static, hemostatic properties [3,4]. Recently, halloysite nanotubes (HNTs) a two-layered aluminosilicate have attracted increasing interest as an inorganic filler and reinforcement materials for polymers. Due to having a nontoxic property and low cost, HNTs and their composites have been studied for biomedical applications such as bone cement, tissue engineering, drug delivery, and enzyme immobilization, among others. Dexketoprofen trometamol (DT) is an active optical isomer of ketoprofen. DT is used as an agent for the treatment of painful musculoskeletal disorders such as back pain and osteoarthritis, dental or postoperative pain and dysmenorrhea [3]. In this regard, the aim of this study is to design drug carrier material that performed controlled release and to examine drug release properties. For this purpose, dexketoprofen-loaded beads were prepared using chitosan and pure halloysite nanotube, and drug release profiles were examined by using UV-Vis spectrophotometry. Besides, drug release kinetics were studied using the Korsemeyer Peppas model, Higuchi model, and zero and first order kinetic model. Moreover, morphological and physicochemical properties of the prepared composite beads were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR).

Keywords: Dexketoprofen, drug delivery systems, halloysite nanotube, chitosan

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➢ *ORAL PRESENTATION*

The role of natural compound Apigenin in the treatment of high-grade hepatocellular carcinoma: Results of in vitro experiments on SNU-449 cell line

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Abstract

Aim-Background: The role of natural compounds during the treatment of cancer has paramount importance. Apigenin is a flavonoid that is being studied for its antineoplastic and inflammatory effects. The role of apigenin as an antitumor agent or antitumor immunity in high-grade hepatocellular cancer is unknown. The aim of the present study was the evaluate the effects of Apigenin on proliferation, cell invasion, and cell viability in hepatocellular cancer (HCC) cell line SNU-449 which derived from a hepatitis B virus-infected donor.
Materials and methods: To evaluate the antiproliferative and antimetastatic effects of Apigenin in HCC, we performed cell viability (MTT) assay was performed at 24, 48, 72nd-hour intervals and at six different concentrations (between 2.5-100 µM) of Apigenin. The minimum effective concentration on cell viability was chosen and later three procedures were performed at the designated dose at 48th hour of exposure to Apigenin. In addition to MTT assay, Sulforhodamine B (SRB), colony formation, and wound healing assays were performed. All results are expressed as median (IQR= interquartile range).
Results: MTT assay showed that 5µM at 72 hours was the effective minimum dose. Absorbance in 5 µM and untreated groups were 0.581(IQR:0.26) and 0.67(IQR: 0.049); respectively($p>0.05$). SRB assay showed that there was no difference between Apigenin and the null treated group (0.54[IQR:0.7] versus 0.381[IQR:0.365]; $p>0.05$). Colony formation assay showed that there was a modest difference between the Apigenin treated and untreated cells (74% surviving fraction relative to control). Wound areas of Apigenin and control group were 528366(IQR:691200) and 528861(IQR:523150)μm^2; respectively($p>0.05$). Wound closure rates were similar between the Apigenin and control group (59.5[IQR:36.9]%versus 59.75[IQR15.4]%; $p>0.05$).
Conclusions: The antiproliferative and antimetastatic effects of Apigenin is not prominent in HCC. The antitumor effects may be due to antitumor immunity. Studies must be directed towards the induction of immune response in tumor microenvironment.

Keywords: Apigenin, Antimetastatic treatment, Antiproliferative effects, Hepatocellular cancer, SNU-449 cell line

➢ *ORAL PRESENTATION*

The antiproliferative effects of PEITC on high grade Hepatocellular Cancer: A preclinical approach

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Abstract

Aim-Background: High Grade hepatocellular carcinoma (HCC) has a high risk of recurrence following resection or liver transplantation. HCC is a tumor that has high resistance to conventional chemotherapeutics. Phenethyl isothiocyanate (PEITC) is a novel antineoplastic agent that is being studied in many cancers. Therefore, there is need for discovery of novel antitumor agents. The aim of the present study is to evaluate the antitumor efficacy of PEITC on high grade HCC cell line SNU-449.
Materials and Methods: SNU-449 was obtained from the ATCC stock and cultured according to the company protocol. Cell viability (MTT) assay was performed at 24, 48, 72th hour intervals to determine the minimum effective concentration of PEITC in SNU-449. This concentration was used in cell proliferation (SRB), colony formation and wound healing assays. The later two analysis was performed after 48 hours of incubation with the effective dose of PEITC. All results are expressed as median (IQR= interquartile range).
Results: The MTT assay showed that antitumor efficacy of PEITC started from 10µM at 72 hours; absorbances in 10µM and control group were 0.431(IQR:0.458) and 0.67(IQR:0.049); respectively ($p<0.05$). Since this was the minimum effective dose of PEITC, we used this concentration to perform other procedures. The results of SRB assay in PEITC and control were 0.581(IQR:0.789) and 0.381(IQR:0.365), respectively ($p>0.05$). The colony formation assay surviving fraction of PEITC treated cells was 19.35% relative to the untreated cells. Wound area of PEITC treated cells and the control group were 1982061(IQR: 269014) μm^2 and 528861(IQR:523150) μm^2; respectively($p<0.05$). Wound closure percent in PEITC and control groups were 10.35% (IQR:10.3) and 59.75%(IQR:15.4); respectively ($p<0.05$).
Conclusion: PEITC seems to decrease cell viability and cell invasion process in high grade hepatocellular carcinoma cell line SNU-449. It is a good candidate for a novel antineoplastic agent. Intra cellular effects of this agent need further research.

Key words: Hepatocellular Cancer, PEITC, Antineoplastic therapy, SNU-449 cell line

➢ *ORAL PRESENTATION*

Immobilization of lipase on bionanocomposite support as a green sustainable material

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Abstract

The modern chemical industry is moving towards greener chemistry with the production of bio-products, utilizing the selectivity and specificity of enzymes. Free enzymes are not preferred in the industry due to the separation cost, recovery difficulties, and losses in re-use. Therefore, the immobilized enzyme is preferred instead of the free enzyme in reactions catalyzed by enzymes. Lipase enzyme is the most commercially used enzyme in industrial and biotechnological applications. Lipases are environmentally friendly biocatalysts with many advantages due to their high selectivity, activity in solvent-free conditions, and their biodegradability. In recent years, bionanocomposite carriers have been preferred as an enzyme support material because they are environmentally friendly and suitable for green chemistry. The biggest advantages of bio-composite materials are biodegradable and biocompatible. They improve the chemical and mechanical properties of enzymes when used as immobilized enzyme support. In this study, chitosan and functional multi-walled carbon nanotube were selected as bio-nano composite support material. In this experiment, the chitosan prepared at certain conditions was 30°C at 570 rpm for 24 hours, then functional multi-walled carbon nanotube added and dissolved at the same conditions for 1 hour. The prepared solution has been turned into bio-nano composite beads, and the lipase of TL 100 L (*Lipozyme TL 100 L*) has been immobilized. Tris-HCl and Phosphate are used as a buffer solution. The efficiency of immobilization was determined by the Bradford method. Some parameters that affect the yield of immobilization have been examined such as temperature (25 -45 ° C) and pH (6 -7.5). The activities of free and immobilized enzyme were determined by the method of olive oil hydrolysis. Lipase, which has been immobilized for this new and original bionanocomposite support developed, can be considered as an alternative to commercial enzymes as they provide re-use in their catalytic reactions.

Keywords: Lipase, bionanocomposite, chitosan, functional multi-walled carbon nanotube, immobilization.

➢ *ORAL PRESENTATION*

Ethnobotanic features of natural plants used by inhabitants in Bozova (Şanlıurfa)

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Abstract

The aim of this study to determine for which purposes the inhabitants of Bozova (Şanlıurfa) use plants and demonstrate importance of these plants for ethnobotanical features. As result of field research 131 genera and 170 taxa belonging to 50 families (109 species, 35 subspecies, 24 varietes) were identified. 1 of them belongs to Fungi kingdom, Ascomycota divisio; the others belong to Plantae kingdom, Spermatophyta divisio, Gymnospermae (1 taxon) and Angiospermae (169 taxa) subdivisio. By number of taxa they contain, top families in the area are; Fabaceae (31), Asteraceae (24), Lamiaceae (14), Apiaceae (10), Brassicaceae (10), Poaceae (6) and Malvaceae (5). By number of species they contain, top species in the area are; *Trifolium* (9), *Trigonella* (6), *Convolvulus* (4), *Astragalus* (3), *Centaurea* (3), *Euphorbia* (3) and *Medicago* (3). According to chorology of taxa; 56 are Irano-Turanian, 14 are Mediterranean, 7 are East Mediterranean, and 3 are Euro-Siberian elements. Chorology of 91 taxa are not known. When life forms of taxa are evaluated; 19 are phanerophytes, 6 are chamaephyte, 56 are hemicryptophytes, 79 are therophytes, 8 are geophytes, 2 are parasite and 1 is mushroom. The taxa determined from the research area are used for different purposes, for example; 61 for food, 48 for medical purpose, 35 for fodder, 9 for roofage, 8 for household goods, 7 for spice/flavoring, 5 for games, 5 for fuel, 5 for ornament, 4 for amulet/incense, handicraft (natural painting, broom, woodwork), 3 for fragrance, 2 for against evil eye, 2 for painting and it's seen that others are used for different purposes. Besides 11 of them are identified as harmful, while 7 are known only by their names.

Keywords: Ethnobotany, Vernacular name, Bozova, Şanlıurfa, Turkey.

➢ *ORAL PRESENTATION*

Is 1.25-Dihydroxyvitamin D friend or foe for hepatocellular carcinoma?

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Abstract

Objectives and Background: Active form of vitamin D (1.25-Dihydroxyvitamin D) usage in various diseases confuses the researchers about its effects on cells nowadays. In this study, we aimed to determine the effect of 1.25-Dihydroxyvitamin D on hepatocellular carcinoma cells. Methods: 250 nM 1.25-dihyrdroxyvitamin D is treated to HepG2 hepatocellular carcinoma cells at the 48th hour. After this treatment, total RNA was isolated and gene expressions of Ki-67, MMP-2, MMP-9, HIF-1α, hTERT and piR-823 expressions were determined by RT-PCR. Results: According to our obtained data, Ki-67, hTERT and piR-823 expressions were upregulated after treatment ($p<0.001$), while MMP-2, MMP-9 and HIF-1α gene expressions were downregulated ($p<0.001$). Conclusion: However, in some diseases and cancers 1.25-Dihydroxyvitamin D is useful for treatment, our gene expression data indicated that 1.25-Dihydroxyvitamin D has not beneficial effect on hepatocellular carcinoma cells. However, previously studies suggested 1.25-Dihydroxyvitamin D has a protective role against carcinogenesis, recent studies indicated non-beneficial effect of 1.25-Dihydroxyvitamin D possibility during cancer cases. Our study is specially to emphasize 1.25-Dihydroxyvitamin D negative impact on hepatocellular carcinoma cells according to the gene expressions of proliferation and adhesion markers. Moreover, this negative impact was also supported by observing the expression of oncogenic piRNA region.

Keywords: 1.25-Dihydroxyvitamin D, Adhesion, Hepatocellular Carcinoma, Proliferation, piRNA

➢ *ORAL PRESENTATION*

Heamatological Alterations Induced by Sulfoxaflor in Swiss Albino Mice (*Mus musculus*) and Ameliorative Effect of Sulfated Polysaccharide Fucoidan

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Abstract

Neonicotinoids, a novel set of systemic neuro-active pesticides, are one of the highly growing insecticides in agriculture and veterinary fields. Sulfoxaflor is the first commercial insecticide from the new chemical class sulfoximines and it is classified in neonicotinoid insecticides. Neonicotinoids exhibited low mammalian toxicity and have a relatively low risk for nontarget organisms. However, in recent studies, it has been determined that neonicotinoids cause different toxic effects in mammals. Fucoidan is a sulfated polysaccharide isolated from microalgae, largely made up of L-fucose and sulfate groups. Fucoidan has different bioactive properties such as anticancer, antiviral, anticoagulant, antiinflammatory and antioxidant. The purpose of the present study was to evaluate sulfoxaflor induced alterations in some haematological parameters in male Swiss albino mice (*Mus musculus*) via acute oral exposure and the possible ameliorative effect of the fucoidan against the effects of sulfoxaflor. Mice in sulfoxaflor toxicity groups were administered to sublethal oral dose 15 mg/kg (1/50 of the oral LD_{50}) by intragastric gavage for 24 hours and 7 days. Mice in the amelioration groups were orally administered with 50 mg/kg fucoidan by intragastric gavage for 24 hours and 7 days. RBC, WBC, HGB, MCV, MCH, MCHC, HCT, PLT were analyzed for determining the effects of sulfoxaflor and fucoidan on haematological parameters in the mice. Findings of this study showed that sulfoxaflor caused significant alterations in some haematological parameters and these alterations were ameliorated by fucoidan in mice.

Keywords: Neonicotinoids, Sulfoxaflor, Fucoidan, Heamatological parameters

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➢ *ORAL PRESENTATION*

Effect of Isabella grape (*Vitis labrusca* L.) addition on bioactive, technological and sensorial properties of tarhana

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Abstract

Tarhana is a traditional fermented food which is generally made from wheat flour, yoghurt, yeast, various vegetables and spices. It is commonly dried and ground after fermentation and prepared as a soup to consume. Both alcoholic and lactic acid fermentations result in a unique taste, odor and aroma to the final product. Tarhana can be produced and stored by numerous methods with a wide variety of ingredients to obtain different technological, sensorial and functional properties. Isabella grape, which is grown in the Black Sea region of Turkey known with its special odor and aroma properties. In this study, the addition of isabella grape pulp to tarhana formulation was investigated in terms of *bioactive, sensorial and technological properties. Control, 15% and 30% grape pulp* added tarhana samples were produced. Dry matter, pH, titratable acidity, color (*L*, a*, b**), antioxidant capacity (AC) with DPPH and FRAP methods and total phenolic content (TPC) were followed in doughs before fermentation and in the final products. Color and sensorial properties of tarhana soup were also investigated. While grape pulp ratio increased, *a**, and acidity of the samples increased, pH, *L** and *b** values decreased significantly. AC of the 30% grape pulp added tarhana dough was found about 2 and 2.5 fold higher than control samples in respect to FRAP and DPPH findings. After the drying process, 13-22% decrease in AC was calculated. Similar changes were also observed for TPC of the samples. In the sensory evaluation, panellists reported that the color of the grape pulp-added soups has an undesirable shade of grey, which may be a result of the oxidation and/or degradation of phenolics, but the consistency, odor, taste and acceptability of the enriched samples scored higher. As a result, Isabella grape added tarhana was found to have good sensorial properties with higher bioactive compounds.

Keywords: Isabella, grape, tarhana, antioxidant, phenolic, sensory

➢ *ORAL PRESENTATION*

Beta-karbolin türevi bileşiklerin mesane kanseri hücrelerindeki anti-tümöral etkileri

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Özet

Mesane kanseri özellikle erkeklerde en sık görülen kanserlerdendir. Standart tedavi, Mycobacterium bovis'in zayıflatılması ile elde edilen Bacillus Calmette-Guerin'in intravezikal uygulanmasıdır. Ancak, canlı mikobakteri kullanımı ciddi yan etkilere yol açtığı için daha etkili ve daha az zararlı tedavi arayışları devam etmektedir. Beta-karbolin türevleri olan harmin ve harmol, anti-tümöral ve anti-inflamatuvar özellikleri bilinen, kanser tedavisinde kullanılabilirliği üzerine çok çeşitli çalışmalar yapılan alkaloidlerdendir. Bu çalışmanın amacı, harmin ve harmol bileşiklerinin mesane kanseri hücrelerindeki anti-tümöral etkinliklerini incelemek ve bu etkinin mekanizmasını aydınlatmaktır. Bu amaçla, insan mesane kanseri hücrelerinin (T24) canlılığına harmin ve harmol bileşiklerinin etkileri, MTT yöntemi ile konsantrasyon ve zamana bağımlı olarak incelenmiştir. Migrasyon üzerine etkileri "wound healing" yöntemi ile belirlenmiş; hücrelerdeki morfolojik değişiklikler ışık mikroskopi ile takip edilmiştir. Harmin ve harmol bileşiklerinin kanser hücrelerindeki etki mekanizmalarının anlaşılması amacıyla hücreler NF-kappaB sinyal iletim yolunun bir uyaranı olan TNF-alfa ile uyarıldıktan sonra, prostaglandin E2 (PGE2) düzeyleri ELISA yöntemi ile tespit edilmiştir. Harmin, konsantrasyon ve zamana bağımlı olarak T24 hücrelerinin canlılığını inhibe etmiştir. IC_{50} değeri 11.26 mikromolar olarak hesaplanmıştır. Aynı şekilde, harmol bileşiğinin de MTT yöntemi ile T24 hücrelerinin canlılığını inhibe ettiği; ancak daha yüksek konsantrasyonlarda etkili olduğu gözlenmiştir. IC_{50} değerine göre harminin mesane kanseri hücrelerinin migrasyonuna etkisinin incelendiği çalışmalarda, 10 mikromolar konsantrasyonda bileşiğin 24 saat sonra hücre göçünü %32 oranında inhibe ettiği, daha yüksek konsantrasyonlarda da (20 ve 40 mikromolar) %56 ve %74 oranlarında inhibisyon olduğu tespit edilmiştir. Harmol ile yapılan migrasyon çalışmalarında, 20 mikromolar bileşiğin hücre göçünü 24 saat sonunda %32 inhibe ettiği belirlenmiştir. Harmin, tek başına PGE2 seviyelerini %50 oranında azaltırken, TNF-alfa ile birlikte uygulandığında PGE2 seviyelerinin arttığı tespit edilmiştir. Bazı kanser hücrelerinde konstitütif aktif olan NF-kappaB sinyal iletim yolunun, PGE2 ekspresyonunda artışa neden olduğu, harmin gibi alkaloidlerin NF-kappaB inhibitörü gibi davranarak PGE2 ekspresyonunu düşürdüğü, TNF-alfa eklenmesi ile PGE2 düzeyindeki düşüklüğün geri döndürüldüğü düşünülmektedir. Çalışmalarımız, NF-kappaB sinyal iletim yolu bileşenlerinin ekspresyon düzeylerinin incelenmesi ile devam etmektedir. Bu mekanizmanın aydınlatılması, harmin gibi beta-karbolin türevlerinin tek başına ve kombine ilaç tedavileri şeklinde anti-kanser ajanlar olarak değerlendirilmelerine olanak sağlayacaktır.

Anahtar kelimeler: Mesane kanseri, harmin, anti-tümöral ajanlar, NF-kappaB

➢ *ORAL PRESENTATION*

Metoksi/halokalkon *N*-glikozit bileşiklerinin sentezi ve biyolojik aktivitelerinin incelenmesi

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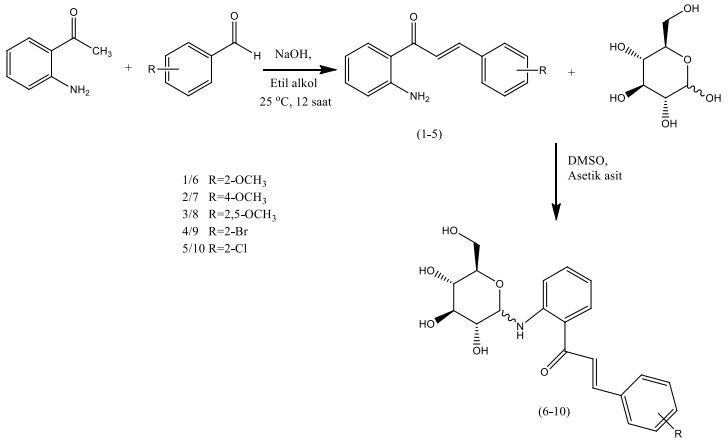
Özet

Kalkonlar birçok biyoaktif bileşiklerin sentezinde başlangıç bileşeni olarak kullanılmaktadır. Kalkon bileşiklerinin antitümor aktivitelerin yanı sıra antitüberküloz ve antifungal kemoterapik etkilere sahip olduğu belirtilmiştir Son yıllarda şekerlerin biyolojik sistemlerdeki öneminin ortaya çıkması üzerine birçok bilim adamı şeker grubu içeren bileşiklerin sentezine yönelmiştir. Literatürde O- ve N-glikozidik heterosiklik bileşikler bilinmekte olup çok geniş yelpazede biyolojik aktvite gösterdikleri rapor edilmiştir Bu tür bileşikler antikanser, antitumor ilaçlarında ve enzim inhibisyonunda önemli etkiye sahiptirler.
Bu çalışmada; bilinen metoksi/halo kalkon bileşikleri (1-5 nolu) ve yeni kalkon *N*-α/*β*-D-glikopiranosid türevlerin (6-10) sentezi gerçekleştirildi. Sentezlenen (6-10) nolu bileşiklerinin dokuz mikroorganizmaya karşı antimikrobiyal aktiviteleri ve üç farklı yöntem (FRAP, CUPRAC ve DPPH) kullanılarak antioksidan kapasiteleri araştırıldı. Ayrıca farklı halojen (-Br, -Cl) ve değişik pozisyonlardaki metoksi gruplarının yapı-aktivite açısından aktiviteye olan etkisi de araştırılmış oldu.Sentezlenen tüm bileşikler spektroskopik olarak NMR (1D: 1H, APT), MS, FT-IR yöntemleri kullanılarak karakterize edilmiştir.



Anahtar Kelimeler: Kalkon, *N*-glikozit, biyolojik aktivite.

➢ *ORAL PRESENTATION*

Covid-19 hastalarında hematolojik parametrelerin tanısal ve prediktif değeri

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Özet

Amaç: Covid-19 hastalarında hematolojik parametrelerin tanısal değerinin belirlenmesi, hastalık şiddeti ile ilişkisinin ortaya konması için, kabul sırasında analiz edilen ilk hematolojik bulguları değerlendirmeyi amaçladık. Yöntemler: Başakşehir Çam ve Sakura Şehir Hastanesi'ne başvuran, Covid-19 tanısı doğrulanan, yatırılarak takip ve tedavisi düzenlenen toplam 93 hasta ve (rRT-PCR) PCR testi negatif 67 kontrol hastasının yaş, nötrofil-lenfosit oranı (NLR), monosit-lenfosit (MLR) oranı, trombosit-lenfosit oranı (PLR) verileri, retrospektif olarak analiz edilerek karşılaştırıldı. Bulgular: Sağlıklı kontrol grubuyla karşılaştırıldığında, COVID-19 hastalarında anlamlı yüksek nötrofil-lenfosit oranı (NLR) (sırasıyla 1,78±0,8-4,77±3,8, p <0,001), monosit-lenfosit oranı (MLR) (sırasıyla 0,23±0,08-0,39±0,24, p <0,001) ve trombosit-lenfosit oranı (PLR) (sırasıyla 117,8±49,0-200,9±110,2, p <0,001) düzeyleri mevcuttu. NLR, MLR ve PLR ve yaş arasında pozitif korelasyon saptandı.
Sonuçlar: COVID-19 hastalarında, anormal periferik kan rutin inceleme sonuçları saptandı. NLR, PLR ve MLR COVID-19 hastalık şiddetini değerlendirmek ve hospitalizasyon ihtiyacı için bağımsız, güvenilir biyobelirteçler olarak düşünülebilir. Bu yüzden, COVID-19 hastalarının prognozunu tahmin etmek için başlangıç hemogram parametreleri önemlidir.

Anahtar Kelimeler: Periferik kan, Nötrofil-lenfosit oranı, Monosit-lenfosit oranı, Trombosit- lenfosit oranı, COVID-19

➢ *ORAL PRESENTATION*

Investigation Of Dietary Impact On Lactobacilli and Microbiota

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Abstract

Lactobacilli are a group of lactic acid producing bacteria which are thought to have protective effects on human gut microbiota due to their probiotic properties. On the other hand, gut microbiota composition is known to mainly depends on diet. It is still unknown which food constituents specifically promote growth and functionality of beneficial bacteria in the intestine. The aim of our study was to determine various nutrients impacts on Lactobacilli which is a flora member of gut microbiota. The nutrients which was chosen are banana, onion, artichoke and succory.
Lactobacilli were cultured on blood agar and incubated for 48 hours at 37^0 degrees. And the bacteria were passaged into the broth medium tubes. On the other hand banana, onion, artichoke and succory juice was extracted. Then 2 mililiters of extracts were taken to the tubes. Later on,the tubes were incubated with the nutrients. Turbidity of the tubes were compared. From the tubes, 0.01ml of samples were again passaged on agar plate to observe the bacterial growth. Colonies were counted on each plate.
As a result, lactobacilli which was treated with artichoke showed more growth then the others. Second one to the artichoke was found to be banana, third one was succory and the last one was onion.
In conclusion, it was found that various nutrients may effect the growth of Lactobacilli which is the member of gut microbiota. Also, preliminary evidences suggests that dietary patterns are associated with distinct combinations of bacteria in the intestine. So, more studies should be done to figure out the impacts on the gut microbiota-modulating effects of diet.

Keywords: Lactobacilli, microbiota, nutrition

➢ *ORAL PRESENTATION*

Investigation of levulinic acid production from Jerusalem artichoke

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Abstract

Levulinic acid (LA) is one of the platform chemicals and used in a wide range of applications in chemical industry [1]. Conventional industrial production of LA from maleic anhydride or furfuryl alcohol. However, low efficiency, high raw material input, due to the more complex production process compared to hydrolysis of biomass, high cost of equipment, waste problems and high costs for catalyst recycling its production of LA in petrochemistry remained limited. [2]. LA price in petroleum-based production is 5-8 $ / kg while it is aimed to reduce the price to 0.09-0.22 $ / kg in the production of LA from lignocellulosic biomass [3]. In this study, production of LA and by-products formic acid, acetic acid and 5 hydroxy methyl furfural (5-HMF) is investigated in the presence of hydrochloric acid from Jerusalem artichoke. Decomposition studies of Jerusalem artichoke are carried out in the batch autoclave reactors in at 180°C and in the presence of aqueous hydrochloric acid solution with a pH of 0.5, in different concentrations of Jerusalem artichoke/catalyst solution feedstock. The concentrations of 0.045 g/mL, 0.068 g/mL and 0.091 g/mL are prepared to examine the effect of concentration on the product yields. The produced amount of LA, formic acid, acetic acid and 5 hydroxy methyl furfural (5-HMF) and sugar compounds are varied with the reaction time of 20, 40, 60 and 120 minutes. Analysis of the aqueous products are done with HPLC and TOC (total organic carbon) analyser. The highest LA yield was found at 0.068 g/mL at 180°C and 0.5 pH as 30.7 % as the highest while 29.5% at 0.045 g/mL with a reaction time of 60 minute. LA yield increases up to 60 min. and decreases at 120 min. of reaction time. Liquefaction efficiency of the biomass is found as greater in low feed concentrations.

Keywords: Hydrolysis, dehydration, rehydration, levulinic acid, biomass

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➢ *ORAL PRESENTATION*

Antimicrobial, Antioxidant and Antiproliferative activities of *Galium aparine*

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Abstract

Plants have come to the fore with their many features since the past. It has been used for different purposes such as food, spice, medicine and shelter. Many studies have shown that plants have high biological activities. In this study, the antioxidant, oxidant, antimicrobial and antiproliferative activities of *Galium aparine* L. plant were determined. Ethanol extracts were extracted from above-ground parts of plant samples in soxhlet device. The antioxidant and oxidant potentials of the extracts were determined using Rel assay kits. Their antimicrobial activities were determined against fungus and bacterial strains using the agar dilution method. Its antiproliferative activities were tested against the A549 cell line. As a result of the studies, TAS value of plant extracts was determined as 5.147 ± 0.237, TOS value as 18.679 ± 0.245 and OSI value as 0.346 ± 0.018. It has been determined that plant extracts are effective against *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606 at concentrations of 50-200 µg/mL. It was also determined that the extracts were effective against *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135, *C. glabrata* ATCC 90030 at concentrations of 50-100 µg/mL. It has been determined that plant extracts have strong antiproliferative activity. As a result, it has been determined that *Galium aparine* has antioxidant, antimicrobial and antiproliferative potential.

Keywords: Antimicrobial, antioxidant, antiproliferative, medicinal plants.

➢ *ORAL PRESENTATION*

Yeni Nitro Sübstitüe Naftalimit Sentezi ve Antioksidan aktivitesi

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Özet

Antioksidanlar, serbest radikallerin salınmasından sonra diğer moleküllerin oksidasyonunu engelleyebilen ve geriye kalanları engelleyebilen moleküllerdir. Antioksidan özelliklere sahip yeni moleküllerin geliştirilmesi aktif bir araştırma alanıdır. Çünkü bu tür moleküller ateroskleroz, felç, diyabet, Alzheimer hastalığı ve bazı kanser türleri gibi birçok kronik hastalık riskini azaltma potansiyeline sahiptir. Kimyasal ve farmasötik endüstrilerde radikal zincir oksidasyon süreçlerini durdurmak için farklı antioksidanlar (C ve E vitaminleri, glutatyon, lipoik asit, butile fenoller, vb.) yaygın olarak kullanılmaktadır. Bu nedenle, etkili sentetik antioksidanları keşfetmeye büyük bilimsel ilgi vardır.
Amid türevleri, birçok biyolojik aktivite sergileyen önemli bir kimyasal gruptur. Örneğin, anilidler, antibakteriyel, anti-fungal, anti-enfeksiyöz ve anestezik gibi biyolojik aktiviteleri nedeniyle tıpta, eczacılıkta, biyolojide ve diğer ilgili alanlarda yaygın olarak kullanılmaktadır. Çeşitli anilidler ayrıca biyoaktif türler (antimikrobiyal, antioksidan ve anterosklerotik ajanlar) olarak geniş uygulanabilirlik bulmuşlardır. Aynı zamanda, sentetik amid türevleri güçlü antioksidan aktivite gösterebilir. Bu çalışmada tekli elektron ve hidrojen atomu verme eğilimi olan 4-nitro süstitüe naftalimit türevi sentezlenmiş ve antioksidan aktivitesi belirlenmiştir. DPPH yöntemiyle hesaplanan IC_{50} değeri literatürde bulunan amit türevleriyle ve standart antioksidan olan C vitamini ile karşılaştırılabilir olarak bulunmuştur.

Anahtar Kelimeler: Naftalimit, DPPH, Antioksidan.

➢ *ORAL PRESENTATION*

MicroRNA expression altered in endometrial tissues of PCOS women

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Abstract

To assess whether normally ovulating women and Polycystic ovary syndrome (PCOS) patients differ in tissue expression of several microRNAs (miRNA) in endometrium. Besides being the most common endocrinological disorder in reproductive age women, PCOS also affects fertility in a significant portion of couples seeking help for infertility. Patients with PCOS usually suffer from anovulation as a subfertility cause. However, PCOS carries the potential to disrupt endometrial intactness and hamper implantation, as well. We investigated the changes in the expression levels of a number of selected microRNAs in endometrial tissue samples of PCOS women and tried to detect an implied relationship. MicroRNAs are small RNA fragments that act as posttranscriptional regulators of various gene targets rather than encoding proteins themselves. miRNAs play certain roles in several biological processes. The abnormal expression of some miRNAs has been associated with a number of disorders. In humans, miRNAs are important in uterine functions, however, the endometrial aspects of PCOS related infertility and its implications in miRNA profiles have not been studied in detail. Endometrial disorders might be associated with the abnormal expression of miRNAs in the uterus. In this study, mir- 155 expression was found to decrease in endometrial tissues of women with polycystic ovary syndrome and lower expression of this mirna has been associated with increased apoptosis, inflammation and decreased cell survival. An inflammatory milieu in the endometrial tissue might be a confounding factor for the failure of embryonic implantation and inflammation supression might prove beneficial in treating subfertility issues in this group of patients.

Keywords: Polycystic ovary syndrome (PCOS), microRNA, infertility, endometrial disorders

➢ *ORAL PRESENTATION*

Solunum sistemi enfeksiyonlarının hızlı tanısında BioFire® FilmArray® Respiratory Panel v1.7'nin değerlendirilmesi

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Özet

Respiratuar sistem enfeksiyonlarının erken tanısı ve tedavisi mortalite ve morbiditenin azaltılması bakımından çok önemlidir. Özellikle pediatrik yaş grubunda, kronik hastalığı olanlarda ve yaşlı hastalarda bu daha da önem arz etmektedir. BioFire® FilmArray® sistemi nested multiplex polimeraz zincir reaksiyonu (PCR) yapan bir filmarraydir. Bu amaçla hastanemize akut solunum yolu enfeksiyonu belirtileri ile başvuran hastalardan alınan nazofarengeal sürüntü örnekleri BioFire® FilmArray® Respiratory Panel v1.7 ile değerlendirilmiştir.
Akut solunum yolu enfeksiyonu belirtileri ile hastanemize başvuran 281 hasta çalışmaya alınmıştır. Hastalardan nazofarengeal swab ile alınan örnekler viral taşıma besiyerine konulup (COPAN swab ve Transport medium, Italy) laboratuvara taşınmıştır. Teste başlamadan önce test poşu yükleme istasyonuna yerleştirilmiştir. Test poşu, numune hazırlama, ters transkripsiyon ve iç içe PCR için gerekli tüm reaktifleri içermektedir. Daha sonra hem hidrasyon solüsyonu hem de 300 µl numune, tampon karışımı ile birlikte poşa enjekte edilmiştir. İlk olarak FilmArray, numuneden tüm nükleik asitleri çıkarmakta, saflaştırmakta ve ardından bir multipleks nested PCR gerçekleştirmektedir. Multiplex nested PCR'dan sonra FilmArray yazılımı bir rapor oluşturmaktadır.
Örneklerin 226'sı pediatrik yaş grubundan alınmıştır. Bunu 34 örnekle COVİD-19 servisleri ve14 örnekle Göğüs Hastalıkları servisleri izlemektedir. Çalışmada 281 hastanın 201'inde solunum yolu enfeksiyonuna neden olan patojenler tespit edilmiştir. 33 hastada aynı anda iki etken, 5 hastada aynı anda 3 etken saptanmıştır. Sıklık sırasına göre ayrı ayrı incelendiğinde örneklerin 82'sinde Human Rhinovirus/Enterovirus, 41'inde Influenza B, 32'sinde Respiratory Syncytial Virus, 25'inde Influenza A, 22'sinde Human Metapneumovirus, 21'inde Adenovirus, 7'sinde Coronavirus NL63 tespit edilmiştir. İkişer örnekte Parainfluenza Virus 1, Parainfluenza Virus 4 ve Bordetella pertussis birer örnekte de Coronavirus 229E, Coronavirus HKU1 ve Parainfluenza Virus 3 saptanmıştır.
BioFire® FilmArray® Respiratory Panel v1.7 ile 45 dakikalık test prosedürü ile kolay, hızlı ve güvenilir sonuçlar alınabilmektedir. Hastalara erken ve hızlı müdahele imkanı sunan bu testin özellikle pediatrik yaş grubunda ve diğer riskli hasta gruplarında rutin kullanımını önermekteyiz.

Anahtar Kelimeler: Biofire filmarray, respiratuar sistem enfeksiyonu, hızlı tanı

➢ *ORAL PRESENTATION*

Synthesis And Characterization of Silver and Gold Nanoparticles Using *Olea europaea* Leaf Extract

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Abstract

The synthesis and characterization of Ag and Au nanoparticles from *Olea europaea leaves* collected the Eastern Black Sea region were investigated in this study. 10 g of dried sample was shaken in 100 mL distilled water containing citric acid (0.1 M) for 90 min at room temperature and extracted in a laboratoary microwave device (Milestone, Start S Microwave, USA) at 4 minutes, 90 W and left cooling. And then the filtrate was collected and used for AgNP and AuNP production. Different portions of extract solution was added to aqueous $AgNO_3$ solution (0,5 mM-1 mM) and the mixture was exposed to a household microwave at 90 W for 1–30 min. Similarly, A certain volume of extract was added to HAuCl4 solution (0,5 mM-1 mM) and exposed to microwave radiation.Uv-Visible spectroscopy is one of the widely used techniques for the characterization of nanoparticles. The progress of the reaction was monitored by measuring the absorbance of the solution at regular intervals of time. Absorption spectra were measured on a Shimadzu UVP-1240 spectrophotometer.

Keywords: *Olea europaea* leaf Extract, Microwave Assisted Extraction, AgNP, AuNP, Uv-Visible.

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➢ *ORAL PRESENTATION*

The importance of plant phenolics in the treatment and prevention of diseases

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Abstract

Plants are very important natural materials that contain the active ingredients of many drugs. They are used in the prevention and treatment of many diseases with the phenolic compounds they produce. Today, interest in plants and their phenolic compounds is increasing day by day. Researchers in different fields of science, such as food manufacturers, food processors, pharmacologists, biologists, dieticians, are researching plant phenolics and plants. In this context, the benefits of plant phenolics containing diets rich in fruits and vegetables are supported by in vitro research. Plants rich in phenolics have been reported to have a protective effect against certain cancers, cardiovascular diseases, osteoporosis, type 2 diabetes, pancreatitis, gastrointestinal problems, lung damage and neurodegenerative diseases. In addition, a wide variety of physiological properties of phenolic compounds such as antioxidant, anti-inflammatory, anti-microbial, anti-allergic, thrombotic, anti-atherogenic, cardioprotective and vasodilatory effects have been investigated. In this study, the importance of plant phenolics in the treatment of diseases is emphasized.

Keywords: Diseases, medicinal plants, plant phenolics, therapy.

➢ *ORAL PRESENTATION*

Investigation of Calcium Pyrophosphate Dihydrate Crystallization Kinetics in the presence of Tartaric Acid

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Abstract

Calcium pyrophosphate dihydrate is a calcium salt of phosphoric acid, which is important for clinical studies due to its pathological effect on the human body. It is well known that calcium pyrophosphate crystallizes in the joints and causes a disease called pseudogout or chondrocalcinosis. Calcium pyrophosphate dihydrate deposits, especially in the knees, elbows, and wrists, cause damage to joints. Many studies in the literature reveal the effects of different drugs used to prevent the formation of calcium pyrophosphate dihydrate. Additive usage is one of the most important techniques to improve crystallization processes' physical properties or solve problems encountered in crystallization processes. There are many studies in the literature on the effect of additives on growth and nucleation kinetics, crystal morphology, and agglomeration rate. Therefore, in our study, calcium pyrophosphate dihydrate crystallization was investigated in an MSMPR type crystallizer in the presence of a selected additive. As an additive, tartaric acid, which is taken into our body during our daily diet naturally because of being an acid of fruit origin and therefore not dangerous for human health, was chosen. Tartaric acid is frequently encountered in processed food products as an additive as well. At the end of each experiment, the particle size distribution was measured to examine the crystallization kinetics. The average particle size of the calcium pyrophosphate dihydrate crystals obtained in the presence of tartaric acid increased, and this increase was almost doubled in high concentrations of tartaric acid compared to pure medium. Estimation of calcium pyrophosphate dihydrate crystallization kinetics was effected using McCabe's □L theory, and the crystal growth rate was found to be dependent on the crystal size. ASL, MJ-2, and M-4 models, which are the size-dependent growth models, were tested to determine the kinetic parameters from experimental results. It was statistically determined that the M-4 model is the best model to characterize the system. The results suggest that low-dose daily intake of tartaric acid is important in the daily diet.

Keywords: Calcium pyrophosphate dihydrate, Crystallization kinetics, Size-dependent growth models, Tartaric acid

➢ *ORAL PRESENTATION*

The fate of microplastics in wastewater treatment plant

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Abstract

Plastic materials and their use for different purposes have an important role in modern societies. Microplastics (MP), which are generally defined as plastic particles with a size smaller than 5 mm, are of concern because they can be harmful to aquatic and terrestrial life. Microplastics were classified by size class (>1 mm, >250 mm, >125 mm, >38 mm, >1.5 mm) and shape (fibers / lines, fragments, films, foams, beads and glitter). Wastewater treatment plants (WWTP) are mentioned as a significant point source of microplastics (MPs) in the aquatic environment. WWTP are regarded as the receptors of MPs derived from industry, agricultural, and domestic wastewater. WWTPs, generally being designed to remove organic matter and nutrients, not for MPs. The behaviour and transport of MPs throughout WWTPs showed that the conventional treatment facilities do not remove plastic materials completely from sewage. At the same time, some previous studies have demonstrated that current WWTP's can remove >95% of MPs from raw wastewater. During the treatment of wastewater, a significant proportion of MP particles are removed in the initial stages of primary and secondary treatment. The removal rate of MPs is affected by the characteristics of MPs such as size, shape, and density. The partitioning of MPs through the settlement processes of wastewater treatment results in the majority becoming entrained in the sewage sludge. The number of plastic particles within sludge samples vary across based on different technologies applied in WWTPs.

Keywords: Microplastic, Wastewater treatment, Sluge, Fate, Transport

➢ *ORAL PRESENTATION*

Sülforafan ve kanser ilişkisi

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Özet

Dünya genelindeki en ölümcül hastalıklardan birisi olan kanser, hücrelerin anormal/kontrolsüz çoğalması ve metastazı ile karakterize olan hastalıkların genel adıdır. Dünya Sağlık Örgütü (WHO) 2030 yılında 13,1 milyon insanın kanserden öleceğini tahmin etmektedir. Yaşa veya ırka dayalı önemli bir ayrım olmaksızın dünya çapında kanser ölüm oranlarındaki artış dikkate alındığında, bu kronik hastalığın insidansını azaltmaya yönelik her türlü çalışma dikkat çekmektedir.

Doğal ürünler yüzyıllardır önemli bir ilaç kaynağı olmuştur. Günümüzde kullanılan ilaçların yaklaşık yarısı doğal ürünlerden elde edilmektedir. Epidemiyolojik çalışmaların meta-analizleri genellikle sebze ve meyve tüketiminin kanser insidansı ve ölüm oranı ile ters ilişkili olduğunu işaret etmektedir.

Brassicaceae (*Cruciferaceae*) familyasında, brokoli (*Brassica oleracea* L var *italica*), Brüksel lahanası (*B. oleracea* var *gemmifera*), lahana (*B. oleracea* var *capitata*) ve karnabahar (*B. oleracea* var *botrytis*) gibi tükettiğimiz birçok sebze yer almaktadır. *Brassica* sebzelerin tüketimi, genel sebze tüketimine göre kanserden korunma ile daha güçlü ilişkili içerisindedir. *Brassica* sebzelerinin kanserden korunma ile ilişkili en çok çalışılan biyoaktif bileşikleri arasında glukozinolatlar (GLS) bulunmaktadır. GLS'ler, biyolojik olarak aktif değillerdir. Kemopreventif etki göstermeleri için hidroliz olmaları gerekmektedir. Bu hidroliz reaksiyonu bitkinin hasadı, kesme, doğrama ve çiğneme gibi hücre zarının zarar görmesiyle salınan mirosinaz adlı endojen bir enzim tarafından gerçekleştirilmektedir. GLS'ler izotiyosiyanat (ITC) ya da indol-3 karbinollere hidrolizlenir.

Bir ITC bileşiği olan sülforafanın (SFN) sentezlendiği glukozinolat türü glukorafanindir. Glukorafanin içeriği brokoli ve brokoli filizi başta olmak üzere karnabahar, beyaz ve kara lahanada bulunmaktadır. In vitro ve in vivo birçok çalışma, SFN'nin kanser gelişiminin birçok adımını etkilediğini bildirmiştir. SFN, faz I enzimlerini baskılanması ve faz II enzimlerini uyarmasıyla kanseri başlangıç aşamasında bloke edebilmektedir. Kanserli hücrelerde ise apoptoz, hücre proliferasyonu ve kanserin daha spesifik aşamalarında yer alan anjiyogenez gibi olayları etkileyebilmektedir.

SFN ile indüklenen apoptoz mekanizmasında insan kolon kanseri hücrelerinde Bax'ın yukarı ve Bcl-2'nin aşağı regülasyonu, prostat kanseri hücrelerinde kaspaz-8 ve kaspaz-9'un aktivasyonu, pankreas kanseri hücrelerinde ise kaspaz-8 ve kaspaz-3'ün aktivasyonu gözlenmiştir.

Anahtar Kelimeler: Sülforafan, *brassicaceae*, glukozinolat, anti-kanser, sitotoksisite, apoptoz

➢ *ORAL PRESENTATION*

Prevention of Fungal Diseases Causing Internal Rots In Pomegranate Fruits By Using Sustainable Methods And Prolonging The Storage Period

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Abstract

In this study, it was aimed to eradicate the fungal pathogen, which is contaminated from the calyx (flower nose) surface before harvesting and causes fruit internal rot during storage, or to prevent its growth by using sustainable methods before storage, and prolonging the storage period in pomegranate (Punica granatum) fruit. For this purpose, four physical methods including flame retardancy, calyx surface (70% alcohol) disinfection, mineral salt application and ultraviolet irradiation, four food preservatives including lactic acid, acetic acid, boric acid and benzoic acid were applied. Nine essential oils were applied, including thyme oil, black seed oil, garlic oil, rosemary oil, myrtle tree oil, eucalyptus tree oil, centaury oil, juniper tree oil and chamomile oil. After application to pomegranates, they were kept in cold storage at 6 ° C on 26.10.2019. First checks on 26.12.2020, second checks on 26.02.2020, third and last checks were made in order to conrol the presence of fungal growth on 26.04.2020. Fungal isolation was made from pomegranates with fungal rot detection and fungal diagnosis was made from the isolates obtained. The flame application among the physical methods was the most effective with 83.33% durability rate. This was foolowed by mineral salt and Calyx surface (70% alcohol) disinfection application with 66.67% fruith durability. The most fruit rot (50%) was observed in the ultraviolet light application method. *Alternaria alternata* was the most common fungal agent isolated from pomagranates expose to physical methods. Lactic acid application methods among the food preservatives, were the most effective with 77.7% durability rate. The most fruit decay was observed in boric and benzoic acid applications with 55.5%. The most common fungal pathogen in this application was *Coniella granati*. In essential oil applications, the highest fruit durability with 77.3% was obtained in thyme essential oil application. The highest rate of fruit decay was detected in rosemary essential oil applications with a rate of 83.33. In this study, Aspergillus niger and Penicillium spp. has been the most isolated fungal pathogens.

Keywords: Hicaze pomegranate, patogen, fruit rot, antifungal, food preservative.

➢ *ORAL PRESENTATION*

A bioactive diterpene in Coffee: Cafestol

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Abstract

Coffee contains various bioactive substances that can be included alkaloids such as caffeine and trigonelline, phenolic acids, diterpenes such as cafestol, lignans, flavonoids. Coffee has two diterpenoids named cafestol and kahweol which involve in increasing cholesterol, but only caffeine in coffee has pharmacological effects. The amount of cafestol and kahweol in coffee depends on the method of brewing. The most of amount these substances release when was contacted boiled water. Soluble dietary fiber contents of brewed coffee are significant. Surveys have shown dose-dependent effect of cafestol on elevating serum cholesterol levels. Boiled coffee contains the highest concentrations such as Scandinavian-style and Turkish-style while, trace amounts see in instant, drip-filtered and percolated coffees. Nowadays, scientists report they have identified this compound that contribute to human health benefit. Researchers say that this knowledge could someday help them develop new medications to better prevent and treat the disease.

Keywords: Coffee, Cafestol, Bioactivite, Health.

➢ *ORAL PRESENTATION*

The New Trend in Food Technology: The Using Fields and the Processing of *Citrus* Wastes

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Abstract

Citrus, known for its beneficial effects on health for centuries, belongs to the genus citrus of the family Rutaceae. Different citrus fruits have different characteristics in terms of type, variety, quality and degree of maturity. Citrus wastes contain soluble sugar, starch, fiber including cellulose, hemicellulose, lignin and pectin, ash, fat and protein and some bioactive compounds. As a result of the processing of citrus wastes, some citrus by-products that can be used in pharmaceutical, nutraceutical, food, health drinks and cosmetic fields occurs. These citrus by-products are supposed to be economical and renewable resource. Thus, the cost of the formulated products is reduced and the use of synthetic agents is limited along with keeping the environment from dangerous of pollution. Also, these natural bioactive compounds, which are believed to significantly protect people from many diseases, are effectively used in the medicinal as well as therapeutic formulations. Green consumerism, the use of friendly compounds such as citrus extract for food preservation, is the trend in food technology. Extracts from citrus have been successfully used in some food products to limit contamination, and to prevent yeast spoilage. Biocitro, citrus extract, would be added to some foods as a natural preservative. Developing novel techniques to explore various applications of the chemicals derived from the citrus wastes is the main searching topic on recent investigations.

Keywords: *Citrus*, Citrus waste, Food technology.

➢ *ORAL PRESENTATION*

Diversity of Wild Medicinal Mushrooms in Turkey

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Abstract

Mushrooms are very important natural source of foods and medicines. More than 600 mushroom species are used for food or medicine due to their pharmacologically active substances and essential nutrients. Medicinal mushroms are those which produce medically active compounds and they have been used as medicine since ancient time. Because of its suitable habitats, vegetation and climate for fungal growth, Turkey is very rich in terms of mushrooms diversity. The knowledge on the studies on mushroom diversity of Turkey is based on a study period of more than 100 years. The number of these studies has increased significantly over the last two decades and about 2300 mushroom species have so far determinated from Turkey. This study is based on the published literature on Turkish wild medicinal mushrooms between 1915 and 2020. 155 medicinal mushroom species which were reported from Turkey are listed. The systematic classification of the species is performed and they are given in alphabetical order alongwith the distribution throughout to country. A total of 155 mushroom species that produce medically significant metabolites have so far been reported from Turkey. 7 of them belongs *Ascomycota* and 148 to *Basidiomycota*. Short notes, distributions, morphological and ecological features of Turkish medicinal mushrooms are presented and discussed briefly.

Keywords: Medicinal mushrooms, biodiversity, Turkey.

➢ *ORAL PRESENTATION*

Antioxidant, antibacterial and antifungal potentials of ethanolic extracts of *Marrubium globosum*

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Abstract

Medicinal plants have been used in many fields from past to present. It has been used in different communities for different purposes such as shelter, heating, food and medicine. Today, many researchers have investigated the pharmacological effects of different plant species. It has been reported as a result of research that they have many biological activities. In this study, the total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), antibacterial and antifungal activities of the ethanol extract of the aerial parts of *Marrubium globosum* Montbret & Aucher ex Benth. were determined. The aerial parts of the plant are powdered. Powder samples were extracted with ethanol in Soxhlet apparatus. TAS, TOS and OSI values of the extracts were determined using Rel assay kits. Antibacterial and antifungal activities were determined by the agar dilution method. As a result of the studies, TAS value of plant extract was determined as 7.677±0.231 mmol/L, TOS value was 12.387±0.083 μmol/L and OSI value was 0.162±0.004. Plant extract was effective against bacterial strains at 50-200 μg/mL concentrations. In addition, the extract was effective against fungus strains at 100-200 μg/mL concentrations. As a result, it has been determined that the aerial parts of *M. globosum* have antioxidant, antibacterial and antifungal potentials.

Keywords: Antioxidant, Antibacterial, Antifungal, Medicinal Plants, Oxidant.

➢ *ORAL PRESENTATION*

Antioxidant activity and Element contents of wild mushroom *Tylopilus felleus* (Boletales)

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Abstract

Mushrooms have been used for different purposes in human history. It has been used in food, medicine, or religious rituals in different communities. In recent studies, it has been reported that mushrooms are important natural resources. In this study, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and element contents of wild mushroom *Tylopilus felleus* (Bull.) P. Karst. were determined. Wild mushroom *T. felleus* samples were collected from Ilgaz Mountain National Park (Kastamonu/Turkey). Dried samples of *T. felleus* mushrooms were extracted with ethanol in soxhlet apparatus. TAS, TOS and OSI values were measured using Rel Assay kits. Element contents were determined using atomic absorption spectrometry. Fe, Zn, Cu, Pb and Ni of mushrooms were determined as 119.57±12.78, 21.07±1.69, 44.10±2.07, 2.89±0.47 and 0.82±0.011, respectively. In addition, TAS, TOS and OSI values were calculated as 3.427±0.147, 20.512±0.266 and 0.601±0.031, respectively. As a result, it was determined that *T. felleus* mushroom has antioxidant potential.

Keywords: Antioxidant, Element contents, Medicinal mushroom, Oxidative stress,

➢ *ORAL PRESENTATION*

Antioxidant and Antimicrobial activities of wild mushroom *Entoloma sinuatum* (Agaricales)

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Abstract

Mushrooms have an important place in human life since ancient times. It has different properties. They play a role in the decay of organic cover in the ecosystem. In addition, edible mushrooms stand out with their nutritious properties. In addition, medicinal mushrooms are used in the prevention and treatment of diseases. Recent research has shown that mushrooms have antioxidant and antimicrobial potential. In this study, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and antimicrobial activities of *Entoloma sinuatum* (Bull.) P. Kumm. mushroom was determined. *E. sinuatum* samples were collected from Belgrad forest (İstanbul/Turkey). Samples were extracted with ethanol in a soxhlet apparatus after drying. TAS, TOS and OSI values were measured using Rel Assay kits. The antimicrobial activities of the extract were tested against bacteria and fungus strains by the agar dilution method. TAS, TOS and OSI values were found to be 2.641±0.152, 6.582±0.226 and 0.251±0.015, respectively. In addition, ethanol extract of mushroom was effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 400 µg/mL concentrations, *Enterococcus faecalis* and *Escherichia coli* at 200 µg/mL concentrations and against *Candida albicans* and *C. tropicalis* at 50 µg/mL concentrations. As a result, it was determined that *E. sinuatum* has antioxidant and antimicrobial potential.

Keywords: Antimicrobial, Antioxidant, Medicinal mushroom, Oxidant

➢ *ORAL PRESENTATION*

Research on the Hover flies (Diptera: Syrphidae) fauna of Adıyaman province

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** This study was producted from Msc thesis of first author

Abstract

Hoverflies are often big and attractively coloured flies and many species mimic bees and wasps. Adults are commonly found on flowers and are important pollinators. The larvae of some 40% of the species are zoophagous, mostly feeding on aphids, which makes them especially valuable for agro-ecosystems (Ssymank et al., 2008). There are more than 6200 species of Syrphidae all over the world in about 300 genera, and 3-4 not yet consensual subfamilies (Young et al., 2016). According to Sarıbıyık (2014), there are 314 species under 73 genera belonging to Syrphidae family in Turkey. This study was based on adult hover fly specimens which were collected from Adıyaman province between 2019 and 2020. Specimens were collected from different locations of Adıyaman province by using insect net. Obtained specimens were killed in ethyl acetate killing jars and were transferred to dry storage. Adult specimens obtained from the field were brought to the entomology laboratory and were stretched in accordance with the standard museum methods and kept in the Entomology Laboratory of Gaziantep University Biology Department for diagnosis. Specimens were identified by examining their morphological characters under a stereo microscope, according to literatures. As a result, 10 genera and 11 species (*Chrysotoxum intermedium, Episyrphus balteatus, Eristalinus taeniops, Eristalis arbustorum, E. tenax, Helophilus hybridus, Melanostoma mellinum, Metasyrphus corollae, Sphaerophoria scripta, Syritta pipiens, Xanthogramma pedissequum*) belonging to three subfamilies were identified from Adıyaman province. In the study, adult, head and abdomen photographs were presented for each species. Also, zoogeographic distributions of the species were given. All the specimens were kept in Entomology Museum of Biology Department, Gaziantep University after storage conditions were provided.

Keywords: Hover flies, Syrphidae, Fauna, Adıyaman**.**

➢ *ORAL PRESENTATION*

Çekirdeklik kabak çeşitlerinin farklı gelişme dönemlerindeki yağ özellikleri ile soğuk pres yöntemiyle elde edilen yağların kalitelerinin belirlenmesi

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Bu yayın Gizem Çağla Dülger'in doktora tezinden üretilmiştir.

Özet

Bu araştırma, Edirne koşullarında, 2014 ve 2015 yıllarında yetiştirilen Palancı, VD1sn8 ve VD1sn6 genotipleri ile Nusem ticari hibrit çeşit olmak üzere dört farklı çekirdek kabaklarının, çekirdek oluşumundan son hasat zamanına kadar üç farklı dönemde; yağ verimi, yağ asidi bileşimi, tokoferol ve sterol kompozisyonlarının değişiminin belirlenmesi amacıyla yapılmıştır. Ayrıca son hasat döneminde elde edilen kabak çekirdeklerinden soğuk pres yöntemi ile elde edilen yağların bazı kalite özellikleri de incelenmiştir. Olgunlaşma periyodu boyunca kabak çekirdeklerinin yağ verimlerinin arttığı, nem oranlarının azaldığı tespit edilmiştir. VD1sn8 genotipine ait soğuk pres kabak çekirdeği yağlarının; yağ miktarı, yağ asidi bileşimi, tokoferol ve sterol miktarları ile renk değerleri açısından diğer çeşitlerden farklı olduğu tespit edilmiştir. Kabak çekirdeği çeşitlerinin ortalama kurumaddede yağ oranları %37,21-42,07; yağ asidi bileşiminde oleik asit oranları %39,59-46,26; linoleik asit oranları %33,73-40,14, palmitik asit oranları %11,04-13,49, stearik asit oranları %5,61-6,23 arasındaki değerlerde bulunmuştur. Kabak çekirdeği çeşitlerinin toplam tokoferol miktarları 678,68-1212,25 mg/kg; toplam sterol miktarları 3058,2-3928,9 mg/kg; toplam fenolik madde miktarı ise 291,5-508 mgGAE/kg olarak tespit edilmiştir. Soğuk pres kabak çekirdeği yağlarının antioksidan aktiviteleri, DPPH yöntemiyle; 0,27-0,42 µmol Troloks/g yağ; ABTS+ yöntemiyle; 0,08-0,17 µmol Troloks/g yağ olarak tespit edilmiştir. Soğuk pres kabak çekirdeği yağlarının S. aureus, S. enteritidis, E. coli ve A. parasiticus NRRL 2999 test mikroorganizmaları üzerine etkili olduğu ancak L. monocytogenes ve A. parasiticus NRRL 465 üzerine etkilerinin ise olmadığı tespit edilmiştir.

Anahtar Kelimeler: kabak çekirdeği, soğuk pres yağ, tokoferol, sterol, antioksidan aktivite, antimikrobiyal aktivite

➢ *ORAL PRESENTATION*

Aflatoxins: A Serious Health Risk to Human

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Abstract

Aflatoxins, the fungal metabolites produced by some strains of Aspergillus flavus and Aspergillus parasiticus, are one of many natural occurring mycotoxins that are found in soils, foods, humans, and animals. Aflatoxins are found in grains, nuts, dairy products, tea, spices and cocoa as well as animal and fish feeds. As the health consequences of high levels of aflatoxins are considered as a major problem, the international community and many individual countries have set strict limits for acceptable aflatoxin levels. Limited doses are not harmful to humans or animals; however, these limits are often greatly exceeded. Chronic toxicity caused by aflatoxins comprises immunosuppressive and carcinogenic effects and particularly affects the liver. When mycotoxins contaminate foods, they cannot be destroyed by normal cooking processes. However, there are methods such as hazard analysis of critical control points (HACCP) to keep final food products safe and healthy. HACCP is a process control system which promotes food safety from farm to table by reducing biological, chemical and physical agents. In this study, we review the Aflatoxin occurrence, prevention and health effects.

Keywords: Aflatoxin, Occurrence, Prevention, Health.

➢ *ORAL PRESENTATION*

The Importance of Dietary Plant Phenolic Compounds in Nutrition and Health

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Abstract

Phenolic compounds with antioxidant properties are secondary metabolites found in large amounts in plants. Fruits, vegetables, cereals, dry legumes, and beverages like coffee and tea are the major sources of phenolic compounds which are essential parts of human diet. Due to their antioxidant activities, the numbers of phenolic compounds found in natural foods can prevent many health disorders and reduce the risk. Investigations have strongly suggested that long-term consumption of plant foods provides protection against diseases associated with oxidative stress, such as cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. In this study, we review the substantial information on the origin, mode of action and health benefits of dietary plant secondary metabolites owing to their antioxidant properties.

Keywords: Antioxidants, Dietary plants, Health, Nutrition, Phenolic compounds

➢ *ORAL PRESENTATION*

Quality Control and Analysis of Herbal Medicinal Products

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Abstract

Nature has been one of the main resources for maintaining health and treating diseases since the first man. Despite such an old history, in recent years there has been an increasing trend towards natural healing products, including phytotherapy. However, this approach to natural products shows a great change according to the results of scientific research. The most important of these change expectations is the standardization of products through their effective components. This is particularly important in terms of observing a reproducible biological response. On the other hand, determining the limit values of these toxins with analytical techniques in products is extremely important in terms of the quality and reliability of traditional herbal medicinal products in order to reduce possible health risks due to contamination such as microbiological, industrial toxins, heavy metals, agriculture and animal drugs. In this context, in the quality control of starting materials, identification tests in pharmacopoeia monographs, purity control, reference substances and determination of solvents and residues in drug preparation, microbiological control, determination of specifications of excipients in dosage form are made. The control of the finished traditional herbal medicinal product, which contains the herbal drugs or preparations with the specifications in the pharmacopoeia monographs as starting material and produced according to the GMP rules, is complemented by physico-chemical analyzes, qualitative and quantitative tests, the standardized and adjusted extract amount in the dosage form and the control of standardized excipients and stability tests. . The licensing of herbal medicinal products that meet these criteria is carried out by the relevant Ministries of Health in the world as in our country. Although side effects are minimally defined, this does not mean that they are completely harmless. For this reason, it is an important factor that the practitioners are physicians and that the products are given under the supervision of pharmacies and pharmacists who are competent in their field.

Anahtar Kelimeler: Herbal medicinal product, standardization, chromatographic analysis, quality control, stability, phytovigilance

2.2. FULL-TEXT PAPERS

➢ *ORAL PRESENTATION*

Solid Polymer Electrolyte: A Review

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Abstract

Polymer electrolyte has attracted great interest for the next generation of electrochemical devices such as batteries, superconductor and dye sensitized solar cell due to high density and more safety. In this review, we summarized the fundamental properties, material and preparation techniques of different type polymer electrolyte including gel polymer electrolyte, composite polymer electrolyte and solid polymer electrolyte. Due to the low conductivity problem, we also reviewed methods to increase the conductivity of polymer electrolyte. Those including doped with single salt, double salt system and ionic liquid.

Keywords: Polymer electrolyte; conductivity; salt; ionic liquid

Introduction

Overview of electrolyte

Generally electrolyte consist of salt and solvent. It is a vital part in electrochemical devices because it provides ionic conductivity and hence provides charge to each electrodes in the cells (Cheng Zhong, Yida Deng, Wenbin Hu, Jinli Qiao 2015). Basically, batteries are made from anode, cathode and electrolyte. The electrolyte can then be described as inert material in the batteries, hence it is compulsory to demonstrate the stability on the electrode surface (Lin et al. 2017). There are several nature of electrolyte which are :

i) the concentration of the ion and solvent;
ii) the ion species and its size;
iii) the interaction among ion and solvent;
iv) the interaction between the electrolyte and electrode;
v) the potential window.

There are several types of electrolyte as reported in literature. **Figure 1** shows the classes of electrolyte. As shown in the figure, electrolyte are mainly categorized as liquid electrolyte, solid electrolyte and quasi solid state electrolyte. Liquid electrolyte can be classed into aqueous and non-aqueous electrolyte whereas for solid or quasi solid state electrolyte, it can be divided into two main classes which are organic and inorganic electrolyte (Cheng Zhong, Yida Deng, Wenbin Hu, Jinli Qiao 2015).

Polymer Electrolyte

Polymer can be divided into two major type which is synthetic and natural polymer (Singh et al. 2013). Polymer electrolyte are defined as solid ionic conductor formed by the dissolution of salts in suitable high molecular weight polymer (Vincent, 1987).In the past three decades, a wide number of different classes of polymer electrolytes (PEs) have been proposed for implementation in a variety of applications ranging from energy-conversion devices (e.g., secondary batteries, fuel cells, dye sensitized solar cells), to super capacitors, sensors, and actuators.

In general, PEs should allow a facile and selective migration of only the desired ions; the best PEs show a high ionic conductivity and transference numbers close to 1. These characteristic, together with an enhanced chemical and electrochemical stability and adequate mechanical properties, could lead to the development of secondary

lithium batteries capable of an improved efficiency and cyclability with respect to the current state of the art (Bertasi, 2015).

The first fabrication of solid electrical conductor polymer/salt complex was reported by Wright in 1975, utilizing poly (ethylene oxide) (PEO) as a host polymer and the semi crystalline structure of its complexes (Vito et al., 2011). From the practical standpoint, PEO itself is not an ideal electrolyte, thus proper manipulations was needed to prevent its crystallization and to extent the elastomeric phase which is favourable to high ionic conductivity. Herewith, the first effort to tailor the insulating property of PEO was done by incorporating with two different types of ionic salt namely, sodium and potassium thiocyanates in order to develop a high conducting PEs (Shanmukaraj et al. 2020).

Moreover, since its development in the 70s, PEs application and role in industry has widely grown. The role of the PEs in these applications generally is to (Vito et al., 2011):

(i) separate two electrodes;
(ii) provide good electronic insulation; and
(iii) allow a fast and selective transport of the desired ions.

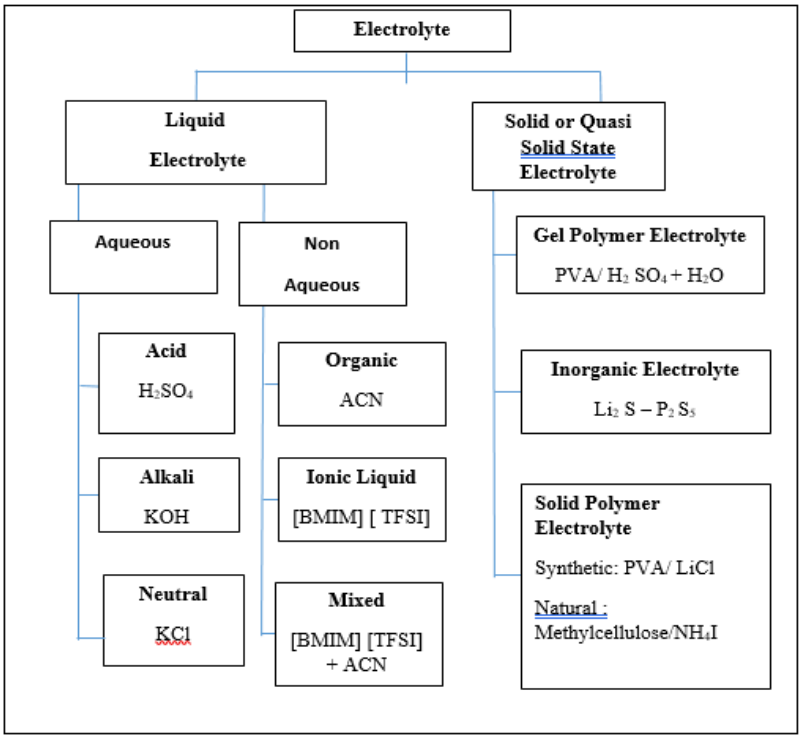

Figure 1: Classification of electrolytes for electrochemical devices (Cheng et al., 2015).

Today, the label "polymer electrolytes" refers to any macromolecular or supramolecular nano-aggregate systems characterized by a significant ionic conductivity, usually higher than approximately 10^{-7} Scm^{-1} (Wright 1998).

The choice of PEs used in modern applications, including high energy density batteries, electrochromic devices, fuel cells and sensors, has been proved by learning their morphological, structural and electrical properties(S. B. Aziz and Abidin 2014). On the other hand, the choice of polymer hosts for PEs largely depends on two factors:

(i) the existence of polar (functional) groups with large power of sufficient electron donor to form coordination with cations; and
(ii) a low hindrance to bond rotation (S. B. Aziz and Abidin 2013).

Polymer electrolytes can be divided into few different types; normally solid polymer electrolytes (SPEs), gel polymer electrolytes (GPEs) and composite polymer electrolytes (CPEs) (Zhou et al. 2019)

Gel Polymer Electrolyte

The development of Gel polymer electrolyte (GPEs) systems usually consist of two steps. Firstly, a salt is doped into a polar or ionic liquid to produce an ion-conducting solution and then, an inert polymeric solution is added to donate an acceptable mechanical stability (Di Noto et al. 2011). GPEs have attracted particular attention due to several advantages such as their low volatility, safety and high thermal stability. Commonly, Poly(vinylidenefluoride) (PVdF) is used as the polymer host in gel polymer electrolytes, because the it consist of strongly electron-withdrawing functional group (eCeF). Additionally, PVdF-based polymer electrolytes can be highly anodic stable. According to Stephan and Nahm (Manuel Stephan et al. 2006), PVdF has obtained a dielectric constant of 8.4, which assists for greater ionization number of salt and thus provides higher in number of charge carriers. Besides that, the chemically unstable of fluorinated polymers leading to poor interfacial behaviour with lithium metal anode. Hence it increased its safety purposes because of the reaction between lithium and fluorine results in the formation of LiF owing to poor safety, hazards ultimately.

From literature, researchers have direct their attention to study the poly(vinylidene fluoride-hexa fluoropropylene) (PVdF-HFP) (88:12) copolymer This copolymer obtained an amorphous phase of hexa fluoroporpylene (HFP) which assist to provide space and entrapped the large amount of liquid electrolytes while the PVdF crystalline phase provide a mechanical support for the polymer matrix. Further, studies on PVdF-HFP gel electrolytes are progressing (Saikia and Kumar 2004).

Nevertheless, all gel polymer hosts lose their mechanical strength when they are plasticized. Also, the gain in ionic conductivity is accompanied by a loss of mechanical strength adversely and also drives to poor compatibility with the lithium electrodes and this high reactivity of lithium metal results in serious problems in terms of battery cyclability and eventually safety. In a way to remain the mechanical properties of polymer gel electrolytes, the gel films have to be hardened either by chemical or by physical curing (high energy radiation) and this results in high processing costs.

Composite Polymer Electrolyte

Composite polymer electrolyte (CPEs) is a subcategory of polymer electrolytes with an idea of incorporating electrochemically inert fillers into polymer matrices. Generally, particulate fillers such as zirqonium oxide (ZrO_2), titanium oxide (TiO_2), and aluminium oxide ($Al_2 O_3$) have high surface area and hydrophobic fumed silica were incorporated into the polymer matrices (Manuel Stephan et al. 2006).

In addition, the function of the doped composite material in electrolyte system is to improve the electrochemical and morphological properties of polymer electrolytes. It has been well known that the addition of ceramic material such as fillers can improves their interfacial properties in contact with the lithium electrode and enhanced the conductivity of polymer hosts. This increases the ionic conductivity which can be explained by the enhanced degree of amorphocity of the polymer chain or hindered recrystallization (Appetecchi et al., 2000).

There are two classes of the ceramic fillers for polymer electrolyte which are active and passive. The active materials classes are participated in conduction process e.g. Li_2N, $LiAl_2O_3$ while the inactive classes of materials do not involve in the lithium transport process such as Al_2O_3, SiO_2, MgO . The selection of fillers between active and passive components is quite subjective (Stephen and Nahm, 2006).

Solid Polymer Electrolyte

Solid polymer electrolyte (SPEs) can be produced by dissolving inorganic salts in functional (polar) polymer.The interactions of polar groups of polymers with metal ions from salt are mainly resulting from electrostatic forces and accordingly the formation of coordinating bonds(S. B. Aziz et al. 2018) There are some important factors that may have effect on the polymer-metal ion interactions, such as chemical properties of the functional groups attached to the polymer backbone, distance and composition between functional groups, degree of branching, molecular weight, chemical properties and charge of metal cation, and counter ions). The cations are able to move from one coordinated site to another when subjected to an electric field. This is due to the weak coordinate of the

cations to sites along the polymer chain(S. B. Aziz et al. 2018) (Shujaheedan et al., 2018). SPEs had been used over liquid polymer electrolytes because it possesses a few advantages (Kuila et al., 2007):

(i) flexible;
(ii) compact;
(iii) laminated solid-state structures;
(iv) no leakage;
(v) low discharge in batteries;
(vi) relax elasticity under stress conditions;
(vii) available in different geometries; and
(viii) easy processing.

3.0 Ways To Improve Conductivity of Polymer Electrolyte

Solid polymer electrolyte (SPE) can overcome the drawbacks of liquid electrolyte such as, free from leakage, rugged fabrication, more stable and have longer shelf life. Nonetheless, the disadvantages of SPE is that it has low ionic conductivity at ambient temperature (Rosli, 2010). Since conductivity is the main focus for polymer electrolytes, many researchers have conducted the studies to improve its conductivity. There are a few examples of polymer elctrolytes being doped with salts in order to improve its conductivity. **Table 1** shows the previous work of polymer salt system.

Table 1: Polymer electrolyte doped with various salt

Electrolyte	Conductivity	References
chitosan - NH_4NO_3	2.53 x 10^{-5} Scm^{-1}	(Majid and Arof 2005)
chitosan - $NH_4CF_3SO_3$	8.91 × 10^{-7} S cm^{-1}	(Khiar, Puteh, and Arof 2006)
methylcellulose – $NH_4CF_3SO_3$	1.14 x 10^{-4} Scm^{-1}	(S. Misenan et al. 2018)
chitosan – PVA- NH_4I	3.73×10^{-7} S cm^{-1}	(Buraidah and Arof 2011)

According to Li et al.,mixed salt system contains mixed cations and mixed anions exhibits better ionic conductivity than single salt systems as the addition of second component will hinder the formation of aggregates and clusters, thus increasing the mobility of the ionic carriers. The mixed-salt systems were found to have larger amorphous phase content than either of the single-salt systems and more potential charge carriers. The mixing of salts suppresses the crystalline nature in the polymer matrix and contributes to a dramatic effect upon the lithium motion and micro viscosity of the amorphous phase (Li et al. 2018). **Table 2.** shows the previous report of polymer electrolyte with double salt system.

Table 2.: The previous research of polymer electrolyte with double salt system.

Electrolyte	Conductivity	References
PVA – K^+I^- –$Bu_4N^+I^-$	0.13 x 10^{-1} Scm^{-1}	(M. F. Aziz et al. 2013)
P(VP-co-VAc) – KI- TPAI	1.90 × 10^{-3} S cm^{-1}	(Ming, Ramesh, and Ramesh 2016)
PEGDA – LiTFSI - LiBOB	1.0 ×10^{-3} S cm^{-1}	(Li et al. 2018)

Another way to improve conductivity of polymer electrolyte is by doping with ionic liquid (ILs). ILs has good ionic conductivity, low volatility and also wide electrochemical potential window. They can act as plasticizer and ionic conductor in polymer electrolyte. ILs can be divided in two different type which are, protic ILs and aprotic ILs. Protic ILs is a group of ILs that contain protonated species, thus they become proton conducive while the aprotic ILs is vice versa. Most ILs are in aprotic class, thus they are not proton conducive (Ketabi, Decker, and Lian 2016).

Ionic liquids have been receiving increased attention in various disciplinary areas due to their unique physicochemical properties, such as high thermal stability, negligible vapor pressure, non-flammability, relatively

high ionic conductivity, and good electrochemical stability. It is salt that has the melting point below 100 °C thus it is in molten state at room temperature (Chaurasia, Singh, and Chandra 2011).

The addition of ILs into polymer matrix can increased the ionic conductivity due to high ionic concentration in the SPE membrane. A study conducted by doping chitosan with 14 different anion of 1-ethyl-3-methylimidazolium cation ([C2mim]+) ionic liquid have shown that the anion in the ionic liquid can affecting on the thermal, morphological, and electrochemical properties of the SPE (Leones et al. 2017). Juan et al., 2014 has studied different cation of trifluorosulfonly imide (TFSI) ionic liquid in gel polymer electrolyte. The three different cations were pyridine, imadazolium and phosphonium based. From the ionic conductivity, voltammetry and impedance data, it showed that electrical properties of gel polymer electrolyte relies on the species of ILs cation (Tafur and Fernández Romero 2014). **Table 3** shows the several pervious study of doped ionic liquid in the polymer electrolyte system.

Table 3: Shows the previous work of polymer electrolyte – ionic liquid system.

Electrolyte	Conductivity	References
PEMA/PVdF–HFP–LiTf BMIMTF	4.86 x 10^{-5} S cm^{-1}	(Sim, Yahya, and Arof 2016)
PGMAdoped -bmimTFSI	1.86 x 10^{-6} S cm^{-1}	(Radzir et al. 2015)
Chitosan-glycerol –C_2MIMSCN	1.61 x 10^{-3} Scm^{-1}	(Leones et al. 2017)
Chitosan-starch-$BMIMNO_3$	2.26 x 10^{-4} Scm^{-1}	(Shaffie et al. 2019)
Chitosan-methylcellulose-BMIMTFSI	1.51x 10^{-6} Scm^{-1}	(M. S. M. Misenan, Shaffie, and Khiar 2018)

Another method to increase the conductivity of polymer electrolyte is by plasticization. A study conducted by Kadir and coworker (Kadir, Majid, and Arof 2010) showed that plasticized chitosan–PVA blend polymer electrolytes shows the conductivity was significantly enhanced to the order of 10^{-3} Scm^{-1} by addition of NH_4 NO_3 salt and ethylene carbonate as plasticizer. This was attributed to the increase in the number of mobile ions and in the ionic mobility of the ions (Kadir, Majid, and Arof 2010). Moreover, addition of plasctizers to biodegradable blend polymer host structure leads to increase in amorphous content thus increases the conductivity (Yahya and Arof 2004). A high value of dielectric constant of plasticizer could solvate more salts, thus increases the number of free mobile ions that contribute to conductivity of polymer electrolyte (Noor et al., 2011). There are several factors that improve the electrical conductivity of polymer electrolytes upon adding plasticizer which are (Pradhan, Choudhary, and Samantaray 2008).

(i) increasing the amorphous phase content;
(ii) dissociation ion aggregates; and
(iii) lowering the glass transition in solid polymer electrolytes;

One of the examples of plasticizer is glycerol as it improves it conductivity due to presence of hydroxyl groups According to Kadir et al. (2010), by adding plasticizer like ethylene carbonate (EC) which has relatively low viscosity. It will decrease the local viscosity around the charge-transporting ions and increases ionic mobility (Kadir, Majid, and Arof 2010).

Kuila and coworkers (2007) had successfully prepared a PEO composite polymer electrolyte using perovskite type $LaMnO_3$ as nano filler with poly(ethylene glycol) (PEG) as plasticizer. As a result, it is proven that upon adding plasticizer to the polymer electrolyte, the conductivity improved approximately 5 times as compared to pure PEO. A maximum conductivity is achieved at value of 2.60 x 10^{-4} Scm^{-1} with 30 wt% of PEG as plasticizer at room temperature compared to pure PEO with conductivity of 1.05 x 10^{-6} Scm^{-1}(Kuila et al. 2007).

Conclusion

In conclusion, we reviewed the progress of polymer electrolyte including gel polymer electrolyte, composite polymer electrolyte and solid polymer electrolyte. The transformation trend of electrolyte from liquid-state to solid-state with energy density and safety merits as well as smart functionalities. Nonetheless, low ionic

conductivity of polymer electrolyte at ambient temperature. In our opinion, novel polymer electrolyte with high conductivity should be explored including doped with various salt and ionic liquid.

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➢ *ORAL PRESENTATION*

Liquid Chromatographic-Mass Spectrometric Analysis of Diclofenac Based Cu(II) Complex

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Abstract

Coordination compounds have an important place in the structure of living things, pharmacology, biological reactions such as electron transfers and mechanisms as the co-factor of most enzymes. Copper, which has many functions in the human body, also has many benefits. Copper is involved in the work of the central nervous system and the formation of free energy in the body, plays a role in the structure of many enzymes and the synthesis of protein in the body. In its deficiency, anemia, energy and fatigue, weakness of the immune system, iron deficiency, loss of appetite and similar disorders are observed. In our study, mixed ligand copper 2-diclofenac complex, which has very few examples in the literature, was investigated. In this study, new molecules created by replacing the diclofenac based copper (II) complex with axial ligands in different solvents with solvent molecules were studied. UV-VIS and FT-IR measurements of new compounds in different solvents were taken. In addition, mass verification of new compounds formed by liquid chromatographic-mass spectrometric measurements was performed. When the results obtained from the measurements made with different devices are compared, it is seen that the results support each other.

Keywords: Coordination compounds, Diclofenac based Cu(II) complex, Liquid Chromatographic-Mass Spectrometric Analysis

1.Introduction

Coordination compounds are formed as a result of the entanglement of a central atom with a number of neutral or negatively charged atoms or groups, which are called ligands and have electron excess.

There are some important coordination compounds in the structure of living things. The most important biological complex of Fe (II) ions is hemoglobin. Chlorophyll substance, which is found in the structure of plants and gives them green color, is a coordination compound with the central atom magnesium. Coordination compounds also play an important role in pharmacology. Coordinated bonding is valid in the mechanism of action of some drugs.

Copper is a transition metal found in abundance in the human body and plays some roles in human physiology. Copper, which has many functions in the human body, also has many benefits. Copper plays a role in the work of the central nervous system, in the formation of free energy in the body, in the structure of many enzymes and in protein synthesis. In case of copper deficiency in our body; Anemia, fatigue, weakness in the immune system, iron deficiency, loss of appetite and similar disorders are seen.

2.Liquid Chromatography-Mass Spectrometry System

Electrospray ionization-mass spectrometry (ESI-MS) is one of the popular and powerful methods used to identify and determine organic and inorganic ions, especially preferred as a detector for ion chromatography and HPLC. The most characteristic feature of this method is that it has a soft ionization. Therefore, metal-organic complexes can also be detected (1-7).

High selectivity and simultaneous analysis of multiple species are also attractive features of this method. Transition metals as copper, iron and zinc, play a vital role in the metabolism of living organisms. ESI-MS is used because

of the identification of metal complexes in a wide variety of samples, its high selectivity, sensitivity and smooth transition from solution to gas phase. (8-9).

3.Experimental Study

The diclofenac-based copper complex we used in our study, [Cu $(dicl)_2(vim)_2$], was synthesized by Assoc. Dr. Sevim Hamamcı Alisir (10). FT-IR and UV spectra of the synthesized complex were obtained in methanol, ethanol and acetonitrile solvents. Mass confirmations were made using liquid chromatography-mass spectrometry. In Figure 1, the shape and formula of the complex we used in our study are given.

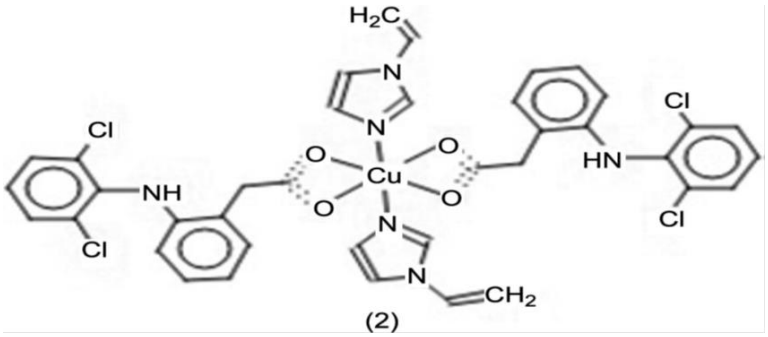

Figure1: Bis{2-[2-(2,6-dichloroanilino)phenyl]acetato-κ 2O,O'}bis(1-vinly-1H-imidazole- κN3) copper(II) ,[$Cu(C_{14}H_{10}Cl_2NO_2)_2(C_5H_6N_2)_2$], [$Cu(dicl)_2(vim)_2$]

For Liquid Chromatography-Tandem Mass Spectrometer analysis, Thermo Scientific brand TSQ Quantum Access Max model LC-MS/MS device was used at Hitit University Scientific Technical Application and Research Center (HÜBTUAM) and analyzes were performed at HÜBTUAM.

UV measurements of the complexes were made with Thermo Scientific Evolution Array UV-VIS Spectrophotometer. FTIR measurements of the complexes were made using the Perkin Elmer Spectrum Two device.

Table 1: Sample Information

Name of the product	Synthesis products		
Product Material	Liquid		
Solvent Program	Minute	% A (Methanol-ethanol-acetonitrile)	
	0	100	
	3	100	
Solvent Flow Rate	0,2 mL/min		
Column oven temperature	25 °C		
Column Features	-		
Injection volume	20 µL		
Analysis Time	3 min		
Standards	Synthesis products		

Table 2: Analysis Test Conditions

MS/MS Analysis Conditions	
Capillary Temperature	300 ºC
Vaporizer Temperature	350 ºC
Sheeth Gas Pressure (Arb)	25
Aux Gas Pressure (Arb)	10
Spray Voltage (Positive Polarity)	4400
Spray Voltage (Negative Polarity)	2500
Discharge Current (Positive Polarity)	4
Discharge Current (NegativePolarity)	4
Full Scan Mode	100-1500 m/z
Ion Mode	Negative/Positive Polarity
Ionization Source	ESI

4. Results and Discussion

In our study, it was investigated whether the synthesized diclofenac-based copper complex, when dissolved in different solvents, replaced the ligands with the solvent. Therefore, the behavior of the complex in methanol, ethanol and acetonitrile was studied. The mass spectrum of the complex synthesized in the study was taken in both positive and negative ESI mode in different solvents. The mass spectrum obtained from the complexes was compared using methanol, ethanol and acetonitrile. In Figures 2, 3 and 4, mass spectra obtained in methanol, ethanol and acetonitrile are given.

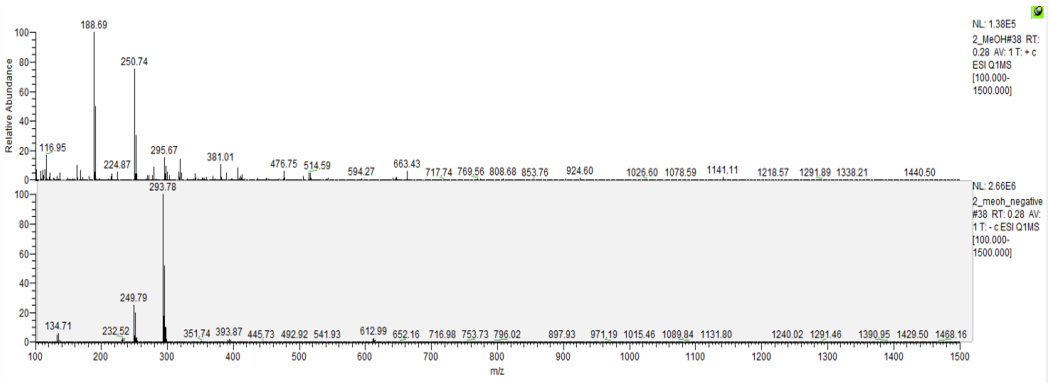

Figure 2. Mass spectrum of the complex of $[Cu(dicl)_2(vim)_2]$ in positive and negative ESI mode in methanol.

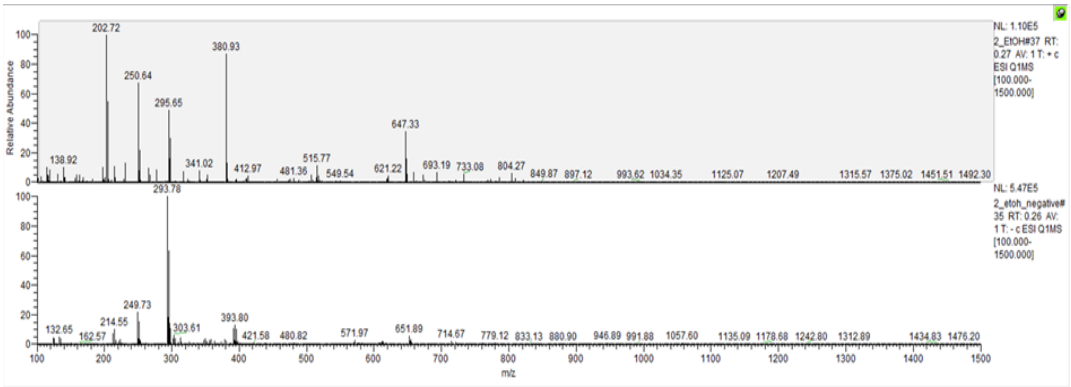

Figure 3: Mass spectrum of the complex of $[Cu(dicl)_2(vim)_2]$ in positive and negative ESI mode in ethanol

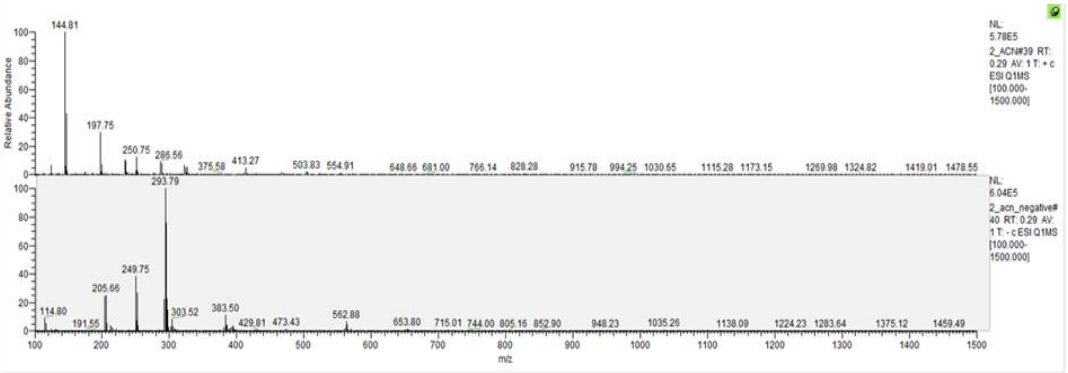

Figure 4: Mass spectrum of the $[Cu(dicl)_2(vim)_2]$ complex in acetonitrile in positive and negative ESI mode.

When the mass spectrum of the synthesized $[Cu(dicl)_2(vim)_2]$ complex obtained in positive and negative ESI mode in methanol is examined, it is seen that the complex does not replace the solvent in either mode. When the mass spectrum obtained in positive and negative ESI mode of the synthesized $[Cu(dicl)_2(vim)_2]$ complex in ethanol is examined, it is seen that three ligands change with the solvent in negative ESI mode. When the mass spectrum obtained in positive and negative ESI mode of the $[Cu(dicl)_2(vim)_2]$ complex in acetonitrile is examined, it is seen that three ligands change with the solvent in negative ESI mode.

The UV spectra obtained in methanol, ethanol and acetonitrile for $[Cu(dicl)_2(vim)_2]$ complex are given in figures 5, 6 and 7.

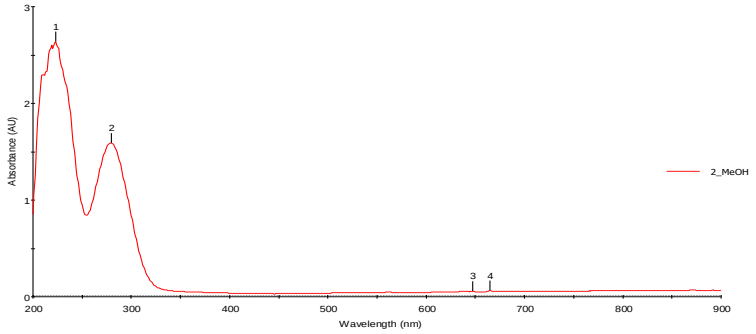

Figure 5: UV spectrum obtained in methanol for $[Cu\ (dicl)_2(vim)_2]$ complex

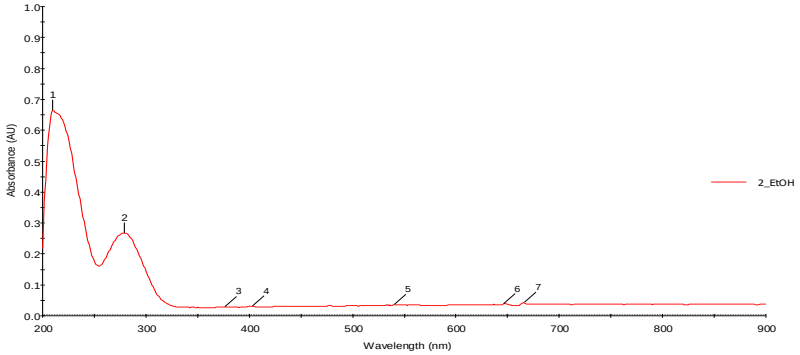

Figure 6: UV spectrum obtained in ethanol for $[Cu\ (dicl)_2(vim)_2]$ complex

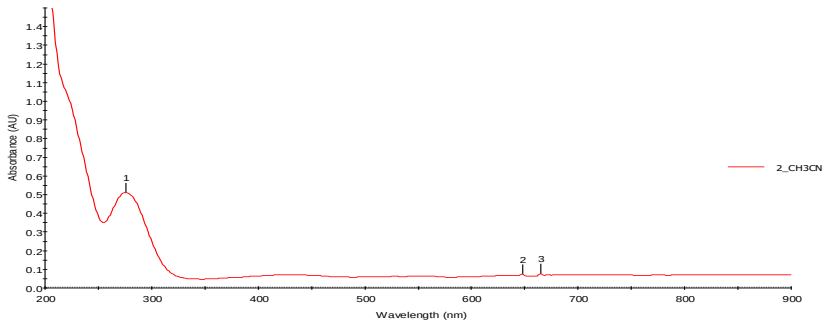

Figure 7: UV spectrum obtained in acetonitrile for [Cu (dicl)$_2$(vim)$_2$] complex

When the UV spectra of [Cu(dicl)$_2$(vim)$_2$] complex obtained in different solvents are examined; peaks are seen at 647.20 nm and 665.20 nm. In copper metal, there were shifts in d-d transitions due to differences in polarity of solvents.

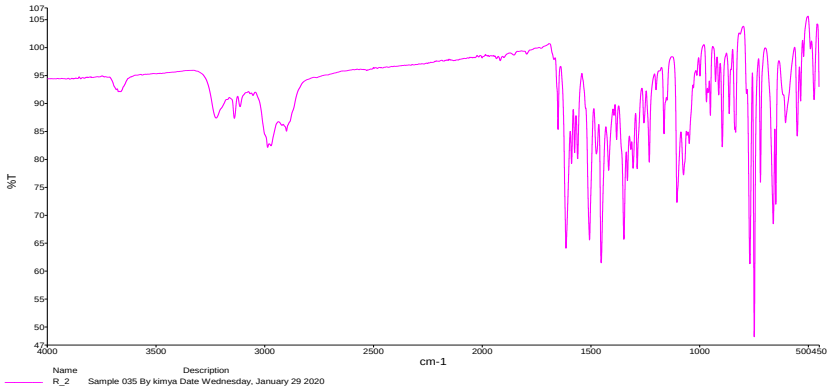

Figure 8: IR spectrum of [Cu(dicl)$_2$(vim)$_2$] complex

When the IR spectrum was examined, medium intensity C-O peak at 1231 cm^{-1}, aromatic C= C peak at 1574 cm^{-1}, aromatic C-H peak at 3140 cm^{-1}, medium intensity C-N peak at 1103 cm^{-1}.When the IR spectrum of the complex was examined, it was seen that the obtained values were compatible with the literature.

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➢ *ORAL PRESENTATION*

Characterisation of biodegradable Zn alloy for temporary implant applications

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Abstract

In this work, biodegradable Zn alloy was manufactured for scaffold and temporary implant applications. Samples with interconnected porous structure were manufactured by powder metallurgy based space holder method. Zinc is an alternative to Mg as a biodegradable metal. Zn can have appropriate biodegradation rate than Mg. Recent studies confirm that the Zn is nontoxic and can provide osseointegration. Zn alloys have low melting temperature, machinability and reactivity. Zn-Mg alloys were manufactured by using Zn, Mg powders. Powder mixtures were ball-milled. Carbamide powders were used as a space holder. Samples were immersed into the water and then space holder was removed. Sintering was done at 400 ºC for 1 hour under argon. Corrosion rates of the samples were lower than the Mg alloys. Biodegradation was investigated by weight loss and metal release measurements. Zn ion release was lower than the upper limit for humans. Alloying was improved the strength of Zn. Osseointegration and biocompatibility properties were improved with Mg. Mechanical properties were investigated by destructive compression tests and non-destructive ultrasonic tests.

Keywords: Zn alloy; Biodegradable implant; Tissue engineering; Powder metallurgy; Temporary implant

1. INTRODUCTION

Porous scaffolds are employed in the tissue engineering. Scaffold provides mechanical support for the seeded cells and determines the shape of the developed tissues. Scaffold allows the transportation of the body fluids by their inter-connected pores and provides tissue growth. Scaffold materials should have an exact and definite biodegradation rate inside the body. Polymer materials have low strength, low wear resistance and also release toxic reagents, while ceramics or bioactive glasses are very brittle. Biodegradable metal-based scaffolds are commonly made from two major classes, Mg-based alloys or Fe-based alloys. But, biodegradation rate of the Mg alloys is very high in the physiological conditions. Mg alloys also produce very high hydrogen gas during the biodegradation in the body fluids. On the other hand, corrosion rates of the Fe-based alloys are low. Hence, it is important to design and develop novel alloys for the scaffold applications (Zhao ve ark., 2016; Seyedraoufin ve Mirdamadi, 2013; Li ve ark., 2014).

As the standard electrode potential of the pure Zn is lower than the pure Fe and higher than the pure Mg, which means Zn is nobler than Mg, Zn-alloy based scaffolds can have more appropriate (lower) biodegradation rate than Mg (Yang ve ark., 2017). Corrosion process of the Zn does not produce hydrogen gas due to the high hydrogen over-potential of the Zn. Zn is important and essential trace element for humans. Daily recommended intake (upper limit) of the elemental Zn is about 40 mg. Recent studies confirm that the Zn alloys are nontoxic and can provide osseointegration (bone formation) and cell adhesion. In addition, Zn alloys have good machinability, low melting point and low oxygen reactivity (suitable for die casting) than the Mg alloys (Vojtech ve ark., 2011; Zhao ve ark., 2017; Bowen ve ark., 2015).

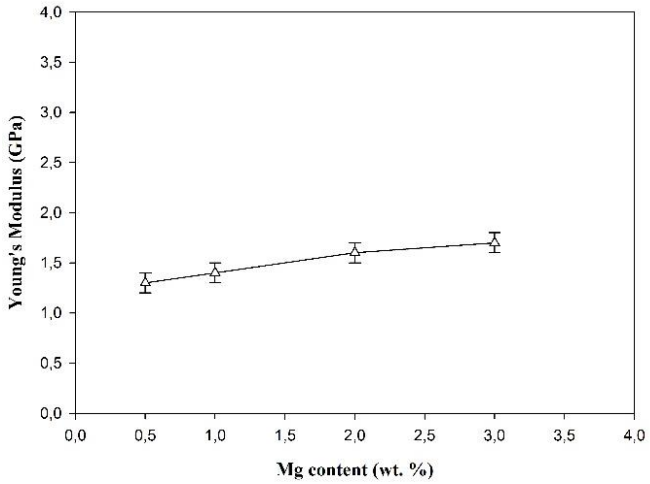
In this study, open-cell biodegradable Zn-(0.5-3%)Mg alloy foam was manufactured for scaffold applications. Disadvantage of Zn is its poor strength. Alloying with Mg improves the strength of Zn. Osseointegration and biocompatibility was improved with the Mg.

2. MATERIALS AND METHOD

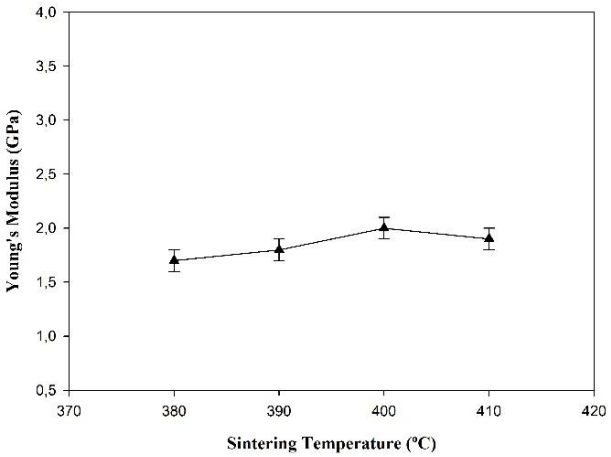
In the experiments, Zn-(0.5-3%)Mg alloys were manufactured by using metal powders with particle sizes of 34 µm. Mg powders were introduced to the Zn powder. At the beginning, powder mixtures were ball-milled with zirconia balls with a rotational speed of 400 rpm. In the powder metallurgy method, carbamide powder in the range of 710-1000 µm was employed (70 %) as a pore former. Mean particle size of carbamide was 860 µm. Mixtures were moulded at 190-200 MPa into cylindrical specimens with diameters of 12-20 mm and heights of 15-17 mm. Carbamide was removed in water and then the samples were sintered at 400-410 ºC. Simulated body fluid (SBF) was prepared from chemicals (Merck) according to the literature. The pH of the SBF was 7.40. Electrochemical corrosion tests were done using a potentiostat (Interface 1000, Gamry). Initially, open-circuit potential (OCP) was conducted for 7200 seconds. Tafel tests were employed to determine the polarization resistances and corrosion rates. The samples were dipped into the SBF for in vitro biodegradation tests. Solution volume to sample surface area ratio was constant. Weight loss values were determined by gravimetric method. After different periods, the samples were removed from the solution. Weight loss was computed.

3. RESULTS AND DISCUSSION

Biodegradable Zn-Mg alloy foams were manufactured for temporary implant applications. Figure 1 shows the effect of a) Mg content, b) sintering temperature on the elastic modulus



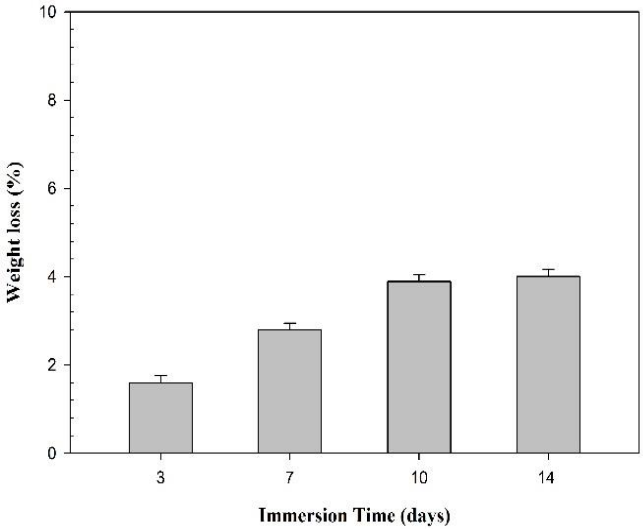
(a)



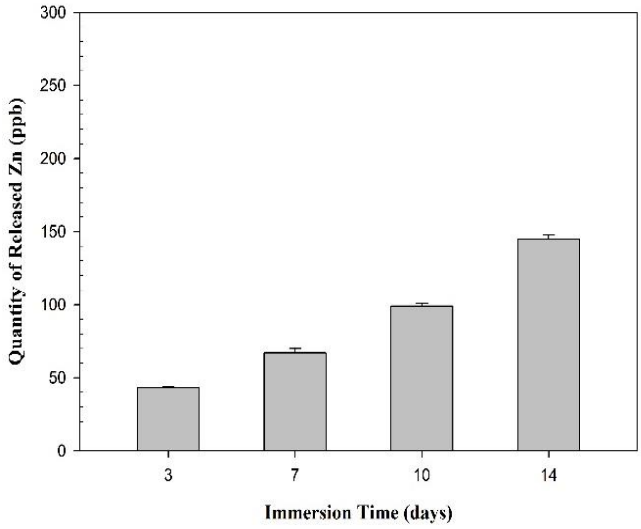
(b)

Figure 1. Effect of a) Mg content, b) sintering temperature on the elastic modulus

Figure 2 shows the effect of the immersion period inside the SBF on the weight loss and Zn ion release. Weight loss was raised with time. The precipitates are consisted of zinc oxyde (ZnO), zinc hydroxyde ($Zn(OH)_2$), and calcium phosphates. Weight loss was about 2-4 %. Metal release amount was lower than the daily upper limit of 40 mg/day for the Zn. Low metal release of the alloy was attributed to the surface oxide (ZnO).



(a)



(b)

Figure 2. Effect of immersion time on the a) weight loss and b) Zn ion release.

4. CONCLUSIONS

In this work, biodegradable Zn-Mg alloy foams were manufactured for scaffold material for tissue engineering. Highly porous samples with interconnected pores were manufactured by powder metallurgy method. Corrosion tests were performed in SBF. The results demonstrated that the corrosion rates of the alloys were lower than the traditional Mg-based scaffold materials. Biodegradation behaviour was investigated. Zn ion release amounts were negligible than the daily recommended upper limit for humans. Osseointegration and biocompatibilities were improved with addition of Mg.

Acknowledgements

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➢ *ORAL PRESENTATION*

Gençlerin Kemik Sağlıklarını Koruma Konusundaki Farkındalıklarının İncelenmesi

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Özet

Sağlıklı yaşam için osteoporozdan korunmak mümkündür. Bunun için genç yaşlarda kemik sağlığı konusunda bilgili olmak ve önlem almak gereklidir. Çalışmada gençlerin kemik sağlığını koruma konusunda farkındalıklarını ortaya çıkarmayı amaçladık. Toplam 200 üniversite öğrencisinin katıldığı çalışmaya 147 (%73,5)' i erkek, 53 (%26,5)'i kız öğrenci katıldı. Yaşları (18-26) arasında değişen, ortalama (20,7±1,78) yaşa sahip katılımcıların süt ve süt ürünü kullanımına ilişkin verdikleri cevaplar (%54,5) evet, (%32) hayır, (%13,5) kararsızım şeklindeydi. Kalsiyum ve D vitamini kullanımı sorgulandığında (%15)'i evet, (%84,5)'u hayır, (%0,5)'i kararsızım cevabını verdi. Düzenli egzersiz yapar mısınız sorusuna (%33,5) evet, (%55,5) hayır, (%11) kararsızım cevapları verildi. Cinsiyetle ezersiz yapma arasında karşılaştırma yapıldığında istatistiksel olarak anlamlı fark bulundu (p=0,004). Erkekler daha fazla egzersiz yapan cinsiyetti. Yeterli güneş ışığına maruziyetleri sorgulandığında ise (%47,5) evet, (%31,5) hayır, (%21) kararsızım cevabı alındı. Çalışma grubunun kemik sağlığını koruma ile ilgili olarak katılımcıların yarısı süt ve süt ürünü tüketmekteydi, düzenli egzersiz yapma, güneş ışığından yararlanma, Ca ve D vitamini kullanma oranları düşük bulundu.

Anahtar Kelimeler: Kemik, koruma, Vitamin D, Kalsiyum, Öğrenci, Sağlık

GİRİŞ

Güncel yaşamda ülkelerdeki endüstrileşmede artış sebebi ile ve düzensiz beslenme, egzersiz yapmama, olumsuz yaşam koşulları, bilinçsizlik gibi sebeplerle bireylerde kemik sağlığı ile ilişkili sorunlar artmaktadır. Bu sorunlar ise tıp alanında farmakolojik ve tıp etiği sorunlarını da artırmaktadır. Bu bağlamda sağlık çalışanları tarafından bireylerde kemik sağlığının korunmasına dönük girişimlere ihtiyaç vardır (Sindel D.2013).

Düzenli egzersiz, düzenli beslenme, yeterli güneş ışınlarına maruziyet, yeterli kalsiyum ve D vitamininin sağlığa faydalı etkileri olduğu geçmişten günümüze tıp alanında gündem konudur. Günümüzde kemik sağlığı açısından önemleri açıktır. Kemik sağlığı tıp alanında tartışılan önemli bir farmakolojik etik sorunlardır (Malone 2008). Hemşireler ve hekimler bireylerin sağlığını koruma, geliştirme ve hastalıkların tedavisine yönelik uygulamalarda güneş ışığından faydalanma ve D vitamini alımı, egzersiz ve süt ürünlerinin alımı konularında tarih boyunca farklı roller üstlenmişlerdir (Dağhan 2016).

Materyal Metot:

Amaç: Çalışmada gençlerin kemik sağlığını koruma konusunda farkındalıklarını ortaya çıkarmayı amaçladık.

Araştırmanın Niteliği: Tanımlayıcı, kesitsel araştırma olarak yapılmıştır.

Araştırma Soruları:

1. Öğrenciler beslenmelerinde süt ve süt ürünlerine yeterince yer veriyor mu?
2. Öğrencilerin düzenli fiziki aktivite alışkanlığı var mı?
3. Öğrenciler yeterli Kalsiyum ve D vitamini desteği alıyor mu ?
4. Öğrenciler yeterince güneş ışığından yararlanıyor mu ?
5. Cinsiyet değişkeni; öğrencilerin fiziksel aktivite, süt ürünü kullanma, Kalsiyum D vitamini alma ve güneş ışığından yaralanma durumunu etkiliyor mu?

Evren Örneklem:

Bu çalışmanın evrenini; Ekim-Aralık 2018 tarihleri arasında anket uygulaması gerçekleştirilen bir Üniversitenin Edebiyat, İngiliz dili ve Uygulamalı Bilimler Fakültelerinde eğitim gören öğrencileri oluşturmaktadır. Çalışmada örneklem seçimine gidilmemiş olup araştırmaya katılmak için gönüllü erkek ve kız cinsiyette toplam 200 öğrenci katılmıştır.

Yöntem:

Üniversitesi öğrencisi gençler çalışma hakkında bilgilendirilerek, "Katılımcı Görüşme İzin Formu"nu imzalayan katılımcılara, anket uygulanmıştır. Anket soruları; evet, hayır kararsızım cevaplarını içeren şıklardan oluşmaktadır.

Verilerin Değerlendirilmesi:

Elde edilen veriler için, SPSS for Windows 21.0 programı kullanılmıştır. Yüzde ve ki kare istatistiksel yöntemlerle analiz edilmiştir. p değeri 0,05'in altında olanlar anlamlı olarak kabul edildi.

Etik Boyut:

T.C. Kütahya Dumlupınar Üniversitesi Rektörlüğü Etik Kurulunun 25/05/2018-E.25586

Sayı : 56120658-050.02 karar 4 de çalışmanın etik kurul izni alınmıştır.

Çalışma yürütülürken her aşamasında etik kurallara uyulmuştur.

Bulgular:

Tablo 1: Tanımlayıcı özellikler

Yaş (ort±SS)	20,7±1,78
Boy (santimetre) (ort±SS)	174,2±8,75
Kilo (kilogram) (ort±SS)	68,8±12,5
Cinsiyet	Kadın: 53 (%26,5) Erkek: 147 (%73,5)

Ort±SS: Ortalama±Standart sapma

Toplam 200 öğrencinin katıldığı çalışmaya 147 (%73,5)' i erkek, 53 (%26,5)'i kız öğrenci katıldı. Yaşları (18-26) arasında değişmekte, yaş ortalamaları (20,7±1,78) idi.

Tablo 2: Cinsiyet ile egzersiz karşılaştırması

	Egzersiz var	Egzersiz yok	Kararsız	Toplam	*p=0,004
Erkek	59 (%40,1)	74 (%50,3)	14(%9,5)	147 (%73,5)	
Kız	8 (%15,1)	37 (%69,8)	8 (%15,1)	53 (%26,5)	
Toplam	67 (%33,5)	111 (%55,5)	22 (%11)	200	

Katılımcıların düzenli egzersiz yapar mısınız sorusuna (%33,5) evet, (%55,5) hayır, (%11) kararsızım cevapları verildi. . Cinsiyetle ezersiz yapma arasında karşılaştırma yapıldığında istatistiksel olarak anlamlı fark bulundu (p=0,004). Erkekler daha fazla egzersiz yapan cinsiyetti.

Tablo 3: Cinsiyet ile süt ve süt ürünleri tüketme karşılaştırması

	Süt ürünü tüketme var	Süt ürünü tüketme yok	Kararsız	Toplam	*p=0,089
Erkek	77 (%52,4)	53 (%36,1)	17 (%11,6)	147 (%73,5)	
Kız	32 (%60,4)	11 (%20,8)	10 (18,9)	53 (%26,5)	
Toplam	109 (%54,5)	64 (%32)	27 (%13,5)	200	

Katılımcıların süt ve süt ürünü kullanımına ilişkin verdikleri cevaplar (%54,5) evet, (%32) hayır, (%13,5) kararsızım şeklindeydi.

Tablo 4: Cinsiyet ile Ca ve Vit D kullanma karşılaştırması

	Ca ve Vit D kullanma var	Ca ve Vit D kullanma yok	Kararsız	Toplam	*p=0,834
Erkek	22 (%15)	124 (%84,4)	1 (%0,7)	147(%73,5)	
Kız	8 (%15,1)	45(%84,9)	0	53(%26,5)	
Toplam	30 (%15)	169(%84,5)	1(%0,5)	200	

Katılımcıların kalsiyum ve D vitamini kullanımı sorgulandığında (%15)'i evet, (%84,5)'u hayır, (%0,5)'i kararsızım cevabını vermiştir.

Tablo 5: Cinsiyet ile güneş ışığından yararlanma karşılaştırması

	Güneş ışığından yararlanma var	Güneş ışığından yararlanma yok	Kararsız	Toplam	*p=0,331
Erkek	73 (%49,7)	42 (%28,6)	32 (%21,8)	147(%73,5)	
Kız	22 (%41,5)	21 (%39,6)	10 (%18,9)	53(%26,5)	
Toplam	95 (%47,5)	63 (%31,5)	42 (%21)	200	

Katılımcıların yeterli güneş ışığına maruziyetleri sorgulandığında ise (%47,5) evet, (%31,5) hayır, (%21) kararsızım cevabı alındı.

Tartışma:

Çalışmamızda katılımcıların düzenli egzersiz yapar mısınız sorusuna (%33,5) evet, (%55,5) hayır, (%11) kararsızım cevapları verildi. Bu sonuç bireylerin egzersiz konusunda duyarlılıklarının yetersiz olduğunu göstermektedir. Hareketsiz yaşam, bireylerin kemik sorunları yaşamalarına sebep olan etkenler arasında yer almaktadır. Bu risk faktörünün ortadan kaldırılması kemik problemlerinin önlenmesi açısından önemlidir (Dinçer 2008).

Çalışmamızda gençlerin cinsiyetleri ile egzersiz yapma durumları arasında karşılaştırma yapıldığında istatistiksel olarak anlamlı fark bulunmuştur (p=0,004). Erkekler daha fazla egzersiz yapan grup olarak belirlenmiştir. Genç bayanların konuya duyarlılıklarının artırılmasına gereksinim olduğu ortaya çıkmıştır. Bayan cinsiyeti erkeklere göre kemik sağlığı yönü ile öncelikle düşünülmesi gereken olgu olarak kabul edilmektedir. Risk faktörlerinin ortadan kaldırılması kemik sağlığının korunması bakımında önemlidir (Dinçer 2008). Bayanlarda menarşa geç girme, menapoza erken ulaşma, 6 aydan uzun süren amenore, sık ve kısa aralıklı doğum, ooferoktomi operasyonları, iyatrojenik menapoz, çok doğum kontrol hapları kullanmak gibi sebepler bayanları kemik sorunları konusunda daha riskli hale getirmektedir (Xu Ni, 2017). Konu ile ilgili toplumun bilinçlendirilmesi önemlidir (Agrawal 2013). Bayan cinsiyeti ve gençler tıpta özellikli olan bireyler olarak görülmekte, bu bireylerin sağlık hizmeti imkanlarının sağlanmasında yetersizlik sorunlarından dolayı tıp etiğinin adalet ilkesine dönük duyarlı sağlık hizmetine gereksinim duymaktadırlar ve öncelikli olarak hizmete ulaşmaları sağlanması gerektiği vurgulanmaktadır. (Ögenler 2016).

Çalışmamızda katılımcıların süt ve süt ürünü kullanımına ilişkin verdikleri cevaplar (%54,5) evet, (%32) hayır, (%13,5) kararsızım şeklindeydi. Bu sonuç bireylerin beslenme konusunda duyarlılıklarının yetersiz olduğunu göstermektedir. Bireylerin beslenme alışkanlıkları bireylerin kemik sağlığını etkileyen önemli faktörlerdendir (Dinçer 2008)..

Çalışmamızda yer alan gençlerin Kalsiyum ve D vitamini kullanımı sorgulandığında (%15)'i evet, (%84,5)'u hayır, (%0,5)'i kararsızım cevabını vermiştir. Gençlerin konu ile ilgili farkındalıklarının yetersiz olduğu görülmüştür. Kalsiyum ve D vitaminin kemik sağlığının korunmasında önemli görevleri bulunmaktadır. Kemik sorunlarının önlenmesi amacıyla 1000 mg kalsiyum ve 600 IU D vitamini önerilmektedir. Son yıllarda kalsiyum takviyesi, güvenliği, kalsiyum ve D vitamininin uygun dozu ile ilgili çeşitli görüşler bulunmaktadır. Başka çalışmalarda da Ca kullanma düzeyi düşük olarak bulunmuştur (Sindel 2013, Altın 2014). Çalışmalarda sağlık okuryazarlığı yüksek olanların düşük olanlara göre, kullandığı ilaçlar hakkında bilgi sahibi olanların olmayanlara göre, reçetesiz ilaç almayanların alanlara göre ilaç uyumu yüksek bulunmuştur (Yılmaz 2018, Etemadifar 2013).

Hasta ilaç uyumsuzluğu; bireylerin yanlış ya da olmayan ilaç bilgisi; yanlış inanışlar, yanlış beklentiler, kendi kendine tedavi gibi sorunlar olarak tanımlanmıştır (Sürmelioğlu 2015, Aydın 2012). D vitamininin kalsiyum düzeyi ve kemik mineralizasyonunu düzenleme etkisinin yanı sıra, endokrin sistemde (diyabet, insülin direnci ve obezite gibi hastalıklar) ilişkisi tanımlanmıştır (Autier 2007). Çalışmalar D vitamininin kanser, kalp hastalıkları, kırıklar ve düşme, otoimmun hastalıklar, grip, tip-1 ve tip-2 diyabet ve depresyonu önleyici rolü olduğunu desteklemektedir (Holick 2008, Kemp 2011, Nair 2012). Sonuçlarımız araştırmalarla benzerlik içermektedir.

Çalışmamızda gençlerin yeterli güneş ışığına maruziyetleri sorgulandığında (%47,5) evet, (%31,5) hayır, (%21) kararsızım cevabı alınmıştır. Çalışmalar birçok hastalık üzerinde güneş ışığının olumlu etkilerinden bahsetmektedir (Yang 2011). Güneşe verilen fizyolojik yanıt, D vitamini üretiminin de ötesindedir. Cilt güneş ışığına maruz kaldığında nitrik oksit salınmakta, vazodilatasyon gerçekleşmekte ve kan basıncı düşmektedir (Baggerly 2015). Bir çalışmada adölesan dönemlerinde yılda iki kez ve daha fazla güneşe maruz kalan bayanlarda mortalite, yılda bir ya da daha az güneşe maruz kalanlara göre daha düşük bulunmuştur (Yang, Lof 2011). Sonuçlarımız araştırmalarla benzerlik göstermektedir.

Sonuç:

Çalışmamızda gençlerin kemik sağlığını koruma ile ilgili olarak yarısı süt ve süt ürünü tüketmekteydi, düzenli egzersiz yapma, güneş ışığından yararlanma, Ca ve D vitamini kullanma oranları düşük bulunmuştur.

D vitaminini yetersiz alımını önlemek için kolların ve bacakların haftada iki kez saat 10.00-15.00 arasında 5-30 dakika güneş ışığına maruz kalması yeterli olmaktadır (12). D vitamini güneşten kaçınılması gerektiği belirtilen zaman diliminde ciltte sentezlenebildiği için, eksikliğini önlemek amacıyla güneşlenmek yerine, gerekli olan D vitamininin besinler ve diyet takviyeleri yolu ile alınması da öneriler arasındadır (4).

Erken tanının kemik sağlığı konusunda yarar getireceği düşünülmektedir. Kemik hastalıkları ile mücadelede esas olan primer korumadır. Toplumun her kesimini kapsayan risklerin erken belirlenmesini ve tanılanmasına yönelik stratejiler her yönüyle kazanç anlamına gelir. Multidisipliner yaklaşımlar sorunun çözümünü kolaylaştırır. Başta Sağlık sektörü olmak üzere, resmi otorite, yerel yönetimler, eğitim, medya işbirliği başlıca paydaşlardır. Farmakolojik ve etik açıdan genç bireylerin konuya farkındalıklarının artırılmasına dönük programların planlanmasına gereksinim olduğu belirlenmiştir.

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➢ *ORAL PRESENTATION*

Sonikasyon yöntemi ile elde edilmiş *Pistacia terebinthus* meyve ekstrelerinin antimikrobiyal aktivitesinin belirlenmesi

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Özet

Pistacia terebinthus (menengiç) ülkemizde genellikle Güney ve Güneydoğu Anadolu bölgesinde yetişmekte olan herdem yeşil bir bitkidir. Bu çalışmada, değişik çözücülerle hazırlanmış menengiç meyve ekstrelerinin test bakterileri üzerinde antibakteriyel aktivitesi araştırılmıştır. *P. terebinthus* meyvelerinin ekstreleri sonikasyon yöntemi kullanılarak elde edilmiştir. Farklı çözücülerle (etanol, metanol, hekzan, aseton, diklorometan, su) hazırlanan ekstrelerin *Enterococcus faecalis* ATCC 29212, *Staphlococcus aureus* ATCC 25923 ve *Yersinia enterocolitica* ATCC 11175 mikroorganizmalarına karşı antimikrobiyal aktivitesi araştırılmıştır. Antimikrobiyal aktivite disk difüzyon ve mikrodilüsyon yöntemi kullanılarak belirlenmiştir. Disk difüzyon yönteminin sonuçlarına göre menengiç meyve ekstreleri *E. faecalis* ATCC 29212 üzerinde 10.07-16.08 mm, *S. aureus* ATCC 25923 üzerinde 16.72-6.21 mm ve *Y. enterocolitica* ATCC 11175 üzerinde 18.54-7.48 mm inhibisyon zon çapı göstermiştir. Ekstrelerin MBC değerleri ise bu bakteriler için sırasıyla 80 mg/mL, 2.5-20 mg/mL ve 5-80 mg/mL olarak kaydedilmiştir. Çalışmanın sonucunda *P. terebinthus* meyve ekstrelerinin test edilen tüm mikroorganizmalar üzerinde antimikrobiyal aktivitesinin olduğu belirlenmiştir. *P. terebinthus* meyve ekstrelerinin gıda ve sağlık sanayilerinde doğal antimikrobiyal madde kaynağı olarak kullanım potansiyeli taşıdığı tespit edilmiştir.

Anahtar Kelimeler: Menengiç, sonikatör, meyve, antibakteriyel aktivite

Determination of the antimicrobial activity of *Pistacia terebinthus* fruit extracts obtained by sonication method

Abstract

Pistacia terebinthus (menengiç) is an evergreen plant that generally grows in the South and Southeastern Anatolia regions in our country. In this study, antibacterial activity of menengiç fruit extracts prepared with different solvents on test bacteria was investigated. The extracts of *P. terebinthus* fruits were obtained using sonication method. The antimicrobial activity of the extracts prepared with different solvents (ethanol, methanol, hexane, acetone, dichloromethane, aquous) against *Enterococcus faecalis* ATCC 29212, *Staphlococcus aureus* ATCC 25923 and *Yersinia enterocolitica* ATCC 11175 microorganisms was investigated. Antimicrobial activity was determined using disk diffusion and microdilution method. According to the results of the disk diffusion method, menengiç fruit extracts showed an inhibition zone diameter of 10.07-16.08 mm on *E. faecalis* ATCC 29212, 16.72-6.21 mm, on *S. aureus* ATCC 25923 and 18.54-7.48 mm, on *Y. enterocolitica* ATCC 11175. MBC values of the extracts were recorded as 80 mg/mL, 2.5-20 mg/mL and 5-80 mg/mL for these bacteria, respectively. As a result of the study, it was determined that *P. terebinthus* fruit extracts had antimicrobial activity on all microorganisms tested. It has been determined that *P. terebinthus* fruit extracts have the potential to be used as a natural antimicrobial source in food and health industries.

Keywords: Menengiç, sonicator, fruit, antibacterial activity

Bu çalışma ilk yazarın yüksek lisans tezinden üretilmiştir.

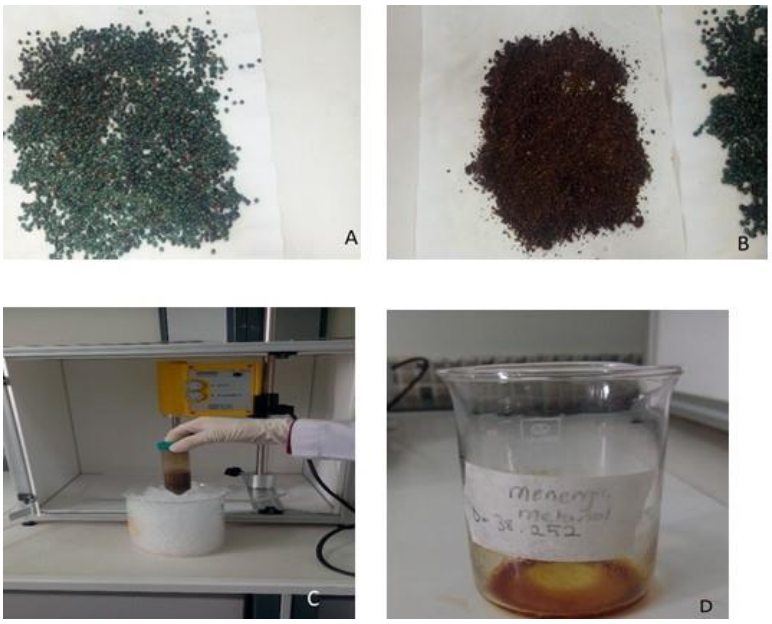
GİRİŞ

Pistacia terebinthus (Menengiç), Anacardiaceae (Sakız ağacıgiller, antepfıstığıgiller) familyasının bir üyesi olan bu ağaç türü Asya ve Akdeniz'e özgü bir bitki olup ülkemizde güney kesimlerde yaygın olarak yetişmektedir (Davis, 1970). *P. terebinthus* çok reçineli bir kokuya sahiptir ve meyveleri küçük mavimsi yeşildir. Ülkemizin bazı yörelerinde yapılan menengiç sabunları ve giderek daha popüler hale gelen menengiç kahvesi, son yıllarda bu bitkinin ticari değerini artıran uygulamalar olarak görülmektedir (Gülsoy ve ark., 2013). Menengiç, antimikrobiyal, antienflamatuar, sitotoksik ve antioksidan potansiyele sahip bir bitkidir (Ciftci, ve ark, 2009). Pistacia türleri, özellikle flavonoitler ve diğer fenolik bileşenler sebebiyle antioksidan, antienflamatuar ve sitotoksik aktivitelerin yanı sıra antimikrobiyal aktiviteye sahip olduğundan araştırmacıların dikkatini çekmiştir (Algan, 2019). *P. terebinthus* bir terebentin kaynağı olarak bilinmekte ve birçok ülkede geleneksel bir ilaç olarak da kullanılmaktadır. Örneğin İran'da bitkinin yakılmasıyla üretilen duman, dezenfektan ve hava temizleyici olarak kullanılmaktadır (Mohagheghzadeh ve ark, 2010). Ayrıca astım gibi çeşitli hastalıkların geleneksel tedavi yöntemi olarak kullanılmaktadır ve Türkiye'de ise bronşit için antiseptik olarak kullanılabildiği literatürlere geçmiştir (Demirbüker-Kavak ve ark, 2010). Bu çalışmada, menengiç meyvelerinden sonikasyon yöntemiyle çeşitli çözücülerle hazırlanmış ekstrelerin *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923 ve *Y. enterocolitica* ATCC 11175 üzerindeki antibakteriyel etkisinin araştırılması hedeflenmiştir.

MATERYAL VE METOD

Ekstrelerin Hazırlanması

Adıyaman ilinde bulunan bir aktardan temin edilen menengiç meyve örnekleri açık havada kurumaya bırakılmıştır. Daha sonra kuruyan örnekler toz haline getirilmiştir. Toz haline getirilmiş örnekten 20 gr alınarak üzerine 30 mL çözücü (etanol, metanol, hekzan, diklorometan, aseton, su) ayrı ayrı eklenmiştir. Çözücü eklenmiş olan örnekler 10'ar dakika ve 3 tekerrür olarak buz üzerinde sonikatörde ekstrakte edilmiştir. Ekstraksiyon işlemi tamamlandıktan sonra süpernatant kısmı bir behere alınarak çözücünün uçması beklenmiştir. Ekstre hazır hale geldikten sonra kullanılıncaya kadar kuru koşullar altında 4°C'de muhafaza edilmiştir (Şekil 1).



Şekil 1. Menengiç meyve ekstrelerinin hazırlanışı

A. Menengiç meyvelerinin kurumaya bırakılması
B. Menengiç meyvelerinin öğütülmüş hali
C. Menengiç meyvelerinin sonikatörde ekstrakte edilişi
D. Menengiç meyve ekstresinin çözücüsü uçurulmuş hali

Antimikrobiyal Aktivitenin Belirlenmesi

Disk Difüzyon Yöntemi

P. terebinthus meyve ekstrelerinin test mikroorganizmalar üzerinde antimikrobiyal aktivitesini belirlemek için disk difüzyon yöntemi kullanılmıştır. *E. faecalis* ATCC 29212 bakterisi Tryptic Soy Broth, *S. aureus* ATCC 25923 ve *Y. enterocolitica* ATCC 11175 bakterilerinin Nutrient Broth besiyerinde ve 37°C'de kültürleri yapılmıştır. Daha sonra, kültürleri yapılan mikroorganizmalar iki defa serum fizyolojik ile yıkanarak 0.5 Mcfarland standartına göre ayarlanmıştır. Konsantrasyonu ayarlanan mikroorganizmaların uygun katı besiyerlerine ekimi yapılmıştır. Ekim yapılan petrilere 3 tekerrür olacak şekilde steril diskler yerleştirilmiştir. Disklerin üzerine 20 µL (2000 µg/disk) menengiç meyve ekstreleri damlatılarak 24 saat 37 °C'de inkübasyona bırakılmıştır. İnkübasyon sonrasında disklerin çevresinde oluşan inhibisyon zonları kumpas ile ölçülerek kaydedilmiştir.

Minimal inhibisyon (MİK) ve bakterisidal (MBK) konsantrasyonlarının belirlenmesi

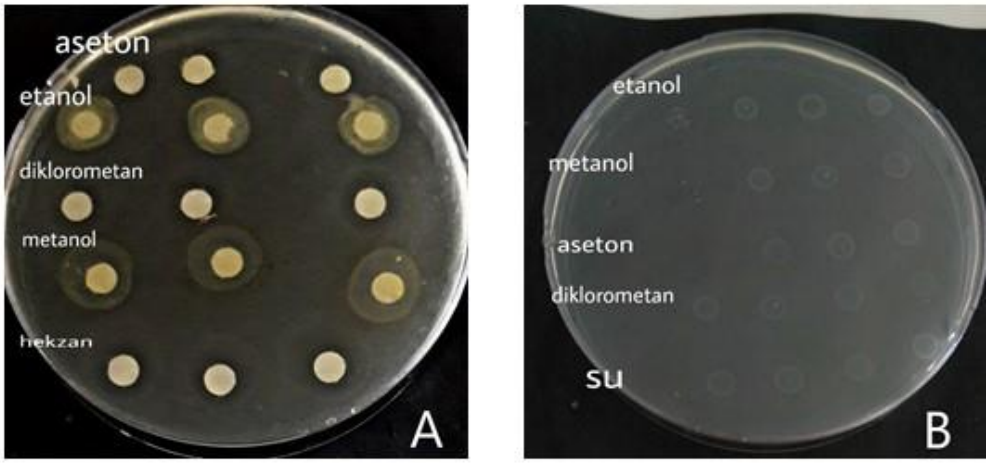
P. terebinthus meyve ekstrelerinin test mikroorganizmaları üzerine MİK ve MBK değerini belirlemek için mikrodilüsyon yöntemi kullanılmıştır. Ekstre ve seçtiğimiz mikroorganizma için uygun besiyeri bulunan her tüpe 0.5 Mcfarland'a göre hazırlanmış test mikroorganizmaları eklenmiş ve karıştırılmıştır. Daha sonra tüpler 24 saat 37°C'de inkübe edilmiştir. İnkübasyon sonrası sıvı besiyerlerinde üremenin olmadığı tüp MİK değeri olarak kaydedilmiştir. Daha sonra bu tüplerdeki örnekler uygun katı besiyerine spot ekim yöntemiyle ekilmiş ve petriler 24 saat 37°C'de inkübe edilmiştir. İnkübasyon sonunda katı besiyerinde üremenin olmadığı konsantrasyon MBK değeri olarak kaydedilmiştir.

BULGULAR VE TARTIŞMA

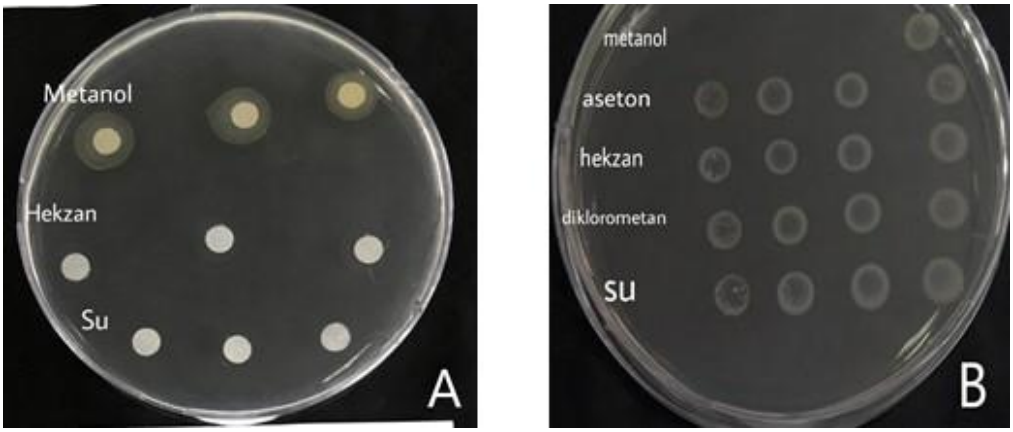
Hastalık, hatta ölüm veya önemli ekonomik kayıplara neden olan mikroorganizma kökenli patojenler tüm dünyada önemli sorunlar oluşturmaktadır. Bunlardan biri olan *Y. enterocolitica*, çevrede ve gıdada yaygın olarak bulunmakta olup kontamine besinler, bu mikroorganizmanın insanlara bulaşmasında önemli bir rol oynamaktadır. *Y. enterocolitica*' nın büyüme sıcaklığı ve pH sınırları çok geniştir. Gıdaların depolandığı buzdolabı sıcaklığında (+4°C) rahatlıkla gelişebilmektedir. Bu özelliklerinden dolayı halk sağlığı açısından önemlidir (Sağun ve Ergün, 1996). *E. faecalis*, hem sağlıklı insanlarda ve hayvanlarda kommensal bir organizma olarak hem de özellikle endokardit olmak üzere birçok hastalığa neden olan Gram-pozitif bir koktur (Minogue, 2014). İnsanlarda epitelyal yüzeylere kolonize olan stafilokok türlerinden özellikle *S. aureus*, sepsis, nekrotizan, pnömoni, toksik şok sendromu, deri ve mukoza enfeksiyonları gibi çeşitli enfeksiyonlara neden olmaktadır (Güldaş ve ark, 2013). Çalışmamızda *P. terebinthus* meyve ekstrelerinin *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923 ve *Y. enterocolitica* ATCC 11175 mikroorganizmaları üzerindeki antibakteriyel aktivitesi mikrodilüsyon ve disk difüzyon yöntemi kullanılarak belirlenmiştir. Menengiç meyve ekstrelerinin tüm test mikroorganizmalarına karşı antimikrobiyal aktivite gösterdiği gözlenmiştir (Tablo 1). Disk difüzyon sonuçlarına göre en yüksek inhibisyon zon çapını *E. faecalis* ATCC 29212 mikroorganizması üzerinde 16.08 mm ile etanol ekstresi, *S. aureus* ATCC 25923 mikroorganizması üzerinde 16.72 mm ile metanol ekstresi ve *Y. enterocolitica* ATCC 11175 mikroorganizması üzerinde 18.54 mm ile su ekstresi göstermiştir. Menengiç meyve ekstrelerinin, *E. faecalis* ATCC 29212 mikroorganizması için MİK ve MBK sonuçları 20-40 mg/mL ve 80 mg/mL, *S. aureus* ATCC 25923 mikroorganizması için 5-10 mg/mL ve 2.5-20 mg/mL, *Y. enterocolitica* ATCC 11175 için ise 5-20 mg/mL ve 5-80 mg/mL olarak tespit edilmiştir (Şekil 2-4).

Tablo 1. Menengiç meyve ekstrelerinin antimikrobiyal aktivitesi

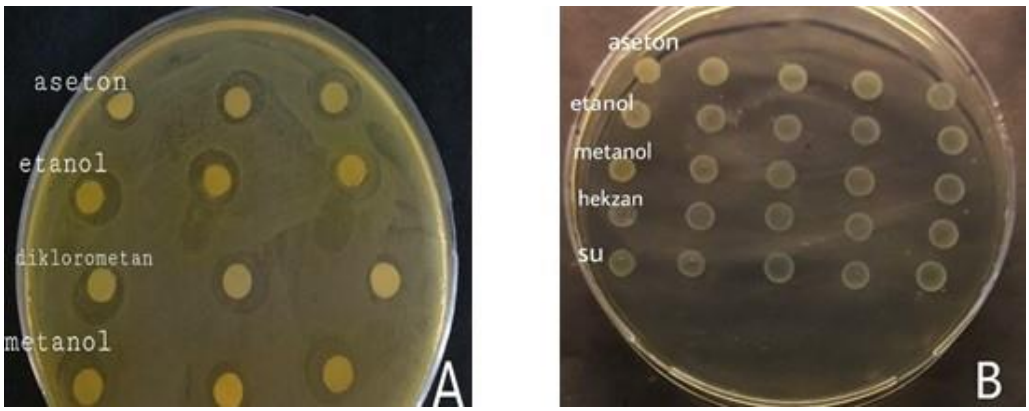
Ekstreler	İnhibisyon zon çapı (mm)			MİK (mg/mL)			MBK (mg/mL)		
	E. faecalis ATCC 29212	*S. aureus* ATCC 25923	*Y. enterolitica* ATCC 11175	*E. faecalis* ATCC 29212	*S. aureus* ATCC 25923	*Y. enterolitica* ATCC 11175	*E. faecalis* ATCC 29212	*S. aureus* ATCC 25923	*Y. enterolitica* ATCC 11175
Aseton	14.74±1.77	13.48±0.79	7.48±1.62	40	20	10	80	20	20
Etanol	16.08±1.39	13.76±0.11	14.92±0.34	40	10	20	80	10	40
Metanol	10.07±1.21	16.72±0.91	15.99±0.88	20	2.5	5	80	2.5	5
Diklorometan	14.57±0.58	11.00±0.99	11.93±0.51	20	20	20	80	20	40
Hekzan	14.46±1.09	12.03±0.56	13.88±0.72	40	10	20	80	20	80
Su	10.65±0.55	6.21±0.71	18.54±0.88	20	20	10	80	20	20



Şekil 2. Menengiç meyve ekstrelerinin *Y. enterocolitica* ATCC 11175 üzerinde antibakteriyel etkisi
A. Aseton, etanol, diklorometan, metanol, hekzan çözücüleri ile hazırlanan ekstrelerin disk difüzyon sonuçları.
B. Etanol, metanol, aseton, diklorometan, su çözücüleri ile hazırlanan ekstrelerin MBK sonuçları.



Şekil 3. Menengiç meyve ekstrelerinin *S. aureus* ATCC 25923 üzerinde antibakteriyel etkisi
A. Metanol, hekzan, su çözücüleri ile hazırlanan ekstrelerin disk difüzyon sonuçları.
B. Metanol, aseton, hekzan, diklorometan, su çözücüleri ile hazırlanan ekstrelerin MBK sonuçları.



Şekil 4. Menengiç meyve ekstrelerinin *E. faecalis* ATCC 29212 üzerinde antibakteriyel etkisi
A. Aseton, etanol, diklorometan, metanol çözücüleri ile hazırlanan ekstrelerin disk difüzyon sonuçları.
B. Aseton, etanol, metanol, hekzan, su çözücüleri ile hazırlanan ekstrelerin MBK sonuçları.

Hacıbekiroğlu ve ark. (2014) tarafından yapılan bir çalışmada, *P. terebinthus* meyvesinin hekzan, etanol, etanol-su, diklorometan çözücüleri ile hazırlanan ekstrelerin bazı mikroorganizmalara (*Streptococcus pyogenes* ATCC 19615, *S. aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231) karşı antimikrobiyal aktivitesi araştırılmıştır. 100 mg/mL konsantrasyonda makro sıvı dilüsyon yöntemi kullanarak yaptıkları bu çalışmanın sonucunda hekzan ve diklorometan ekstresi hiç aktivite göstermezken, etanol ekstresi en yüksek aktiviteyi *E. coli* karşı 25 mg/mL, en düşük aktiviteyi ise *S. aureus* ATCC 25923 ve *P. aeruginosa* ATCC 27853 üzerinde 80 mg/mL göstermiştir. Yaptığımız çalışmada, MİK sonuçlarına göre hekzan ve diklorometan ekstreleri *E. faecalis* ATCC 25923 üzerinde 20-40 mg/mL, *S. aureus* ATCC 25923 üzerinde 10-20 mg/mL ve *Y. enterocolitica* ATCC 11175 üzerinde 20 mg/mL değerleri belirlenmiştir. Etanol ekstresi ise en yüksek aktiviteyi *S. aureus* ATCC 25923 mikroorganizmasına karşı 10 mg/mL olarak göstermiştir. Ayrıca disk difüzyon yönteminde hekzan ve diklorometan ekstreleri *E. faecalis* ATCC 25923 üzerinde sırasıyla 14.48-14.57 mm, *S. aureus* ATCC 25923 üzerinde sırasıyla 12.03-11.00 mm ve *Y. enterocolitica* ATCC 11175 üzerinde sırasıyla 13.88-11.93 mm inhibisyon zon çapı sergilemiştir.

Başka bir çalışmada ise *P. terebinthus* meyvesinin aseton-su karışımı (%39 su-%61 aseton) kullanılarak elde edilen ekstresi disk difüzyon yöntemi ile bazı bakterilere (*S. aureus, Listeria monocytogenes, Salmonella typhimurium* ve *Escherichia coli* O157:H7) karşı antimikrobiyal aktivitesi araştırılmıştır. 0.5 mg/disk konsantrasyonu kullanılan çalışmada en yüksek inhibisyon zon çapı *S. typhimurium* bakterisine karşı 15.25 mm olarak tespit edilmiştir. *S. aureus'a* karşı 9-13 mm, *L. monocytogenes*'e karşı 11.25-14.25 mm, *E. coli* O157:H7 ye karşı 8.75-11.5 mm olarak sonuçlar kaydedilmiştir (Durak ve Uçak, 2014). Yaptığımız çalışmada ise 2 mg/disk konsantrasyon kullandığımız disk difüzyon sonuçlarına bakıldığında aseton ekstresi en yüksek *E. faecalis* ATCC 29212 mikroorganizmasına karşı 14.74 mm olarak ölçülmüştür.

SONUÇ

Yaptığımız çalışmada, *P. terebinthus* meyve ekstrelerinin gram pozitif *E. faecalis* ATCC 29212 ve *S. aureus* ATCC 25923, gram negatif *Y. enterocolitica* ATCC 11175 mikroorganizmalarına karşı antimikrobiyal aktivite gösterdiği belirlenmiştir. Çalışmamızın sonuçları, doğala yönelimin daha da arttığı günümüzde, *P. terebinthus* meyvesinin doğal antimikrobiyal madde kaynağı olarak gıda ve sağlık sanayilerinde kullanım potansiyelinin olduğunu göstermiştir.

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➢ *ORAL PRESENTATION*

Çeşitli zeytin meyvesi ve yaprak ekstrelerinin balık orijinli *Vibrio anguillarum* ve *Streptococcus agalactiae* patojenlerine karşı antibakteriyel etkinliği

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Özet

Son yıllarda, dünya çapında balık yetiştiriciliği ve kültür balıkçılığı ticaretinin gelişmesine paralel olarak, balıklarda enfeksiyöz hastalıklar artış göstermektedir. Bunun sonucunda balık hastalıkları akuakültür sektöründe sınırlayıcı faktörler arasında yer almaya başlamıştır. Bu çalışmada, İzmir'den toplanan Ayvalık Yağlık ve Manzanilla çeşidi zeytin meyvesi ve yaprağından çeşitli çözücülerle hazırlanan (su, etanol, metanol ve aseton) ekstrelerin balık bakteriyel patojenleri olan *Vibrio anguillarum* M1 ve *Streptococcus agalactiae* Pas.Ins. 55118 'e karşı antimikrobiyal aktivitesi araştırılmıştır. Hazırlanan ekstrelerin antimikrobiyal aktivitelerini belirlemede disk difüzyon metodu kullanılmıştır. Ekstrelerin minimum inhibisyon konsantrasyon (MİK) ve minimum bakterisidal konsantrasyon (MBK) değerleri mikro-dilüsyon yöntemi ile belirlenmiştir. Disk difüzyon metodunun sonuçları, *V. anguillarum* M1 ve *S.agalactiae* Pas.Ins. 55118 patojenlerine karşı test edilen tüm ekstrelerin Ayvalık Yağlık çeşidinde 12.51-15.46 mm ve Manzanilla çeşidinde 9.02-16.56 mm inhibisyon zonları ile antibakteriyal aktivite gösterdiği tespit edilmiştir. Test edilen Ayvalık Yağlık ve Manzanilla zeytin meyvesi ve yaprak ekstrelerinin MİK ve MBK değerleri 10-80 mg/ml aralığında kaydedilmiştir. Sonuçlar, İzmir' den toplanan Ayvalık Yağlık ve Manzanilla çeşidi zeytin meyvesi ve yaprak ekstreleri, test edilen iki balık bakteriyel patojenlerinin büyümesi üzerinde antibakteriyel bir etkiye sahip olduğunu göstermiştir. Özellikle, akuakültürde görülen bazı bakteriyel enfeksiyonlar hem ekonomik kayıplara neden olmakta hem de halk sağlığı açısından tehlike oluşturmaktadır. Bu çalışmada, Ayvalık Yağlık ve Manzanilla çeşidi zeytin meyvesi ve yaprak ekstrelerinin akuakültür sektöründe doğal antibakteriyel kaynağı olarak kullanılabilme potansiyeli olduğu tespit edilmiştir.

Anahtar Kelimeler: İzmir, zeytin meyvesi, zeytin yaprağı, antimikrobiyal aktivite, balık patojeni

Antibacterial activity of various olive fruit and leaf extracts against fish-originated *Vibrio anguillarum* and *Streptococcus agalactiae* pathogens

Abstract

In recent years, parallel to the development of the worldwide fish farming and aquaculture trade, infectious diseases in fish increases. As a result, fish diseases have started to be among the limiting factors in the aquaculture sector. In this study, the antimicrobial activity of the extracts prepared with various solvents (aquaus, ethanol, methanol and acetone) from olive fruits and leaves of Ayvalık Yağlık and Manzanilla varieties collected from İzmir was investigated against the fish bacterial pathogens *Vibrio anguillarum* M1 and *Streptococcus agalactiae* Pas.Ins. 55118. Disk diffusion method was used to determine the antimicrobial activities of the extracts prepared. The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of the extracts were determined by micro-dilution method. Results of the disk diffusion method showed that all extracts tested against *V. anguillarum* M1 and *S. agalactiae* Pas.Ins. 55118 pathogens showed antibacterial activity with 12.51-15.46 mm inhibition zones in Ayvalık Yağlık variety and 9.02-16.56 mm in Manzanilla variety. The MIC and MBC values of Ayvalık Yağlık and Manzanilla olive fruit and leaf extracts tested were recorded in the range of 10-80 mg/ml. The results showed that the olive fruit and leaf extracts of Ayvalık Yağlık and Manzanilla varietes collected from İzmir had an antibacterial effect on the growth of the two fish bacterial pathogens tested. Especially, some bacterial infections seen in aquaculture cause both economic losses and a danger to public health. In this study, it has been determined that Ayvalık Yağlık and Manzanilla variety olive fruit and leaf extracts have the potential to be used as a natural antibacterial source in the aquaculture sector.

Keywords: Izmir, olive fruit, olive leaf, antibacterial activity, fish pathogens

Bu çalışma ilk yazarın yüksek lisans tezinden üretilmiştir.

GİRİŞ

Zeytin (*Olea europaea*) bitkisi, uygarlık tarihi boyunca hem besin hem de ilaç kaynağı olarak önemli bir kullanım alanı oluşturmuştur. En önemli meyve ağaçlarından biri olan *O. europaea*, Türkiye, İtalya, Fransa, Yunanistan, Fas, Filistin, Suriye, İspanya ve Cezayir gibi Akdeniz bölgesine özgü bir bitkidir. *O. europaea* dünya mahsülünün %98'ini oluşturmakla birlikte yaklaşık olarak 8 milyon hektarlık alanı kaplamaktadır (Guinda ve ark., 2004; Pereira ve ark., 2007). Zeytin ve yan ürünlerinin bu kadar geniş alanda yetiştirilmesi hem ekonomik hem de sosyal değerini arttırmaktadır. Yapılan çalışmalarda zeytin meyvesi ve yaprağının, hipoglisemik, hiperkolesterolemik, antihipertansif, anti-bakteriyel ve antioksidan ve kardiyoprotektif etkiye sahip olduğu bildirilmiştir (Gonzalez ve ark.,1992; Hansen ve ark., 1996; Upadhyay ve ark., 2010; Aliabadi ve ark., 2012; Nora ve ark., 2012; Aytul, 2010). Zeytin yaprak ekstreleri, enerji seviyelerini arttırmak, kan basıncını düşürmek ve bağışıklık sistemini desteklemek gibi sağlık yararları da olduğu bildirilmektedir (Covas, 2007; El ve Karakaya, 2009). Ayrıca zeytin yaprakları etkin bir biyolojik kaynak olarak değerlendirildiğinde sağlıklı, güvenli ve alternatif antioksidan kaynağı olmakla birlikte gıda ürünlerinin raf ömrünü de uzatma niteliğindedir (Jemai ve ark., 2009; Boudhrioua ve ark., 2009; Bouaziz ve ark., 2010). Zeytin ağacı yan ürünlerinin ekstreleri, fenolik bileşikler gibi önemli antioksidanları içermesi nedeniyle oksidatif parçalanmaları önleyebildiği için kozmetik, tıp, farmasötik ve gıda endüstrisinde kullanılabilmektedir (Jemai ve ark., 2009; Boudhrioua ve ark., 2009; Bouaziz ve ark., 2010). Balıklar, genellikle yüksek yoğunluklu koşullarda yetiştirildiklerinden dolayı birçok bakteriyel enfeksiyona karşı da hassastırlar. Salgın hastalıklar, ölüm oranlarına ve balık çiftliklerinde maddi kayıplara neden olmaktadır (Figueiredo ve ark., 2006; Hatha ve ark., 2005). Streptokoklar, gram pozitif aerobik mikroorganizmalardır. Balıklardaki enfeksiyonlar genel olarak Lancefield grup B organizmaları (*S. agalactiae* Pas.Ins. 55118) ya da Lancefield antijenlerini eksprese etmeyen *S. iniae* türlerinden kaynaklanmaktadır (Gauthier, 2015). *Vibrionaceae* familyasında yer alan *Vibrio anguillarum* gram negatif bakteriyel bir balık patojenidir. Balıklarda hemorajik septisemiye neden olan vibriozis hastalığının etkenidir. Vibriozis, yaklaşık olarak tüm dünyada kültür balıkçılığı yapan işletmelerde önemli ekonomik kayıplara neden olmaktadır. Bulaşıcı bir hastalık olduğundan dolayı pasifik ve atlantikte yaşayan (salmonları, gökkuşağı alabalıkları, kalkan, levrek, çipura, çizgili levrek, kod, japon, avrupa yılan balığı ve ayu balığı) hem ılık su hem de soğuk su balık türlerini etkilemektedir (Buller, 2004; Toranzo ve ark., 2005). Ülkemizde kültür balığı yetiştiriciliği yapan işletmelerde ciddi ekonomik kayıplara yol açmaktadır (Ekici, 2005; Akaylı., 2001; Çağırgan, 1993). Günümüzde kültür balıkçılığı yapan işletmelerde bakterilerin neden olduğu enfeksiyonları kontrol altına almak için yaygın olarak tercih edilen yöntem antibiyotik kullanımıdır. Ancak gereksiz ve uygunsuz antibiyotik kullanımı su ürünleri yetiştiriciliğinde dirençli bakterilerin gelişmesine neden olmaktadır. Dolayısıyla hastalıkların tam anlamıyla tedavi edilememesi ve direncin yayılması ile hem insanların hem de diğer hayvanların sağlığını tehdit etmektedir (Serrano ve ark., 2005). Balık çiftliklerinde kullanılan antibiyotikler sonucu, *Aeromonas hydrophila, A. salmonicida, Edwardsiella tarda, E. icttaluri, Vibrio anguillarum, V. salmonicida, Pasteurella piscida* ve *Yersinia ruckeri*'de antibiyotik direnci geliştiği bildirilmiştir (Petersen ve ark., 2002). Bu nedenle, antibiyotik kullanımı yerine doğal antimikrobiyal maddelerle alternatif tedavilerin geliştirilmesine ihtiyaç duyulmaktadır. Zeytin meyvesi ve yaprağının antimikrobiyal aktivitesi genellikle insan-gıda ve klinik kökenli mikroorganizmalar üzerine belirlenmiş olup balık patojenlerine etkisi üzerine yapılan çalışmalar çok kısıtlı kalmıştır. Bu çalışmada, İzmir'den toplanan zeytin meyvesi ve yaprak örneklerinin su ürünlerinde bakteriyel hastalıklara neden olan iki balık patojeni (*Vibrio anguillarum* M1 ve *Streptococcus agalactiae* Pas.Ins. 55118) üzerindeki antimikrobiyal aktivitesi araştırılmıştır.

MATERYAL VE METOT

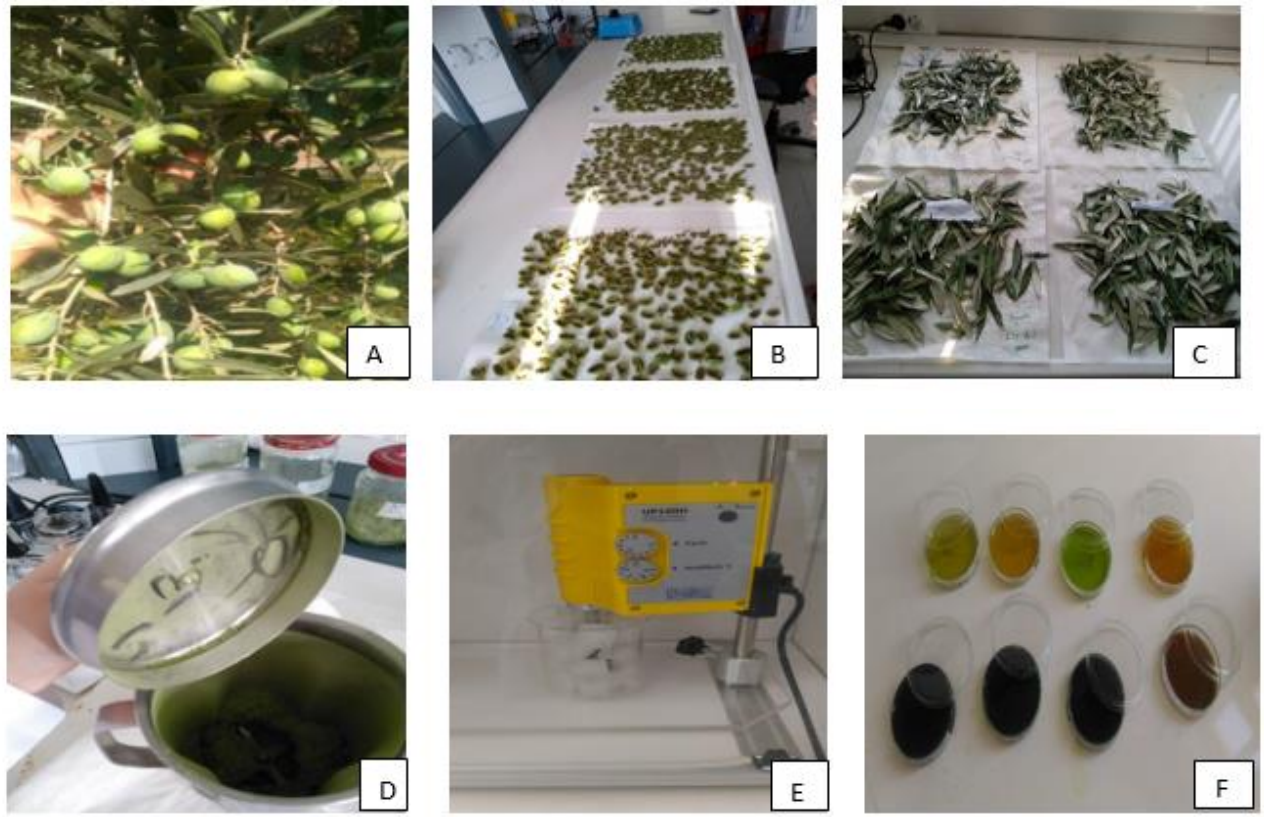
Zeytin meyvesi ve yaprak örneklerinin temini

Zeytin meyvesi ve yaprak örnekleri 26 Eylül 2019 tarihinde Türkiye'nin İzmir ili Zeytincilik Araştırma Enstitüsü' nden Ayvalık Yağlık ve Manzanilla çeşidi temin edilmiştir.

Zeytin meyvesi ve yaprak ekstrelerinin hazırlanması

Zeytin meyvesi ve yaprakları açık havada kurutulduktan sonra toz haline getirilmiştir. 10 gr toz zeytin meyvesi ve yaprak örneklerinden alınarak 30 ml çözücü (su, etanol (%96), metanol ve aseton) ile 10'ar dakikalık sürelerde 3

tekrar şeklinde buz üzerinde sonikatörde ekstrakte edilmiştir. Ekstraksiyon sonrasında süpernatant alınarak çözücüler buharlaştırılmıştır (Şekil 1). Elde edilen ekstre kullanılıncaya kadar kuru koşullar altında 4°C'de muhafaza edilmiştir.



Şekil 1. Zeytin meyvesi ve yaprak ekstrelerinin hazırlanması
(A) Zeytin meyvesi ve yaprakların toplanması
(B-C) Zeytin meyvesi ve yaprakların kurutulması
(D) Zeytin meyvesi ve yaprakların öğütülmesi
(E) Sonikatörde zeytin meyvesi ve yaprak ekstrelerinin hazırlanması
(F) Zeytin meyvesi ve yaprak ekstrelerinin çözücülerinin uçurulması

Kullanılan test mikroorganizmaları

Test mikroorganizmaları olarak balık kökenli patojen *Vibrio angillarum* M1 ve *Streptococcus agalactiae* Pas.Ins. 55118) bakterileri kullanılmıştır. Bakterilerin 24 saatlik aktif kültürleri kullanılarak çalışma yapılmıştır.

Antimikrobiyal Aktivitenin Belirlenmesi

Disk difüzyon yöntemi

Zeytin meyvesi ve yaprak (su, etanol, metanol ve aseton) ekstrelerinin antimikrobiyal aktivitesinin belirlenmesi için disk difüzyon yöntemi kullanılmıştır. Balık orijinli patojen bakterileri *V. anguillarum* M1 ve *S. agalactiae* Pas.Ins. 55118 test mikroorganizmaları olarak kullanılmıştır. *V. anguillarum* %2 NaCl içeren TSB/agar ve *S. agalactiae* Pas.Ins. 55118 TSB/agar besi yerinde sırasıyla 25°C ve 37°C' de kültürleri yapılmıştır. Patojen bakteriler serum fizyolojik ile iki defa yıkandıktan sonra konsantrasyonları 0.5 McFarland'a ayarlanmış ve daha sonrasında uygun katı besi yerine ekimleri yapılmıştır. Ekimi yapılan petrilere steril diskler yerleştirildikten sonra disklere 20 µl (4000 µg/disk) zeytin meyvesi ve yaprak ekstreleri damlatılmıştır. Petriler 24 saat uygun sıcaklıklarda inkübe edilmiştir. İnkübasyon süresi sonunda diskler etrafındaki zonlar ölçülerek kaydedilmiştir. Tüm deneyler üç tekrarlı olarak yapılmıştır.

Minimal inhibisyon (MİK) ve bakterisidal (MBK) konsantrasyonlarının belirlenmesi

Ekstrelerin MİK ve MBK değerleri iki balık patojenine karşı mikro-dilüsyon yöntemi ile belirlenmiştir. Ekstre ve besiyeri içeren her tüpe 0.5 McFarland konsantrasyonunda balık patojenleri eklenmiş ve yavaşça karıştırılmıştır. Daha sonra karışımı içeren tüpler 24 saat uygun sıcaklıklarda inkübe edilmiştir. İnkübasyondan sonra, sıvı besi yerinde gelişmenin olmadığı konsantrasyon MİK değerleri olarak kaydedilmiştir. Tüplerden örnekler alınarak spesifik agar besi yerine spot ekimleri yapılmış ve 24 saat uygun sıcaklıklarda inkübe edilmiştir. İnkübasyon süresinin sonunda, katı besi ortamı üzerinde bakterilerin gelişimini önleyen ekstre konsantrasyonları MBK değerleri olarak değerlendirilmiştir.

BULGULAR ve TARTIŞMA

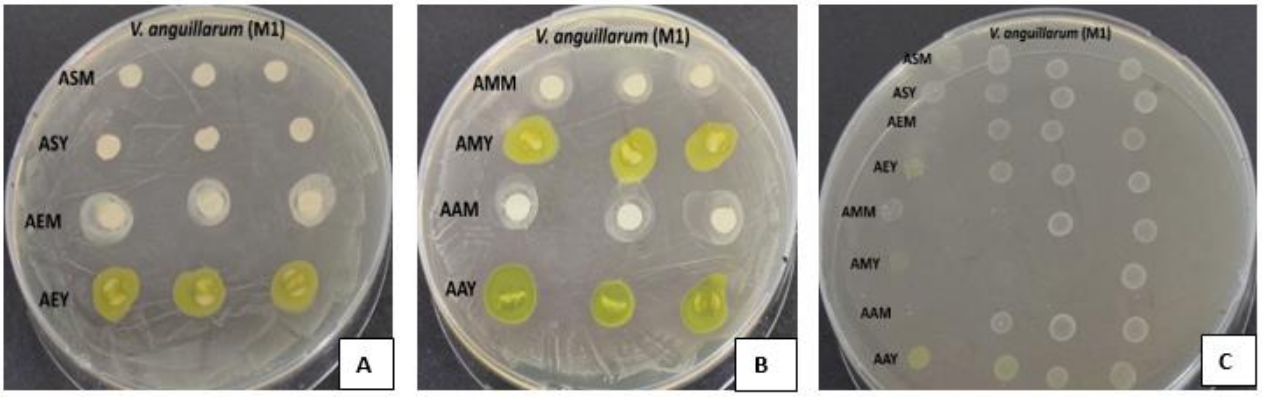
Zeytin meyvesi ve yaprak ekstrelerinin antimikrobiyal aktivitesi disk difüzyon ve mikro-dilüsyon yöntemleri ile belirlenmiştir. Disk difüzyon sonuçlarına göre, Ayvalık Yağlık ve Manzanilla çeşidi zeytin meyvesi ve yaprak ekstrelerin tamamı test edilen her iki balık patojeni *V. anguillarum* M1 ve *S. agalactiae* Pas.Ins. 55118 üzerinde inhibisyon etkisi oluşturduğunu göstermiştir. Ayvalık Yağlık çeşidi en yüksek inhibisyon aktivitesini zeytin yaprak su ekstresinde *V. anguillarum* M1 (15.46 mm) ve *S. agalactiae* Pas.Ins. 55118 üzerinde (13.89 mm) göstermiştir (Tablo 1). Ekstrelerin *V. anguillarum* M1 için MİK ve MBK değerleri ise 20-40 mg/ml ve 10-80 mg/ml olarak tespit edilmiştir (Tablo 1, Şekil 1). *S. agalactiae* Pas.Ins. 55118 için MİK ve MBC değerleri 20-40 mg/ml ve 20-80 mg/ml olduğu belirlenmiştir (Tablo 1, Şekil 2). Manzanilla çeşidi zeytin meyvesi etanol ekstresi *V. anguillarum* M1 üzerinde en yüksek inhibisyon aktivitesini 16.36 mm inhibisyon zon çapı gösterirken, *S.agalactiae* Pas.Ins. 55118 üzerinde zeytin meyvesi aseton ekstresi 16.56 mm inhibisyon zon çapı ile en yüksek antibakteriyel aktivite göstermiştir (Tablo 2). Ekstrelerin, *V. anguillarum* M1 için MİK ve MBK değerleri ise 20-40 mg/ml ve 40-80 mg/ml olarak tespit edilmiştir (Şekil 3). *S. agalactiae* Pas.Ins. 55118 için MİK ve MBK değerleri 10-40 mg/ml ve 10-80 mg/ml arasında değiştiği belirlenmiştir (Şekil 4).

Tablo 1. Ayvalık Yağlık çeşidi zeytin meyvesi ve yaprak ekstrelerinin antibakteriyel aktivitesi

Ekstreler	İnhibisyon zon çapı (mm)		MİK (mg/ml)		MBK (mg/ml)	
	***V.angullurium* M1**	***S. agalactiae* Pas.Ins. 55118**	***V.angulluri um* M1**	***S. agalactiae* Pas.Ins. 55118**	***V.angullu rium* M1**	***S. agalactiae* Pas.Ins. 55118**
Zeytin meyvesi etanol	13.23±1.09	13.78±0.28	20	40	40	80
Zeytin meyvesi metanol	13.86±0.51	13.71±0.52	40	20	80	20
Zeytin meyvesi su	12.79±0.29	13.43±0.62	40	20	80	40
Zeytin meyvesi aseton	13.41±1.51	11.36±0.45	20	20	40	40
Yaprak etanol	13.03±0.45	12.67±0.59	40	40	80	80
Yaprak metanol	14.42±064	13.39±1.76	20	40	10	80
Yaprak su	15.46±1.13	13.89±1.07	40	40	80	80
Yaprak aseton	12.71±1.45	12.51±1.14	20	20	40	40

Tablo 2. Manzanilla çeşidi zeytin meyvesi ve yaprak ekstrelerinin antibakteriyel aktivitesi

Ekstreler	İnhibisyonzonçapı (mm)		MIK (mg/ml)		MBK (mg/ml)	
	***V.angulluri um* M1**	***S. agalactiae* Pas.Ins. 55118**	***V.angullurium* M1**	***S. agalactiae* Pas.Ins. 55118**	***V.angullurium* M1**	***S. agalactiae* Pas.Ins. 55118**
Zeytin meyvesi etanol	16.36± 0.87	16.30±0.23	40	40	80	80
Zeytin meyvesi metanol	10.58±0.32	12.55±0.13	20	40	40	40
Zeytin meyvesi su	14.86±0.53	13.27±1.68	20	40	40	40
Zeytin meyvesi aseton	13.56±1.22	16.56±0.73	20	10	40	10
Yaprak etanol	13.61±0.60	12.93±0.30	20	40	40	80
Yaprak metanol	10.28±0.43	12.33±0.43	20	20	40	40
Yaprak su	12.93±0.50	11.79±0.52	40	40	80	80
Yaprak aseton	9.02±0.42	10.75±0.18	40	40	80	80

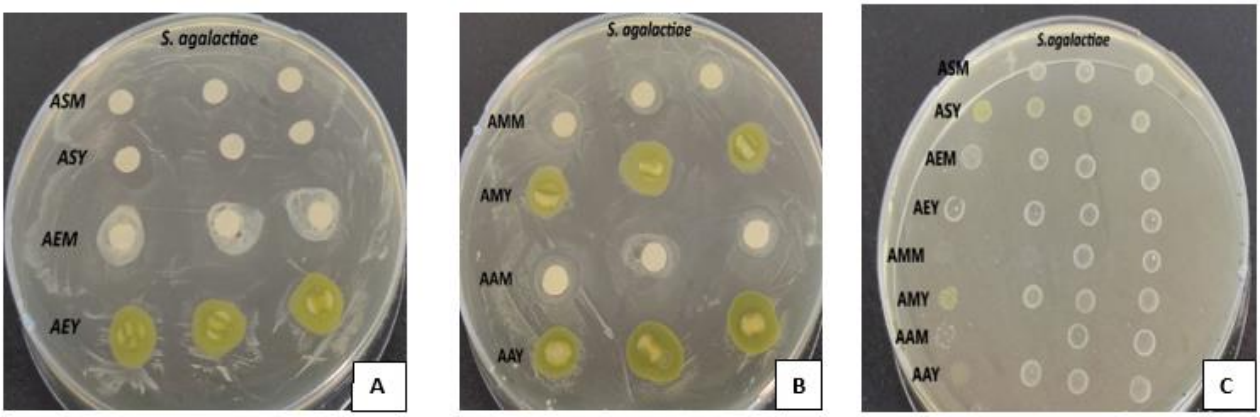


Şekil 1. Ayvalık Yağlık çeşidi zeytin meyvesi ve yaprak ekstrelerinin *V.angullurium* M1 üzerinde antimikrobiyal aktivitesi

(A) Su zeytin meyvesi (ASM), su yaprak (ASY), etanol zeytin meyvesi (AEM), etanol yaprak (AEY) ekstrelerinin disk difüzyon sonuçları

(B) Metanol zeytin meyvesi (AMM), metanol yaprak (AMY), aseton zeytin meyvesi (AAM), aseton yaprak (AAY) ekstrelerinin disk difüzyon sonuçları

(C) Su zeytin meyvesi (ASM), su yaprak (ASY), etanol zeytin meyvesi (AEM), etanol yaprak (AEY), metanol zeytin meyvesi (AMM), metanol yaprak (AMY), aseton zeytin meyvesi (AAM), aseton yaprak (AAY) ekstrelerinin MBK sonuçları

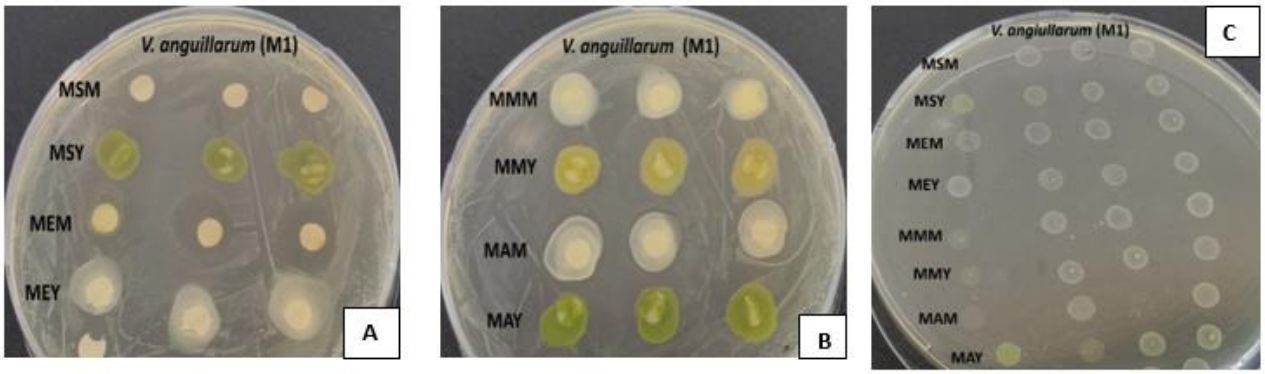


Şekil 2. Ayvalık Yağlık çeşidi zeytin meyvesi ve yaprak ekstrelerinin *S.agalactiae* Pas.Ins. 55118 üzerinde antimikrobiyal aktivitesi

(A) Su zeytin meyvesi (ASM), su yaprak (ASY), etanol zeytin meyvesi (AEM), etanol yaprak (AEY) ekstrelerinin disk difüzyon sonuçları

(B) Metanol zeytin meyvesi (AMM), metanol yaprak (AMY), aseton zeytin meyvesi (AAM), aseton yaprak (AAY) ekstrelerinin disk difüzyon sonuçlar**)**

(C) Su zeytin meyvesi (ASM), su yaprak (ASY), etanol zeytin meyvesi (AEM), etanol yaprak (AEY), metanol zeytin meyvesi (AMM), metanol yaprak (AMY), aseton zeytin meyvesi (AAM), aseton yaprak (AAY) ekstrelerinin MBK sonuçları

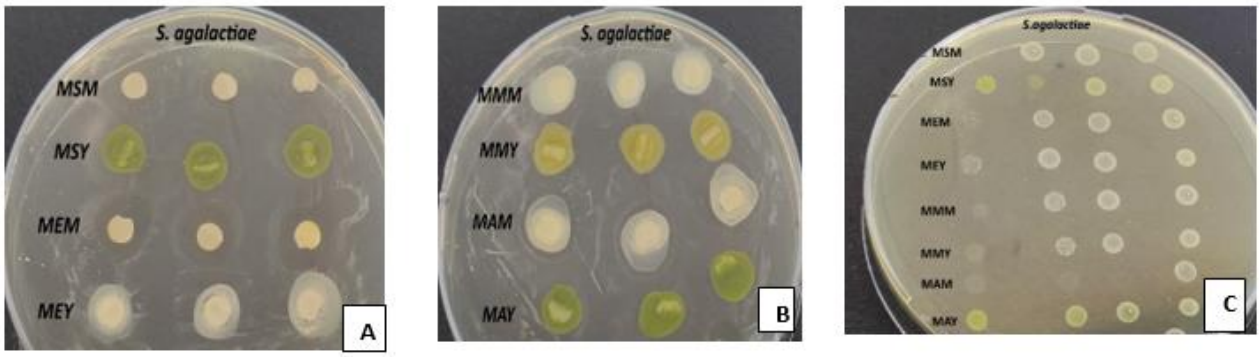


Şekil 3. Manzanilla çeşidi zeytin meyvesi ve yaprak ekstrelerinin *V.angullurium* M1 üzerinde antimikrobiyal aktivitesi

(A) Su zeytin meyvesi (MSM), su yaprak (MSY), etanol zeytin meyvesi (MEM), etanol yaprak (MEY) ekstrelerinin disk difüzyon sonuçları

(B) Metanol zeytin meyvesi (MMM), metanol yaprak (MMY), aseton zeytin meyvesi (MAM), aseton yaprak (MAY) ekstrelerinin disk difüzyon sonuçları

(C) Su zeytin meyvesi (MSM), su yaprak (MSY), etanol zeytin meyvesi (MEM), etanol yaprak (MEY), metanol zeytin meyvesi (MMM), metanol yaprak (MMY), aseton zeytin meyvesi (MAM), aseton yaprak (MAY) ekstrelerinin MBK sonuçları



Şekil 4. Manzanilla çeşidi zeytin meyvesi ve yaprak ekstrelerinin *S.agalactiae* Pas.Ins. 55118 üzerinde antimikrobiyal aktivitesi

(A) Su zeytin meyvesi (MSM), su yaprak (MSY), etanol zeytin meyvesi (MEM), etanol yaprak (MEY) ekstrelerinin disk difüzyon sonuçları

(B) Metanol zeytin meyvesi (MMM), metanol yaprak (MMY), aseton zeytin meyvesi (MAM), aseton yaprak (MAY) ekstrelerinin disk difüzyon sonuçları

(C) Su zeytin meyvesi (MSM), su yaprak (MSY), etanol zeytin meyvesi (MEM), etanol yaprak (MEY), metanol zeytin meyvesi (MMM), metanol yaprak (MMY), aseton zeytin meyvesi (MAM), aseton yaprak (MAY) ekstrelerinin MBK sonuçları

Zeytin meyvesi ve yaprağının balık patojenleri üzerine etkisinin araştırıldığı çalışmalar oldukça sınırlı kalmıştır. Yapılan bir çalışmada, ticari olarak satın alınan zeytin ekstresi, sıcak dumanlanmış gökkuşağı alabalığı (*Oncorhynchus mykiss*) filetolarına uygulanmıştır. Mikrobiyolojik değerlendirme sonuçlarına göre kontrol grubunun raf ömrü 21 gün olarak gözlemlenirken, zeytin yaprağı ekstresi uygulanan grubun 42. günde mikrobiyolojik limit değeri aştığı kaydedilmiştir. Böylece raf ömrü üzerine, zeytin yaprağı ekstresinin önemli ölçüde etkili olduğu belirlenmiştir (Mutlu ve ark., 2016). Yaptığımız çalışmada, İzmir'den toplanan zeytin meyvesi ve yaprak örneklerinden hazırlanan (su, etanol, metanol ve aseton) ekstrelerin test edilen her iki balık patojeni (*V. anguillarum* M1 ve *S. agalactiae* Pas.Ins. 55118) üzerinde yüksek inhibisyon zonu ve düşük MİK ve MBK değerleri ile iyi antimikrobiyal aktivite gösterdiği belirlenmiştir.

SONUÇ

Dünyada hemen hemen birçok ülkede su ürünlerinden balık kolay ulaşılabilir ve diğer protein kaynaklarına göre daha ucuz olması nedeniyle bir besin kaynağı olarak tercih edilmektedir. Kültür balıkçılığı, tüm dünyada olduğu gibi ülkemizde de doğal kaynakların sürdürülebilmesi açısından önem kazanmıştır. Zeytinciliğin oldukça önemli olduğu Ülkemizde zeytin meyvesi ve yapraklarının alternatif değerlendirilme olanaklarının tespiti için yapılan bu çalışmada, İzmir ilinden temin edilen Ayvalık Yağlık ve Manzanilla çeşidi zeytin meyvesi ve yaprak örnekleri balık orijinli gram-negatif *V. anguillarum* M1 ve gram-pozitif *S. agalactiae* Pas.Ins. 55118'e karşı değişik derecelerde antibakteriyel etki gösterdiği belirlenmiştir. Sonuçlar, zeytin meyvesi ve yaprak örneklerinin balık yetiştiriciliğinde yoğun olarak kullanılan antibiyotiklere alternatif doğal koruyucu olarak kullanılma potansiyeli taşıdığını ortaya çıkarmıştır.

TEŞEKKÜR

Zeytin meyvesi ve yaprak örneklerinin teminini sağlayan İzmir Zeytincilik Araştırma Enstitüsü Müdürlüğüne teşekkürlerimizi sunarız.

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➢ *ORAL PRESENTATION*

Çeşitli *Zizyphus jujuba* meyve ve yaprak ekstrelerinin gıda kaynaklı patojenler üzerine antibakteriyel aktivitesinin araştırılması

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Özet

Gıda kaynaklı bakteriyel hastalıklar dünya çapında oldukça büyük problem oluşturmaktadır. 1927 yılında penisilin keşfedilmesiyle bakteriyel hastalıkların önlenmesinde yeni bir çağ başlamıştır. Ancak günümüzde var olan antibiyotiklere kazanılan direnç bakteriyel hastalıklarla mücadelede en büyük zorluk olarak karşımıza çıkmaktadır. Bu çalışmada, *Zizyphus jujuba* (hünnap) meyve ve yapraklarından sıcak su banyosu (SSB) ve sonikatörde (SN) ekstraksiyon yöntemleri ile su ve etanol (%96) ekstreleri hazırlanmıştır. Ekstrelerin gıda kaynaklı *Escherichia coli* ATCC 35218, *Listeria monocytogenes* ATCC 7644 ve *Shigella sonnei* Mu:57'ye karşı antimikrobiyal aktivitesi disk difizyon, minimal inhibisyon konsatrasyonu (MİK) ve minimal bakterisidal konsantrasyonu (MBK) belirleme yöntemleri ile araştırılmıştır. Yapılan çalışmada, en yüksek inhibisyonzon çapı *L. monocytogenes* üzerinde, meyve materyalinde etanol SSB ekstresinde 19.89±0.43 mm inhibisyon zon çapı ile tespit edilmiştir. Hazırlanan ekstrelerin MIK ve MBC değerleri 100-200 mg/mL olarak tespit edilmiştir. Elde edilen veriler, *Z. jujuba* meyve ve yaprak ekstrelerinin gıda ve sağlık sanayilerinde kullanılan sentetik antimikrobiyal maddelere alternatif olarak doğal antimikrobiyal kaynağı olarak kullanılma potansiyeli taşıdığını göstermiştir.

Anahtar Kelimeler: Hünnap, *Escherichia coli, Listeria monocytogenes, Shigella sonnei*

Investigation of the antibacterial activity of various *Zizyphus jujuba* fruit and leaf extracts on foodborne pathogens

Abstract

Foodborne bacterial diseases pose a major problem worldwide. With the discovery of penicillin in 1927, a new era began in the prevention of bacterial diseases. However, resistance to antibiotics available today is the biggest challenge in combating bacterial diseases. In this study, water and ethanol (96%) extracts were prepared from *Zizyphus jujuba* (hünnap) fruits and leaves using hot water bath (SSB) and sonicator (SN) extraction methods. Antimicrobial activity of the extracts against food-borne *Escherichia coli* ATCC 35218, *Listeria monocytogenes* ATCC 7644 and *Shigella sonnei* Mu: 57 was investigated by disk diffusion, minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) methods. In the study, the highest inhibition zone diameter was detected on *L. monocytogenes* with 19.89±0.43 mm inhibition zone diameter with ethanol SSB extract in fruit material. MIC and MBC values of the extracts were determined as 100-200 mg/mL. The data obtained showed that *Z. jujuba* fruit and leaf extracts have the potential to be used as a natural antimicrobial source as an alternative to synthetic antimicrobial agents used in food and health industries.

Keywords: Hünnap, *Escherichia coli, Listeria monocytogenes, Shigella sonnei*

Bu çalışma ilk yazarın yüksek lisans tezinden hazırlanmıştır.

GİRİŞ

Günümüzde bakteriyel kaynaklı hastalıkların çoğalması ve mikroorganizmaların var olan antibiyotiklere kazandığı direnç insanları doğal antimikrobiyal maddeler keşfetme yoluna itmiştir. Uzun yıllardır bitkilerin antimikrobiyal aktiviteleri üzerine yoğun çalışmalar yapılmıştır. Özellikle gıda kaynaklı bakteriyel hastalıklar dünyada yüksek oranda ölüme neden olmaktadır. Gıda kaynaklı bakteriyel hastalığa neden olan patojenler içerisinde *Listeria*

monocytogenes, *Escherichia coli* ve *Shigella sonnei* de yer alır. *L. monocytogenes* gram pozitif, fakültatif anaerobik, spor oluşturmayan, düşük sıcaklıklarda gelişebilme yeteneğine sahip ve birçok mikroorganizmanın inhibe olabileceği tuz ve asit konsantrasyonuna karşı direnç gösteren bir mikroorganizmadır (Luo ve ark., 2017; Rodrigues ve ark., 2017; Hamedi ve ark., 2014). Gıda kaynaklı hastalıklara sebep olan en önemli patojenlerin içerisinde yer alır, en yüksek ölüm oranına sahip bir gıda patojenidir ve yılda yaklaşık 200 milyon dolar maddi kayba yol açabilmektedir (Luksiene ve Paskeviciute, 2011). Enfekte ettiği bireylerde menenjit, septisemi, konjuktivit, deri ve mukoza lokalizasyonları ve kan tablosunda monositoza gibi belirtiler göstermektedir (Yavuz ve Korukluoğlu, 2010). *E. coli* ise gram negatif, spor oluşturmayan bir bakteridir. Enfekte ettiği kişilerde ishal ve idrar yolu enfeksiyonu görülebilmektedir (Fleckenstein ve ark., 2010). *Shigella* infeksiyonlarının yaklaşık %20'sini tavuk eti, balık eti veya deniz ürünlerini içeren salatalar, çiğ olarak tüketilen sebzeler, uygun koşullarda üretilmeyen içme suları gibi etkenler oluşturur (Sağlam ve Şeker, 2016). Kontaminasyon sonrasında insanlarda yaygın olarak ishal görülür. Gıda sanayi insan yaşamında önemli bir yere sahip olduğu için antimikrobiyal maddeler önemli bir yer kaplar. Gıda sanayisinde paketleme ve depolama sırasında bakteri üremesinin engellemek için çeşitli kimsayal maddeler kullanılmaktadır. Ancak kullanılan kimyasal maddelerin olumsuz yan etkileri tüketicilerin doğal antimikrobiyal kaynaklara yönelmelerine neden olmaktadır. *Zizyphus jujuba* (hünnap) Çin halk tıbbında çok yaygın olarak kullanılan bir meyvedir. Hünnap *Rhamnaceae* ailesine aittir, çoğunlukla Asya'nın tropik ve subtropikal bölgelerinde yetişir, binlerce yıldır geleneksel tıpta ilaç olarak kullanılmaktadır (Abd-Alrahman ve ark., 2013). Yapılan çalışmalarda Hünnap meyvesinin immünomodülatör, antioksidan, antitümör, hepatoprotektif ve ayrıca gastrointestinal koruyucu etkilere sahip olduğu bildirilmiştir (Wang ve ark., 2015; Zhang ve ark., 2010; Guo ve ark., 2018). Yapılan literatür taraması sonucunda hünnap meyvesinin içeriğinde bulundurduğu askorbik asit, karotenoidler, fenolik bileşikler ve demir gibi mineraller insan beslenmesinde önemli rollere sahiptir (Promyou ve ark., 2012). Ayrıca yapısında bulundurduğu zengin fenolik bileşikler hünnap meyvesini güçlü bir antioksidan kaynağı yapmaktadır (Koley ve ark., 2011). Bitkilerin yapısında bulunan flavonoidler, tanenler, alkaloid, terpenoid gibi fitokimyasal maddeler birçok enfeksiyonel hastalıkların tedavisinde yaygın olarak kullanılmaktadır (Tariq ve ark., 2011). Hünnap meyvesinin yapısında yüksek miktarda bulunan falavonoidler ve fenolik bileşikler, bitkinin antimikrobiyal özelliğinide ön plana çıkarmaktadır. Ülkemizde de son yıllarda hünnapa olan ilgi giderek artmaktadır. Gıda ve sağlık gibi alanlarında ülkemizde hünnap meyve ve yaprağının doğal antimikrobiyal madde kaynağı olarak değerlendirilme potansiyelini belirlemek amacıyla yapılan bu çalışmada, hünnap meyve ve yaprağının değişik ekstrelerinin gıda kaynaklı patojen mikroorganizmalara karşı antimikrobiyal aktivitesi araştırılmıştır.

MATERYAL VE METOT

Meyve ve Yaprak Örneklerinin Temini

Hünnap meyve ve yaprak örnekleri Hatay ilinden temin edilmiştir.

Ekstrelerinin Hazırlanışı

Hünnap meyve ve yaprakları yıkanıp kurutulduktan (Şekil 1) sonra Waring blendır ile öğütülmüştür. Öğütülen meyve ve yaprak örnekleri sıcak su banyosu ve sonikatör cihazları kullanılarak etanol (%96) ve su ile ekstrakte edilmiştir (Şekil 2). Sonikasyon yönteminde öğütülen meyve materyalleri su ve etanol ile yaprak materyali ise su ile buz üzerinde 10'ar dakika aralıklarla toplamda 30 dakika, sıcak su banyosunda ise meyve materyalleri çözücüler ile 78 ve 100°C de, yaprak materyali ise su ile 100°C'de 36 saat ekstrakte edilmiştir. Ekstrakte edilen örneklerin çözücüleri uçurulmuş ve kuru ekstre elde edilmiştir. Su ve etanol meyve/yaprak ekstreleri uygun konsantrasyonlarda dimetilsülfoksit (DMSO) ile çözüldükten sonra 0.45µm'lik filtre ile steril edilmiştir.

Şekil 1. Hünnap meyve ve yaprakları

Şekil 2: Hünnap meyve/yaprak etanol ve su ekstrelerinin hazırlanışı
A: Sıcak su banyosunda ektraksiyon
B: Sonikatör cihazı ile ektraksiyon

Test Mikroorganizmaları

E. coli ATCC 35218, *L. monocytogenes* ATCC 7644ve *S. sonnei* Mu:57 gıda kaynaklı patojen test mikroorganizmaları kullanılmıştır.

Antimikrobiyal Aktivitenin Belirlenmesi

Disk Difüzyon Testi

Hünnap meyve ve yaprak ekstrelerinin antimikrobiyal aktivitesini belirlenmesinde disk difüzyon testi kullanılmıştır. Test mikroorganizmalarından *E. coli* ATCC 35218 ve *S. sonnei* Mu:57 Nutrient Broth (NB) ve *L. monocytogenes* ATCC 7644iseTryptic Soy Broth (TSB) besi yerlerinde geliştirilmiştir. Test mikroorganizmaları, serum fizyolojik ile iki defa yıkandıktan sonra konsantrasyonları 0.5 McFarland'a ayarlanmış ve katı besi yerlerine inoküle edilmiştir. Ekimi yapılan petrilere steril diskler yerleştirildikten sonra disklere hünnap meyve ve yaprak ekstreleri 20 µL (5 mg/disk) damlatılmıştır. Petriler 24 saat 37°C'de inkübe edilmiştir. İnkübasyon süresi sonunda diskler etrafındaki zonlar ölçülerek kaydedilmiştir. Tüm deneyler üç tekrarlı olarak yapılmıştır.

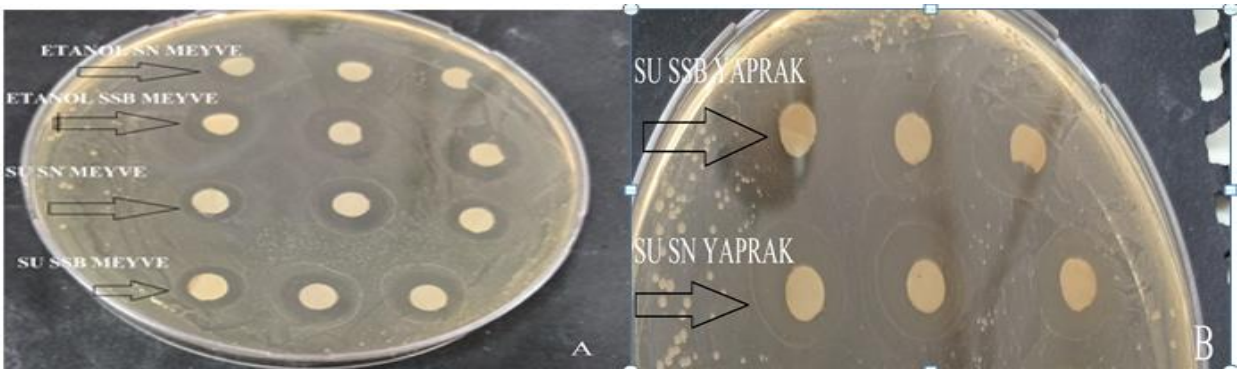
Minimal İnhibisyon (MİK) ve Bakterisidal (MBK) Konsantrasyonlarının Belirlenmesi

Ekstrelerin MİK ve MBK değerleri gıda kaynaklı test mikroorganizmalarına karşı mikrodilüsyon yöntemi ile belirlenmiştir. Ekstre ve besi yeri içeren her tüpe 0.5 McFarland'a ayarlanmış test mikroorganizmaları eklenmiş ve vorteks yapılmıştır. Daha sonra karışımı içeren tüpler 24 saat 37°C'de inkübe edilmiştir. İnkübasyondan sonra, sıvı besi yerinde gelişmenin olmadığı konsantrasyon MİK değerleri olarak kaydedilmiştir. Tüplerden örnekler alınarak spesifik agar besi yerine spot ekimleri yapılmış ve 24 saat 37°C inkübe edilmiştir. İnkübasyon süresinin sonunda, katı besi ortamı üzerinde bakterilerin gelişimini önleyen ekstre konsantrasyonları MBK değerleri olarak değerlendirilmiştir.

BULGULAR VE TARTIŞMA

Hünnap meyve/yaprak su ve etanol ekstrelerinin antimikrobiyal aktivitesi disk difüzyon yöntemi ile belirlenmiştir. Ekstrelerin ayrıca MİK ve MBK değerleri de tespit edilmiştir. Antimikrobiyal aktivitenin belirlenmesinde gıda kökenli patojen test mikroorganizmaları (*E. coli* ATCC 35218, *L. monocytogenes* ATCC 7644 ve *S. sonnei* Mu:57) kullanılmıştır. Disk difizyon deneyinin sonuçlarına göre, meyve ekstrelerinde en yüksek inhibisyon zon çapı *L. monocytogenes* üzerinde 19.89±0.43 mm ile etanol SSB ekstresinde tespit edilmiştir (Şekil 3A). Yaprak materyalinde en yüksek inhibisyon zon çapı 16.25±0.48 mm ile su SN ekstresinde tespit edilmiştir (Şekil 3B). Meyve ekstrelerinde en yüksek inhibisyon zon çapı *E. coli* üzerinde 16.73±0.61 mm ile etanol SN ekstresinde tespit edilmiştir. Yaprak ekstreleri arasında ise en yüksek inhibisyon zon çapı 13.92±0.24 mm ile su SN ekstresinde tespit edilmiştir. Meyve ekstrelerinde, *S. sonnei* Mu:57 üzerinde en yüksek inhibisyon zon çapı 17.33±0.61 mm ile meyve SN etanol ekstresinde tespit edilmiştir (Şekil 4A). Yaprak ekstrelerinde en yüksek inhibisyon zon çapı 15.19±0.75 mm ile su SSB ekstresinde tespit edilmiştir (Tablo 1-2). Ancak, su SSB ekstresi bu mikroorganizma üzerine statik bir etki göstermiştir (Şekil 4B). Bazı hünnap meyve/yaprak su ve etanol ekstrelerinin inhibisyon zon çapı, kontrol grubu olarak kullanılan kanamisin (30 µg) antibiyotiğinin oluşturduğu zon çapından daha yüksek bulunmuştur. Ekstrelerinin MİK ve MBK değerleri 100-200 mg/mL olarak belirlenmiştir. SSB ekstraksiyon

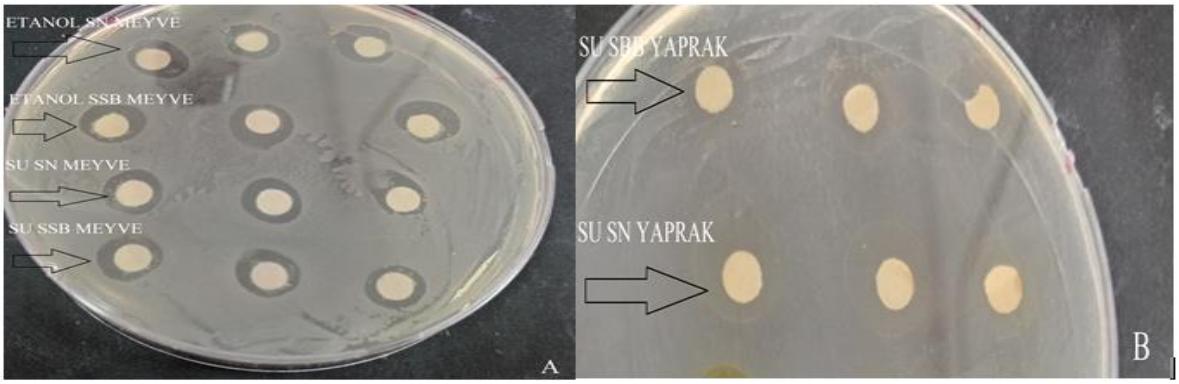
yöntemi ile elde edilen ekstrelerin en düşük MİK ve MBK değeri etanol meyve ekstresinde 100 mg/mL olarak *L. monocytogenes* ATCC 7644'e karşı tespit edilmiştir (Tablo 4-6). SN yöntemi ile elde edilen ekstrelerde en düşük MİK ve MBK değeri etanol meyve ekstresinde *L. monocytogenes* ATCC 7644'e karşı, su yaprak ekstresinde *S. sonnei* Mu:57 mikroorganizmasına karşı 100 mg/mL olarak kaydedilmiştir (Tablo 3-5). Saima ve arkadaşları (2013) yaptığı çalışmada, olgun ve genç hünnap yapraklarının metanolik ekstresinin 50 µL ve 100 µL/disk konsantrasyonlarında *E. coli* mikroorganizması üzerine antimikrobiyal aktivitesi araştırılmıştır. Yaptıkları araştırma sonucuna göre olgun yaprak metanolik ekstresinin inhibisyon zon çapını 20±0.02 mm, genç yaprak metanolik ekstresinin inhibisyon zon çapını 20±0.04 mm olarak kaydetmişlerdir. Bizim çalışmamızda kullandığımız 20 µL/disk konsantrasyonundaki su SN ve SSB yaprak ekstreleri *E. coli* mikroorganizması üzerinde daha düşük inhibisyon zon çapı oluşturmuştur. MİK sonuçlarını ise olgun yapraklarda 47.6±0.05 mg/mL genç yapraklarda ise 97.9±04 mg/mL olarak kaydetmişlerdir. Çalışmalardaki farklı sonuçlar, kullanılan çözücü, ekstraksiyon yöntemi, her bir diske uygulanan etken madde miktarı ve bitkisel materyalin temin edildiği coğrafi bölgenin antimikrobiyal aktivite üzerinde değişik etkiler oluşturmasından kaynaklanabilir. Abd-Alrahman ve arkadaşlarının (2013) yaptığı başka bir çalışmada ise hünnap tohumlarının %50 etanol-su ekstresinin 50-250 ve 1000 µg/mL konsantrasyonlarda *L. monocytogenes* ve *E. coli* üzerinde antimikrobiyal aktivitesini disk difüzyon ve MİK deneyleri yaparak araştırmışlardır. Yapılan çalışmada *L. monocytogenes* üzerine disk difüzon testi 1000 µg/mL konsantrasyonda 16.5 mm olarak, *E. coli* üzerinde 1000 µg/mL konsantrasyonda 17.5 mm ile en yüksek inhibisyon zon çapını kaydetmişlerdir. MİK sonuçlarını *L. monocytogenes* üzerine 51.25 µg/mL, *E. coli* üzerine 52.5 µg/mL olarak kaydetmişlerdir.



Şekil 3: Hünnap meyve/yaprak etanol ve su ekstrelerinin *L. monocytogenes* ATCC 7644 mikroorganizması üzerine antimikrobiyal aktivitesi

A: Meyve SSB-SN ekstrelerinin disk difüzyon sonuçları

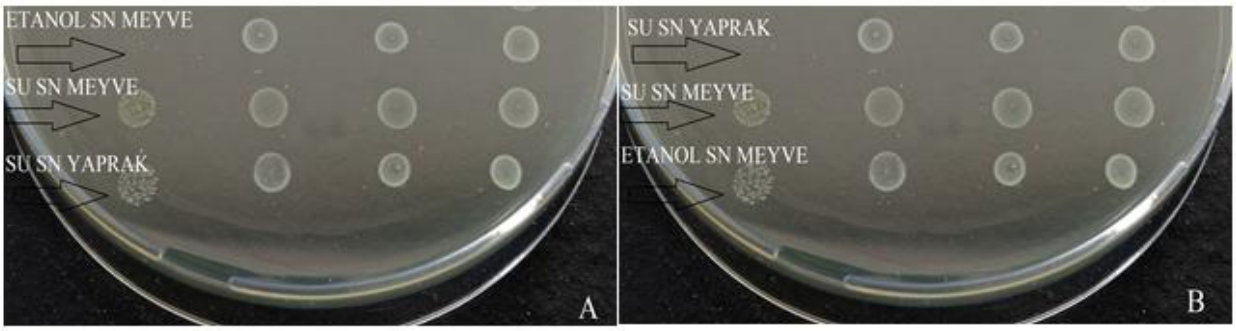
B: Yaprak SSB-SN ekstrelerinin disk difüzyon sonuçları



Şekil 4: Hünnap meyve/yaprak etanol ve su ekstrelerinin *S. sonnei* Mu:57 mikroorganizması üzerine antimikrobiyal aktivitesi

A: Meyve SSB-SN ekstrelerinin disk difüzyon sonuçları

B: Yaprak SSB-SN ekstrelerinin disk difüzyon sonuçları



Şekil 5: Hünnap meyve/yaprak etanol ve su ekstrelerinin antimikrobiyal aktivitesi

A: Meyve/yaprak SN ekstrelerin *L. monocytogenes* ATCC 7644 üzerine etkisi

B: Meyve/yaprak SN ekstrelerin *S. sonnei* Mu:57 üzerine etkisi

Tablo 1: Hünnap meyve/yaprak SSB ekstraksiyon ile elde edilen etanol ve su ekstrelerinin disk difizyon sonuçları

Ekstreler	İNHİBİSYON ZON ÇAPI (mm)		
	Test mikroorganizmaları		
	L. monocytogenes ATCC 7644	*E. coli* ATCC 35218	*S. sonnei* Mu:57
Etanol SSB meyve	19.89± 0.43	16.39± 0.40	17.09± 0.86
Su SSB meyve	16.17± 0.69	14.72± 0.98	15.1± 1.65
Su SSB yaprak	15.80± 1.39	13.36± 0.44	14.04± 0.47
Kanamisin(30 µg) Antibiyotiği	16.81±0.30	11.94±1.88	14.12±1.63

Tablo 2: Hünnap meyve/yaprak SN ekstraksiyon ile elde edilen etanol ve su ekstrelerinin disk difüzyon sonuçları (mm)

Ekstreler	**İNHİBİSYON ZON ÇAPI (mm)**		
	Test mikroorganizmaları		
	L. monocytogenes ATCC 7644	*E. coli* ATCC 35218	*S. sonnei* Mu:57
Etanol SN meyve	19.21±1.48	16.73± 0.61	17.33± 1.20
Su SN meyve	16.14±0.68	15.25± 1.32	15.91± 1.46
Su SN yaprak	16.25±0.48	13.92±0.24	13.06± 0.74
Kanamisin(30 µg) Antibiyotiği	16.81±0.30	11.94±1.88	14.12±1.63

Tablo 3: Hünnap meyve/yaprak SN ekstraksiyon ile elde edilen etanol ve su ekstrelerinin MİK sonuçları

	Minimal inhibisyon konsantrasyonu (MİK) (mg/mL)		
Ekstreler	Test mikroorganizmaları		
	L. monocytogenes ATCC 7644	*E. coli* ATCC 35218	*S. sonnei* Mu:57
Etanol SN meyve	100	200	200
Su SN meyve	100	200	200
Su SN yaprak	200	200	100

Tablo 4: Hünnap meyve/yaprak SSB ekstraksiyon ile elde edilen etanol ve su ekstrelerinin MİK sonuçları

	Minimal inhibisyon konsantrasyonu (MİK) (mg/mL)		
Ekstreler	Test mikroorganizmaları		
	L. monocytogenes ATCC 7644	*E. coli* ATCC 35218	*S. sonnei* Mu:57
Etanol SBB meyve	100	200	200
Su SBB meyve	100	200	200
Su SBB yaprak	200	100	100

Tablo 5: Hünnap meyve/yaprak SN ekstraksiyon ile elde edilen etanol ve su ekstrelerinin MBK sonuçları

	Minimal bakterisidal konsantrasyonu (MBK) (mg/mL)		
Ekstreler	Test mikroorganizmaları		
	L. monocytogenes ATCC 7644	*E. coli* ATCC 35218	*S. sonnei* Mu:57
Etanol SN meyve	100	200	200
Su SN meyve	200	200	200
Su SN yaprak	200	200	200

Tablo 6: Hünnap meyve/yaprak SSB ekstraksiyon ile elde edilen etanol ve su ekstrelerinin MBK sonuçları

	Minimal bakterisidal konsantrasyonu (MBK) (mg/mL)		
Ekstreler	Test mikroorganizmaları		
	L. monocytogenes ATCC 7644	*E. coli* ATCC 35218	*S. sonnei* Mu:57
Etanol SSB meyve	100	200	200
Su SSB meyve	200	200	200
Su SSB yaprak	200	200	200

SONUÇ

Bu çalışmada hünnap meyve/yaprak materyallerinin su ve etanol ekstrelerinin antimikrobiyal aktivitesi gıda kökenli test mikroorganizmalarına karşı tespit edilmiştir. Yapılan çalışmada hazırlanan tüm ekstrelerin test edilen mikroorganizmalar üzerinde antimikrobiyal aktivitesi gözlemlenmiştir. Hünnap meyve ve yaprak ekstreleri gıda ve sağlık sanayilerinde alternatif doğal antimikrobiyal madde olarak kullanım potansiyaline sahip olduğu tespit edilmiştir.

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➢ *ORAL PRESENTATION*

Insight into effects of *Gingko biloba* extract on probiotic properties of *Lactobacillus rhamnosus* GG

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Abstract
Plants that make up the code of life play important roles in the field of health, nutrition, protection, and traditional treatments. Previous researches showed that the high therapeutic effects of *Ginkgo biloba* leaves extract have proven their effects on Alzheimer's disease and dementia. Furthermore, interactions between gastrointestinal microbiota and brain functions are the focus of today. Lactic acid bacteria, which make up an important part of gut microbiota and directly interact with nutrition play a key role in the immune system of the human body. In the present study, the effects of *Ginkgo biloba* leaf extract on *Lactobacillus rhamnosus GG*, which is a well-known probiotic, have been investigated. The aim of this study was to investigate the effects *Ginkgo biloba* leaf extract on the auto-aggregation abilities of *Lactobacillus rhamnosus GG*, its interaction with other pathogenic bacteria (co-aggregation with *Escherichia coli* and *Staphylococcus aureus*), and *in vitro* resistance to the gastric pepsin. Within the scope of the study, bacterial growth kinetics, probiotics auto-aggregation and co-aggregation, pepsin resistance and antioxidant experiments were performed. As a result of the present experiments, significant aggregation, co-aggregation, pepsin resistance and antioxidant results were obtained compared to the control groups. *L. rhamnosus GG* cultures treated with Ginkgo biloba extract showed well growth. Bacterial auto-aggregation was significantly increased at all treatment concentrations. Furthermore, co-aggregation with *E. coli* was increased significantly every hour. Co-aggregation ability with another pathogen, *S. aureus*, was increased significantly at the 1st, 2nd and 3rd hours. It has been found that the extract has positive effects on pepsin resistance and antioxidant effects of the probiotic. In conclusion, modulation of probiotic properties of *L. rhamnosus* GG by *Ginkgo biloba* extract may lead to better indirect health benefits to the host.
Keywords: Aggregation, antioxidant, *Gingko biloba*, pepsin resistance, probiotics.

INTRODUCTION

Plants are a key to human life and used in areas such as traditional treatments, nutrition, and protection. They have inspired many vital studies from the past to the present. The most established traditional therapies in the world are Asian traditional therapies dating back 5000 years. Known as a living fossil, *Ginkgo biloba* is a tree native to Japan, Korea, and China from Asian countries. This tree, which has been living for more than 200 million years and is also known as the maidenhair tree and has survived until today with very little evolution, is thought to belong to the Zhejiang region in eastern China (Singh et al. 2008). Although studies on the biological activities of ginkgo leaves began 20 years ago, its pharmaceutical value has recently been discovered. Pharmacologically active compounds such as flavonoids (kaempferol, quercetin, myricetin, apigenin, isorhamnetin, luteolin and tamarixetin) and terpenes lactones (ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J, ginkgolide M, ginkgolide K, ginkgolide L and bilobalide) have been determined from ginkgo leaves used in Chinese traditional treatment (Van Beek & Montoro 2009; Liao et al. 2011). Studies have shown that leaf extract of *Ginkgo biloba* tree has important effects in the treatment of diseases such as Alzheimer's and age-related memory loss, neurodegenerative diseases, cerebral insufficiency, and neurosensory disorders (Kleinjnen & Knipschield 1992; Oyama et al. 1996; Yucheng et al.1996; Robert et al. 2003; Ralf et al. 2011). In the recent years, while research between gastrointestinal tract (GIT) and brain functions quickly achieve prominence in the scientific world, researchers reported an important link between the gut microbiome and the brain (Carabotti et al. 2015; Amenyogbe et al. 2018; Park, 2018). The GIT is home to microorganisms such as probiotics, pathogens, or commensal which interact closely with the host. The enteric microbiota distributed in the human GIT is unique to each person. However, the phylotypes and amount of bacteria in healthy individuals show similarities. The gut-brain interactions are studied under the

complex term "gut-brain axis" (GBA). Both experimental and clinical studies show that the enteric microbiota has a significant effect on the gut-brain axis (GBA) with these studies, it has been reported that probiotics, which are called microbes that live in the GIT and benefit their host, play an important role in the gut-brain interactions (Saulnier et al. 2013; Foster et al. 2013; Hill et al. 2014; Emge et al.2015). Probiotics, which make up an important part of gut microbiota and directly interact with nutrition play a key role in the immune system of the human body (Billoo et al., 2006; Kim et al., 2006; Lee & Salminen, 1995; Salminen et al., 1996). In the present study, the effects of the extract obtained from the leaves of the *Ginkgo biloba* tree, on growth kinetics, auto-aggregation, and pepsin resistance of probiotic bacterium *Lactobacillus rhamnosus* GG were investigated.

MATERIALS AND METHODS

Extraction of *Ginkgo biloba* leaves

Extraction with ethanol was preferred for simple extraction of the compounds found in ginkgo leaves. 4 g of dried *Ginkgo biloba* leaves were added to 100 mL of 99% ethanol on shaking magnetic stirrer for 24 hours. The mixture obtained after incubation was filtered through filter paper.

Growth of probiotic bacteria in the presence of *Ginkgo biloba* leaf extract and bacterial growth kinetics

Lactobacillus rhamnosus GG, kindly provided from Chr. Hansen, Turkey, was grown in LABSEM medium (Majumder et al., 2011). Probiotic bacteria were treated with different concentrations of ginkgo biloba extract. This assay was performed by measuring with a densitometer every 4 hours for 24 hours. Growth kinetics experiment is most important to determine the minimum inhibitory concentration (MIC) value of the extract used in the treatment. Experimental groups were treated using 125, 250 and 500 µg/mL concentrations of *Ginkgo biloba* leaf extract. Prepared groups were incubated for 24 h at 37°C. Control groups contain only *Lactobacillus rhamnosus* GG and solvent ethanol in LABSEM medium.

Probiotics auto-aggregation

Probiotic cells were grown for 16 hours in the presence of *Ginkgo biloba* leaf extract, and harvested in stationary phase (3200 g, 15 min), followed by washing with Phosphate-buffered saline (PBS) and re-suspended in PBS to OD_{600} 0.5 (Kos et al., 2003). Every hour, 100 µL of upper part of the suspension was taken, mixed with 900 µL of PBS and the absorbance was read using a spectrophotometer. The percentage of auto-aggregation is calculated according to equation 1;

$$Auto - aggregation\%: (1 - \frac{A_t}{A_0}) \times 100$$

where A_t is the absorbance measured after incubation and A_0 is the absorbance measured at 0th hour (Kos et al., 2003).

Bacterial Co-Aggregation

Communities formed by microorganisms of different species clinging to each other and colonizing are known as co-aggregation. It is necessary to determine the co-aggregation effect resulting from the interaction of probiotics with E. coli and S. aureus bacteria, which are pathogenic bacterial strains. Probiotic bacteria are designed as two separate setups as control and treatment groups. *Lactobacillus rhamnosus* GG cells were incubated for 16 hours in the presence of *Ginkgo biloba* leaf extract and the pathogen strains were grown in Nutrient Broth medium, without treatment. All experiment groups harvested in stationary phase (3200 g, 15 min) than washed with PBS and re-suspended in PBS to OD600 0.5 (Kos et al., 2003). Then, 2 mL of probiotic suspension was mixed with 2 mL of either E. coli or S. aureus suspension. For every hour, 100 µL of upper part of the suspension was taken,

mixed with 900 µL of PBS and the absorbance was read using a spectrophotometer. The percentage of co-aggregation is calculated according to equation 2,

$$Co - aggregation\%: (1 - \frac{A_t}{A_0}) \times 100$$

where At is the absorbance measured after incubation and A0 is the absorbance measured at 0th hour (Kos et al., 2003).

Resistance to pepsin of probiotics

Lactobacillus rhamnosus GG was grown at 37ºC for 16 hours as control and treatment groups. Bacterial cells were harvested in stationary phase (3200 g, 15 min) and washed with PBS. The treatment and control groups were incubated for 3 hours at 37ºC in PBS containing 3 mg/mL pepsin. After incubation, the cultures were inoculated on MRS agar and incubated at 37ºC for 48 hours and colonies were counted (Alp et al., 2020).

Antioxidant capacity using DPPH Scavenging Assay

Antioxidant effects of probiotics treated with *Ginkgo biloba* leaf extract were examined using the DPPH scavenging assay. 25 mg/L free-radical DPPH was prepared with methanol. Supernatant (100 µL) obtained after centrifugation and DPPH (100 µL) were added to the 96-well plate. Then, the plate was incubated for 30 min at RT in dark. Absorbance was measured with spectrophotometer at the wavelength of 517 nm. Decreased absorbance, so the remaining amount of DPPH was determined as the amount of free radical scavenging. The results were calculated according to equation 3;

$$Antioxidant\% = \left(\frac{Control_{Abs} - Sample_{Abs}}{Control_{ABS}}\right) \times 100$$ (Brand et al., 1995).

RESULTS AND DISCUSSION

Growth of probiotic bacteria in the presence of *Ginkgo biloba* leaf extract and bacterial growth kinetics

In this study, *Ginkgo biloba* leaf extract were used in concentrations of 125, 250, and 500 µg/mL. The groups treated with *Ginkgo biloba* leaf extract were not inhibited, compared the control group (Figure 1). On other words, the growth of *Lactobacillus rhamnosus* GG was not affected by the extract. Previous studies have found that *Ginkgo biloba* leaf extract has an antimicrobial effect on pathogenic microorganism (Sati & Joshi; 2011). However, it did not show an antimicrobial effect on probiotic bacteria whereas its effect on pathogenic bacteria. The fact that *Ginkgo biloba* leaf extract has no antimicrobial effects on *Lactobacillus rhamnosus* GG suggests that it can have a potential to have selectively positive effects on beneficial bacteria in the intestinal microflora.

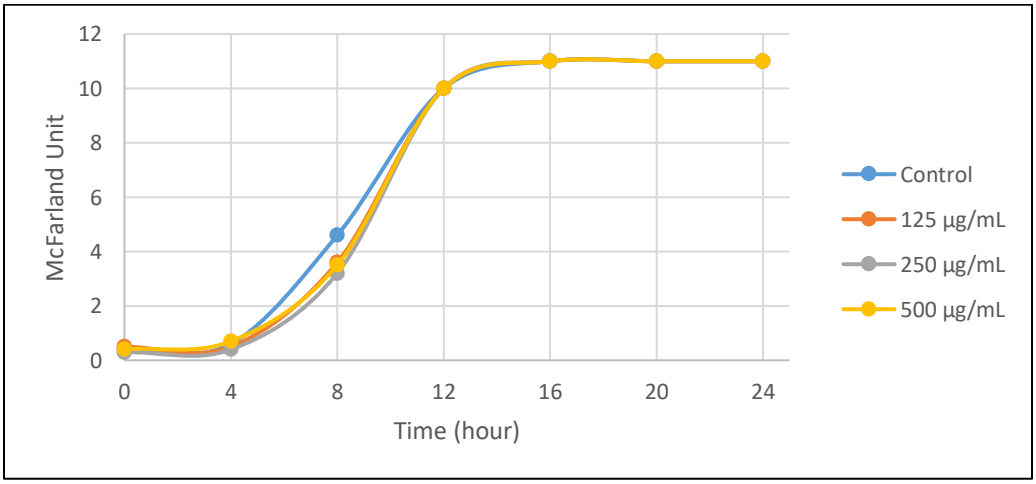

Figure 1: Bacterial Growth Kinetics of *Lactobacillus rhamnosus* GG treated with ethanol extract of *Ginkgo biloba.*

Probiotics auto-aggregation

Although there are many protective mechanisms in the human body, it is often seen that enteric pathogens cause infection by colonizing on microflora present in the gastrointestinal tract (Sekirov et al., 2010). Adhesion to the intestinal epithelial cell is an important precondition for colonizing probiotic strain in gastrointestinal tract (Alander et al., 1997; Freter 1992; Pedersen and Tannock 1989). Thus, adhesion to the gastrointestinal epithelium is an important condition for probiotic bacteria to survive in the gut environment (Boris et al., 1997; Del Re et al., 1998).

The results showed that each concentration (125, 250 and 500 µg/mL) of the extract significantly (p<0.05) increased the auto-aggregation of *Lactobacillus rhamnosus* GG at 1st and 2nd hour when compared the control (Figure 2). There is no significant alteration found at the 3rd hour for the concentrations of 125, 250 and 500 µg/mL. These results could mean *Ginkgo biloba* leaf extract improved the adhesion and live abilities of *Lactobacillus rhamnosus*, and survival in the gastrointestinal tract (Boris et al., 1997; Del Re et al., 1998).

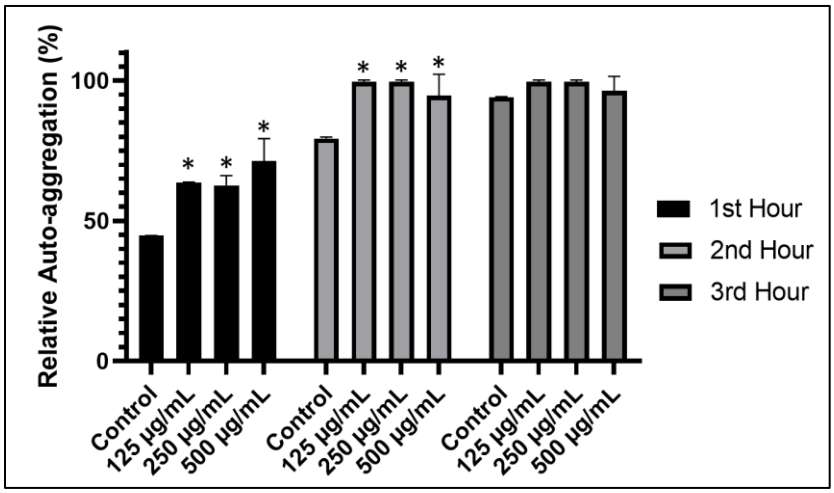

Figure 2: Auto-aggregation of *Lactobacillus rhamnosus* GG treated with ethanol extract of *Ginkgo biloba.* Asterisks (*) indicate that there is significant difference (p<0.05) as compared to control group, according to one-way ANOVA.

Bacterial co-aggregation

The co-aggregation effect of *Lactobacillus rhamnosus* was performed with pathogenic strains *E. coli* and *S. aureus*. The clustering of different microorganism communities is an important detail for the life race in the GIT (Kos et al., 2003). Co-aggregation of *Lactobacillus rhamnosus* GG with *E. coli* was significantly (p<0.05) increased by the extract for each hour of the experiment, as compared to control group (Figure 3A). However, co-aggregation of *Lactobacillus rhamnosus* GG with *S. aureus* just showed a significantly increased at 1st and 2nd hours, compared to control group (Figure 3B). It appears that the "barrier" effect, one of the most important modes for the probiotic effect, can be achieved. The results of the co-aggregation assay indicate that treatment with *Ginkgo biloba* extract could exert its antibacterial effect against pathogenic bacteria by preventing or inhibiting their colonization through increasing the co-aggregation abilities of *lactobacillus rhamnosus* GG.

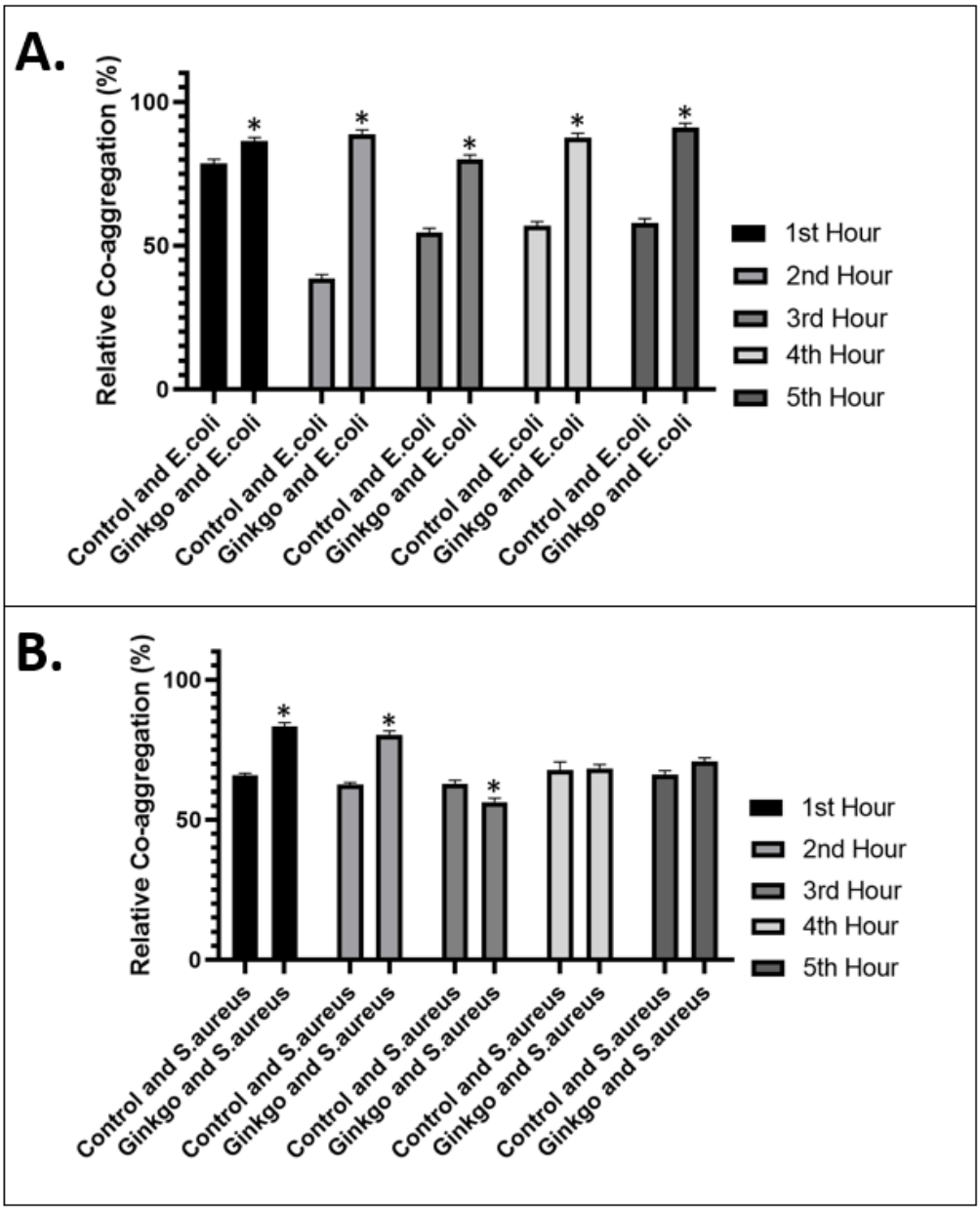

Figure 3: Bacterial co-aggregation of *Lactobacillus rhamnosus* GG with **(A.)** *E. coli* and **(B.)** *S. aureus.* Asterisks (*) indicate that there is significant difference ($p<0.05$) as compared to control group, according to one-way ANOVA.

Resistance to pepsin of probiotics

Foods taken into the body are exposed to various enzymes and salts while passing through the digestive tract, and pepsin is one of these enzymes. When probiotics are taken into the body through diet, they pass through the same digestive tract and therefore resistance to pepsin is important (Alp et al., 2020). Pepsin resistance was 23.4% in the group treated with *Ginkgo biloba* leaf extract, while it was 9.6% in the control group (Table 1). Compared with the control group, *Lactobacillus rhamnosus* treated with *Ginkgo biloba* leaf extract may show higher resistance to pepsin in the digestive tract.

Table 1: Resistance of *Lactobacillus rhamnosus* GG to pepsin.

	CFU/mL	Resistance Percentage
Control	$11.8x10^6$	9.6%
Ginkgo-treated	$28.1x10^6$	23.4%

Antioxidant capacity using DPPH Scavenging Assay

In the present study, we examined the antioxidant property of cell-free supernatant of *Lactobacillus rhamnosus* GG treated with *Gingko biloba* leaf extract. As seen in Figure 4, he results showed that there is no significant difference between the groups (i.e. between *Lactobacillus rhamnosus* GG treated with the extract and the control group). However, this probiotic bacteria showed 19-24% DPPH scavenging activity (Figure 4). Previous studies also showed that *Lactobacillus rhamnosus* GG has antioxidant effects and it is also known that *Gingko biloba* is a highly antioxidant (Bridi et al., 2001).

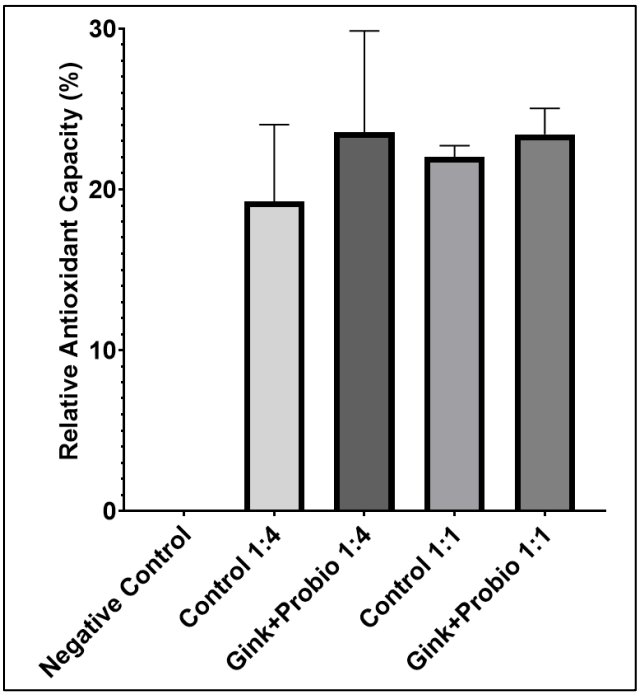

Figure 4: Antioxidant properties of *lactobacillus rhamnosus* GG when treated with *Gingko biloba* leaf extract using DPPH Scavenging Assay.

CONCLUSION

In the present study, growth kinetics, bacterial auto-aggregation, co-aggregation, resistance to pepsin, and antioxidant properties *Lactobacillus rhamnosus* GG, which is a beneficial microorganism, were tested after grown in the presence of *Gingko biloba* leaf extract. *Lactobacillus rhamnosus* GG, when treated with *Ginkgo biloba* leaf extract, showed significant increase in auto-aggregation, co-aggregation and resistance to pepsin. The results of experiments indicate that the vital conditions of the probiotics could be modulated by *Ginkgo biloba* leaf extract, consequently after their adhesion and colonization capabilities. Furthermore, future studies could investigate how *Gingko biloba* leaf extract affect the surface proteins of probiotic bacteria, which are of great importance for bacterial adhesion.

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➢ *ORAL PRESENTATION*

Biyolojik delme işlemi uygulanmış ladin diri ve olgun odununun yoğunluk ve mekanik özellikleri

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Özet

Bu çalışmada, ülkemizin Doğu Karadeniz Bölgesinde yayılış gösteren ve permeabilitesi düşük ağaç türlerinden biri olan Doğu ladini (*Picea orientalis* L.) odununun emprenye edilebilirliğini artırmak amacıyla uygulanan ve biyoteknolojik yöntemlerden biri olan biyolojik delme (bio-incising) işleminin odun yoğunluğu ve mekanik özellikler üzerindeki etkileri incelenmiştir. Bu kapsamda, Doğu ladini diri ve olgun odun kısımlarından elde edilen odun örneklerine (10 x 2,5 x 2,5 cm) biyolojik delme işlemi uygulanmıştır. Bu uygulama sonucunda bir beyaz çürüklük mantarı olan *Physisporinus vitreus*'un ladin diri ve olgun odun kısımlarının yoğunluk ve mekanik özellikleri üzerindeki etkilerini daha iyi anlayabilmek için odun yoğunluk testleri ve liflere paralel basınç direnç testleri yapılmıştır. Elde edilen sonuçlar, *P. vitreus* mantarının ladin odunundaki ağırlık kaybı artışına bağlı olarak olgun odunda göz ardı edilebilir kayıplara neden olduğunu, fakat diri odundaki kayıpların ise önemli düzeyde olduğunu ortaya koymuştur. Ayrıca, *P. vitreus* mantarının % 10 ağırlık kaybına kadar tükettiği kimyasal bileşen ve maddelerin olgun odunda ve diri odunda farklı olma ihtimalinin yüksek olduğunu göstermiştir.

Anahtar Kelimeler: Doğu Ladini, Permeabilite, Biyolojik Delme, Yoğunluk, Liflere Paralel Basınç Direnci.

Density and mechanical properties of biologically incised spruce sapwood and heartwood

Abstract

This study was aimed to enhance the permeability of spruce wood (*Picea orientalis* L.) distributed in the Eastern Black Sea Region of Turkey. Since it possesses low permeability, this study evaluated the effects of bioincising on the density and mechanical properties of spruce wood. Bioincising is one of the most known biotechnological approaches to increase permeability of wood. In this context, bioincising process by employing the white-rot fungus *Physisporinus vitreus* was applied to the wood specimens (10 x 2,5 x 2,5 cm) obtained from the sap- and heartwood portions of spruce wood. Afterwards, wood density and compression strength tests were run on the non-bioincised and bioincised wood specimens to understand the effects the process. The results of density and strength tests showed that bio-incising process increased the mass losses in both sap- and heartwood. In heartwood, the wood density and compression strength values reduced negligibly; however, the decreases in wood density and compression strength in sapwood were significant. It is likely that the chemical components of wood consumed by the fungus up to 10 % of mass loss were different in the sap- and heartwood.

Keywords: *Picea orientalis* L., Permeability, Bio-incising, Density, Compression strength parallel to grain test.

GİRİŞ

Günümüzde emprenyesi güç olan ağaç türlerinin emprenye işlemlerinde yüksek basınç ve yüksek vakum uygulaması ve bu basınç ve vakum değerlerinde uzun süre beklemek gibi prosedürler izlenmektedir. Bu metotların kullanılmasıyla yeterli emprenye madde penetrasyon ve retensiyon seviyelerine ulaşılabilmektedir. Fakat bu metotlar başlangıçtaki yatırım maliyetinin yüksek olmasından dolayı az miktardaki ağaç malzemenin emprenyesi için uygun değildir (Islam ve ark., 2007). Bununla beraber kullanılan metotlarda yüksek basınç uygulanıyor olması odunun mikro yapısında değişikliklere sebep olabilmektedir (Matsumura ve ark.,1999; Drescher ve ark., 2006). Ayrıca bu metotların uygulanabilirliği odunun kurutulması ile yakından ilişkilidir. Kurutma esnasında ladin odun

mikro yapısındaki mevcut kenarlı geçitlerin aspirasyonundan dolayı permeabilitede çok aşırı bir azalma meydana gelmektedir (Ulvcrona, 2006; Lehringer ve ark., 2009a; Yıldız ve ark., 2012). Genellikle permeabilitenin yüksek olması spesifik odun özelliklerini artırmak ve odunu mantarlara, böceklere ve diğer zararlılara karşı korumak için istenen bir özelliktir. Geçmişte permeabilitenin artırılması işlemlerinde enzimler (Durmaz ve ark., 2015), bakteriler (Kobayashi ve ark., 1998; Hansmann ve ark., 2002; Yıldız ve ark., 2012), mavi renk mantarları (Lehringer ve ark., 2010; Danihelová ve ark., 2018) ve odun çürüklük mantarlarından faydalanılmıştır (Schwarze ve ark., 2006; Lehringer ve ark., 2009a; Lehringer ve ark., 2009b; Lehringer ve ark., 2010).
Lehringer ve ark., (2010) yaptıkları çalışmada Avrupa ladini (*Picea abies* (L.) Karst) diri odununda *Physisporinus vitreus* mantar aktivitesinin ve permeabilite artışının diri odunda daha fazla olduğunu, olgun odunda ise kayda değer bir etki göstermediğini ifade etmişlerdir. Bunun aksine, Schwarze ve ark., (2006) *P. vitreus* mantarının hem diri hem de olgun odunda permeabiliteyi artırabildiğini savunmuşlardır. Bahsedilen bu bilgilerden de anlaşıldığı gibi *P. vitreus* mantarı farklı odun kısımlarına bağlı olarak farklı aktiviteler sergilemektedir. Bu yüzden mevcut çalışmada odunun kullanım ömrünün artırılmasında biyoteknolojik yöntemlerin potansiyelini tartışmak amacıyla, ülkemizin Doğu Karadeniz Bölgesinde yayılış gösteren ve permeabilitesi düşük ağaç türlerinden biri olan Doğu ladini (*Picea orientalis* L.)'nin farklı odun kısımlarının yoğunluk ve mekanik özellikler üzerindeki *P. vitreus* mantar aktivitesi tespit edilmeye çalışılmıştır.

1. MATERYAL VE METOD

1.1. Örneklerin hazırlanması ve biyolojik delme işlemi

Bu çalışmada kusur içermeyen odun örnekleri, ülkemizin Artvin yöresinde yetişen Doğu ladininden elde edilmiştir. Kullanılan tüm odun örnekleri doğal değişkenliği minimize etmek amacıyla boyuna yönde ve aynı yıllık halkalara denk gelecek şekilde tam radyal ve teğet yüzeyleri içerecek biçimde elde edilmiştir. Doğu ladininden elde edilen örnekler (10 cm x 2,5 cm x 2,5 cm, uzunluk x genişlik x yükseklik) inkübasyondan önce bir iklimlendirme kabininde 20 ℃ sıcaklık ve % 65 bağıl nemde 2 hafta bekletilmiştir. Daha sonra ladin diri ve olgun odun örnekleri farklı ağırlık kayıpları (% 5-10 ila % 10-15) elde etmek amacıyla 4-8 hafta arasında bir beyaz çürüklük mantarı olan *P. vitreus* FP 90121 mantarına maruz bırakılmıştır.

Biyolojik delme işlemine maruz bırakılan örneklerin fırın kurusu haldeki ilk ve son ağırlıkları tartıldıktan sonra her bir örneğe ait yüzde ağırlık kaybı aşağıdaki formüle göre hesaplanmıştır;

$$WL\ (\%) = [(Wo - W1) / Wo] \times 100 \quad (1)$$

Wo: Örneklerin mantara maruz bırakılmadan önceki fırın kurusu ağırlığı.

W1: Örneklerin mantara maruz bırakıldıktan sonraki fırın kurusu ağırlığı.

1.2. Odun Yoğunluğundaki Değişmeler

Biyolojik delme öncesi ve sonrası toplam 36 adet örneğin her birinde tam kuru yoğunluk değeri aşağıdaki formülden yararlanılarak TS 2472 (1976) standardına göre belirlenmiştir.

$$D^0 = \left(\frac{m^0}{V^0}\right) \quad (2)$$

D_0 = Tam kuru örnek yoğunluğu (g/cm^3)

M_0 =Tam kuru örnek ağırlığı (g)

V_0 = Tam kuru örnek hacmi (cm^3)

$$\%\ Yoğunluk\ değişimi = \left[\frac{D^0 - D^1}{D^0}\right] * 100 \quad (3)$$

D_1 = Biyolojik delme sonrası tam kuru örnek yoğunluğu (g/cm^3)

1.3. Liflere Paralel Basınç Direncinde Değişmeler

Bu çalışmada *P. vitreus* mantarının odunun mekanik direnci üzerinde bir düşüş meydana getirip getirmediğini tespit etmek amacıyla bazı testler gerçekleştirilmiştir. Mevcut çalışmanın başında hazırlanan ve mantara maruz bırakılan örneklerin boyutlarının (2,5 x 2,5 x 10 cm) sadece TS 2595 /1977'de belirtilen liflere paralel çekme direncine uygun olarak (2 x 2 x 3 cm) boyutlarında hazırlanabileceği görülmüştür. Bununla beraber çalışmada biyolojik delme amacıyla kullanılan *P. vitreus* mantarının I. Tip beyaz çürüklük yapması ve odunda öncelikle lignini bozundurmasından dolayı ligninin üzerinde etkili olduğu liflere paralel basınç direnç testinin yapılmasının daha uygun olacağı düşünülmüştür. Bu amaçla ladin diri ve olgun odununa ait kontrol örnekleri, % 5 - 10 ve % 10 - 15 ağırlık kaybı görülen diri - olgun odun örnekleri 2 x 2 x 3 (en x yükseklik x uzunluk) cm boyutlarında kesilmiştir. Bu işlemi takiben toplam 36 örneğin yoğunlukları ve liflere paralel basınç dirençleri Artvin Çoruh Üniversitesi Bilim - Teknoloji Uygulama ve Araştırma Merkezi Mekanik laboratuvarında tespit edilmiştir. Örneklere ait liflere paralel basınç direnç değerleri bir Üniversal Test Cihazı (Zwick) yardımıyla tespit edilmiştir.

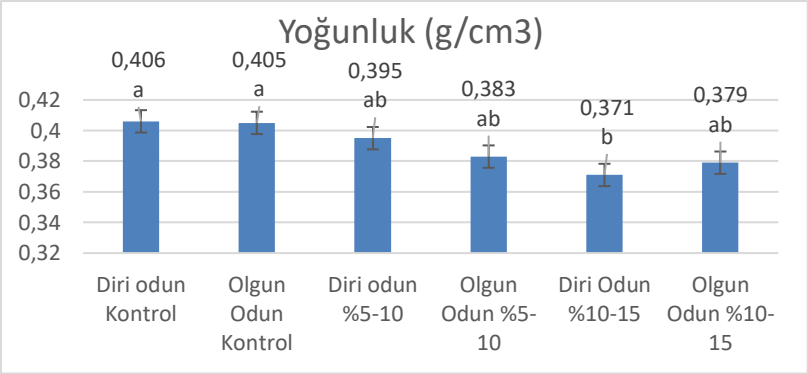
1.4. İstatistiksel Analizler

Hava kurusu yoğunluk değerleri ve liflere paralel basınç direnç değerlerine yönelik tespitler ladin örnek grupları için, diri ve olgun odun ayrımına gidilerek, iki farklı ağırlık kayıpları ve kontrol örnekleri olmak üzere toplam 6 gruba ait veriler kendi içerisinde birbiri ile karşılaştırılmış, bu amaçla istatistiki analiz metotlarından yararlanılmıştır. Burada farklılıkların ortaya konulmasında Tukey testi, ortalamaların karşılaştırılmasında ise varyans analizi kullanılmıştır. Bütün karşılaştırmalarda güven düzeyi % 95 olarak alınmış ve tüm istatistiki değerlendirmeler JMP 5.0.1 paket programı ile yapılmıştır.

2. TARTIŞMA ve SONUÇ

2.1. Meydana gelen ağırlık kayıplarının odun yoğunluğu üzerindeki etkileri

Biyolojik delme öncesi ve sonrası yoğunluk değişimi esas alınarak gruplarda çoklu varyans analizi yapılmış ve gruplar arasında fark olduğu ortaya koyulmuştur (F: 3,853; p<0,05) (Şekil 1).



Şekil 1. Biyolojik delme öncesi ve sonrası ladin farklı odun kısımlarında meydana gelen ağırlık kayıpları arasındaki yoğunluk değişimi.

Şekil 1 incelendiğinde aynı grupta yer alan ladin diri odun ve olgun odun kontrol örnekleri ile farklı grupta yer alan ladin diri odun % 10-15 ağırlık kaybı görülen örnek arasında fark bulunmuştur (p<0,05). Olgun odun kontrol örneğiyle %5-10 ve %10-15 ağırlık kaybı meydana gelen örnekler arasında fark olmamasına rağmen diri odun kontrol örneğiyle %10-15 ağırlık kaybı meydana gelen örnekler arasında fark tespit edilmiştir. Bu da *P. vitreus* mantarının odun yoğunluğunda önem arz eden kimyasal bileşenleri diri odunda olgun oduna nazaran daha fazla degrade ettiğini göstermektedir.

Ladin diri ve olgun odun kısımlarında biyolojik delme sonrası meydana gelen farklı ağırlık kayıplarına bağlı olarak yoğunluk değerindeki yüzde azalışların tespit edilmesiyle elde edilen bulguların yüzde oransal değişiminin verilmesinin daha anlaşılır olacağı düşünülerek elde edilen sonuçlar Tablo 1'de verilmiştir.

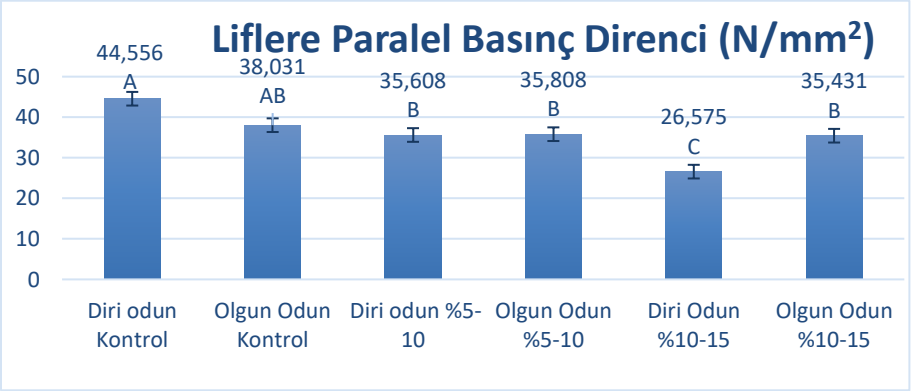
Tablo 1. Ladin diri ve olgun odun kısımlarında biyolojik delme sonrası meydana gelen farklı ağırlık kayıplarına bağlı olarak yoğunluk değişimindeki yüzde azalmalar.

Ağırlık kaybı	Yoğunluk (g/cm^3)		Değişim (%)	
	Diri Odun	Olgun Odun	Diri Odun	Olgun Odun
Kontrol	0,406	0,405	-	-
% 5-10 Ağırlık kaybı	0,395	0,383	-2,71	-5,43
% 10-15 Ağırlık kaybı	0,371	0,379	-8,62	-6,42

Tablo 1 incelendiğinde % 5-10 ağırlık kaybında olgun odunda meydana gelen yoğunluktaki % azalma oranı (% -5,43) > diri oduna (% -2,71) nazaran daha yüksek belirlenmiştir. Fakat % 10-15 ağırlık kaybında ise diri odunda meydana gelen yoğunluktaki % azalma oranı (% -8,62) > diri oduna (% -6,42) nazaran daha yüksek belirlenmiştir.

2.2. Meydana gelen ağırlık kayıplarının liflere paralel basınç direnç değeri üzerindeki etkileri

Biyolojik delme öncesi ve sonrası liflere paralel basınç direnç değişimi esas alınarak gruplarda çoklu varyans analizi yapılmış ve gruplar arasında fark olduğu ortaya koyulmuştur (F: 11,770; $p<0,05$) (Şekil 2).



Şekil 2. Biyolojik delme öncesi ve sonrası ladin farklı odun kısımlarında meydana gelen farklı ağırlık kayıpları arasındaki liflere paralel basınç direnç değişimi.

Şekil 2 incelendiğinde farklı bir grupta yer alan ladin diri odun kontrol (44, 556 N/mm^2) ile aynı gruplarda yer alan olgun odun % 5-10 (35,808 N/mm^2), diri odun % 5-10 (35,608 N/mm^2) ve olgun odun % 10-15 ile yine farklı bir grupta yer alan diri odun % 10-15 (26,575 N/mm^2) arasında istatistik anlamda fark görülmüştür ($p<0,05$). Ayrıca olgun odun kontrol ile farklı bir grupta yer alan diri odun % 10-15 arasında da istatistik anlamda fark görülmüştür ($p<0,05$).

Burada ladin diri ve olgun odun kısımlarında biyolojik delme sonrası meydana gelen farklı ağırlık kayıplarına bağlı olarak liflere paralel basınç direnç değişimindeki yüzde azalmaların tespit edilmesiyle elde edilen bulguların yüzde oransal değişiminin verilmesinin daha anlaşılır olacağı düşünülerek elde edilen sonuçlar Tablo 2'de verilmiştir.

Tablo 2. Biyolojik delme sonrası ladin diri ve olgun odun kısımlarında meydana gelen farklı ağırlık kayıplarına bağlı olarak liflere paralel basınç direnç değişimindeki yüzde azalmalar.

Ağırlık kaybı	Liflere Paralel Basınç Direnci (N/mm^2)		Değişim (%)	
	Diri Odun	Olgun Odun	Diri Odun	Olgun Odun
Kontrol	44,556	38,031	-	-
% 5-10 Ağırlık kaybı	35,608	35,808	-20,08	-5,85
% 10-15 Ağırlık kaybı	26,575	35,431	-40,36	-6,84

Tablo 2 incelendiğinde biyolojik delme sonrası meydana gelen ağırlık kaybı artışına bağlı olarak liflere paralel basınç direnç değişimindeki yüzde azalmanın ladin diri odununda daha yüksek çıktığı görülmektedir. Liflere paralel basınç direnç değişimine bakıldığında % 5-10 ağırlık kaybı görülen örneklerde diri odunda meydana gelen yoğunluktaki % azalma oranı (% -20,08) > olgun oduna (% -5,85) nazaran daha yüksek belirlenmiştir. Aynı şekilde % 10-15 ağırlık kaybı görülen örneklerde diri odunda meydana gelen yoğunluktaki % azalma oranı (% -40,36) > olgun oduna (% -6,84) nazaran daha yüksek belirlenmiştir. Biyolojik delme sonrası meydana gelen ağırlık kaybı artışına bağlı olarak liflere paralel basınç direnç değişimindeki yüzde azalmanın her iki ağırlık kaybında da ladin diri odununda daha yüksek çıktığı görülmektedir. Liflere paralel basınç direnç değerlerindeki düşüşün ligninden kaynaklı olduğu bilgisi göz önüne alındığında, diri odunda % 10-15 ağırlık kaybına kadar lignin tüketiminin önem arz ettiği bir mantar aktivitesinden söz edilebilir.

SONUÇ

Odun yoğunluğu ve liflere paralel basınç direnci test sonuçları, *P. vitreus* mantarının ladin odunundaki ağırlık kaybı artışına bağlı olarak olgun odunda göz ardı edilebilir kayıplara neden olduğunu, fakat diri odundaki kayıpların ise önemli düzeyde olduğunu ortaya koymuştur. Biyolojik delme öncesi ve sonrasında yapılan yoğunluk ve liflere paralel basınç direnç testleri *P. vitreus* mantarının % 10 ağırlık kaybına kadar tükettiği kimyasal bileşen ve maddelerin olgun odunda ve diri odunda farklı olma ihtimalinin yüksek olduğunu göstermiştir. Bu tespitlerin doğru bir şekilde yapılabilmesi içinde *P. vitreus* mantarının odunun anatomik özellikleri üzerindeki etkisine yönelik mikroskobik çalışmalara ve lignin ve karbonhidrat analizi, %1'lik NaOH (Alkali) çözünürlük testi gibi kimyasal analizlere ihtiyaç bulunmaktadır.

TEŞEKKÜR

Bu çalışma ''Biyolojik yöntemle permeabilitesi iyileştirilen odunun bazı özelliklerinin ve bakır dağılımının incelenmesi'' isimli doktora tezinden elde edilen verilerin bir kısmını içermektedir. İstanbul Üniversitesi-Cerrahpaşa Bilimsel Araştırma Projeleri Yürütücü Sekreterliğinin 24880 numaralı projesi ve 115O934 numaralı TÜBİTAK projesi ile de desteklenmiştir.

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➢ *ORAL PRESENTATION*

Bazı *Salvia* türlerinin *in vitro* çimlenmesi ve önemi

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Özet
Salvia cinsi Lamiaceae familyasına ait olup, tıp, kozmetik, aromaterapi gibi birçok amaç için kullanılan türleri içermektedir. Genellikle kabuklarının müsilaj içermesinden dolayı çimlenme potansiyelleri düşüktür. Bu çalışmada, *Salvia* cinsine ait *S. yosgadensis, S. virgata, S ceratophylla, S. sclarea* ve *S. candidissima* türlerinin tohum kabuklarının çimlenme üzerine etkisinin belirlenmesi amaçlanmıştır. Çalışmada bitki tohumları ve tohum kabuğu çıkartılmış embriyo taslağı kullanılmış ve denemeler *in vitro* şartlarda gerçekleştirilmiştir. Tohumların çimlenmesinde *S. ceratophylla* (%0) en düşük çimlenme gösterirken, *S. candidissima* en yüksek çimlenme değerine (%53.3) sahip olmuştur. Ancak embriyo taslaklarının çimlenme oranında ise %93.0'den yüksek çimlenme gözlenmiştir (%40.0 ile *S. virgata* hariç). Çalışmanın sonunda, analiz edilen verilere göre türlerin çimlenme problemlerine ve türlerin kullanım alanlarına değinilmiştir.
Anahtar Kelimeler: *S. yosgadensis, S. virgata, S ceratophylla, S. sclarea, S. candidissima, çimlenme*

In vitro germination and importance of some *Salvia* species

Abstract
Salvia genus belongs to the Lamiaceae family and includes species used for many purposes such as medicine, cosmetics, and aromatherapy. Generally, their germination potential is low because their coats contain mucilage. In this study, it was aimed to determine the effect of seed coats of *S. yosgadensis, S. virgata, S. ceratophylla, S. sclarea* and *S. candidissima* species on germination. Plant seeds and embryo removed seed coat were used as material, and the experiments were carried out *in vitro*. For seed, while *S. ceratophylla* (0%) showed the lowest germination, *S. candidissima* had the highest germination value (53.3%). But, germination rate of embryo was higher than 93.0% (except *S. virgata* with 40.0%). At the end of the study, according to the analysed data, the germination problems and usages of the species were mentioned.
Keywords: *S. yosgadensis, S. virgata, S ceratophylla, S. sclarea, S. candidissima,* germination.

GİRİŞ

Yüzyıllardır doğal ürün olarak kullanılan tıbbi ve aromatik bitkileri farmakoloji, kozmetik, gıda gibi endüstrilerde ham madde olarak kullanımı her geçen gün dünya pazarındaki talebi artış göstermektedir. Ülkemiz gerek coğrafi yapısı gerekse değişik ekolojik koşulları nedeniyle dünyanın çok önemli gen merkezlerindendir. Türkiye florasında yaklaşık olarak 9 753 (3 035'i endemik) doğal tür yayılış göstermekte olup, tür altı taksonlar ilave edildiğinde ise 3 649'u (%31.8) endemik 11 707 takson yer almaktadır Türkiye florasında, en zengin tür sayısına sahip olan Ballıbabagiller olarak bilinen Lamiaceae (Labiatae) familyası ile birlikte 174 familya bulunmaktadır (Güner ve ark., 2012).

Lamiaceae familyası türlerinin çoğu önemli fitokimyasallar bakımından zengin olması nedeniyle tıp, gıda, kozmetik ve parfümeri gibi alanlarda oldukça büyük öneme sahiptirler. Bu familyanın bir cinsi olan adaçayı (*Salvia*), dünyada yaklaşık 900'den fazla türe sahiptir (Abu Darwish, 2014). Tunus'ta 10 tür (Pottier-Alapetite, 1981), Avrupa florasında 36 tür ile temsil edilirken (Hegde, 1972), Türkiye florasında ise yaklaşık %51 endemik olmak üzere 97 adaçayı türü yayılış göstermektedir (İpek ve Gürbüz, 2010).

Doğal floradaki tıbbi özelliklere sahip olabilecek türlerin ekonomiye kazandırılması ve hem üretici hem de tüketici taleplerine göre yüksek verimlilik ve kalitede üretim yapılabilmesi için her şeyden önce ıslah edilerek geliştirilmiş çeşitlerine ve standartlara uygun tohumluk materyaline ihtiyaç vardır (Bayram ve ark., 2010). Tarımsal üretimin

temelini tohum oluşturmaktadır. Bu yüzden, bir bitkinin yetiştiriciliği için tohum çimlenmesi önemli unsurdur. Çimlenme modelleri hakkında ayrıntılı bilgi sadece başarılı yetiştirme için değil, aynı zamanda kurak alanlarda türlerin yetişmesi gibi abiyotik faktörlere tolerans ve dinamiklerinin anlaşılması için de önemlidir. Tohum çimlenmesi içsel dormansi, genotip, olgunluk) ve dışsal faktöre (sıcaklık, tuzluluk, ışık ve nem koşulları gibi) bağlıdır (Jafarinia ve Yazdanbakhsh, 2016; Pająk ve ark., 2019).

Çeşitli tohumların ışık rejimine duyarlılığı türlerine bağlı olup, ışık faktörü fotosentez sürecinde belirleyicisidir. Bazı tohumlar karanlıkta daha iyi çimlenirken, diğer tohumlarda da ışık spektrumuna bağlılık göstermektedir (Jafarinia ve Yazdanbakhsh, 2016).

Büyüme sıcaklığı, çimlenme yüzdesini ve hızını etkiler, su emilimini ve biyokimyasal reaksiyonların akışını engeller (de Souza ve Chaves, 2016). Dormant kuru tohumların, çimlenme sürecininin etkinleştirmesi için suyla beslenmesi gerekmektedir (Jafarinia ve Yazdanbakhsh, 2016). Ayrıca dış su potansiyelinin azalmasıyla tohum çimlenme hızının düştüğü, tohum çimlenmesi için kritik bir su potansiyel değeri olduğu ve altındaki değerde çimlenme olmadığı kanıtlanmıştır (Hadas, 1976). Bunula birlikte, bazı *Salvia* türlerinin tohumlarının özelliklerinden biri, hidrasyon sırasında önemli miktarda müsilaj üretebilmesidir. Bu jel tohumu tamamen sardığından dolayı çimlenme sürecinde dikkate alınması gereken faktörlerden biridir (Geneve ve ark., 2017).

Salvia türleri üzerinde birçok çimlendirme çalışmaları yapılmıştır. Örneğin, *S. officinalis* tohumları üzerine TiO_2 (Titanyum dioksit) uygulamasının 60 mg L-1 konsantrasyonu çimlenme oranını (%94.7) arttırdığı vurgulanmıştır (Feizi ve ark., 2013). Flórez ve ark. (2012) araştırmada *S. officinalis* çimlenmesi üzerinde 125 mT'lik manyetik alanının 24 saatlik uygulama sonucunda çimlenme oranını %69 çıkarttığını bulmuştur. Yücel (2000) *S. cryptantha, S. cyanescens, S. dichroantha, S. tchihatcheffii, S. aethiopis, S. virgata* tohumlarının çimlenmeleri üzerine sodyum klorür (NaCl), potasyum nitrat (KNO_3) ve sülfürik asitin (H_2SO_4) etkilerini incelemiş ve sülfürik asit, hem çimlenme yüzdesini hem de hızını engelleyerek çimlenmeyi tamamen inhibe ettiğini kaydetmiştir. Dastanpoor ve ark. (2013) *S. officinalis* üzerine en yüksek çimlenme oranını (%85.5) 30°C'de 12 saat (sıcaklık x süre) uygulamasında gözlemlemiştir. Gorai ve ark. (2011) *S. aegyptiaca* türünde 30°C'nin daha etkili olduğunu ve artan NaCl uygulamasında çimlenme üzerine engelleyici olduğunu rapor etmiştir.

Ülkemiz, çok sayıda tıbbi bitki türünün doğal yetişme alanı olmakla birlikte, kullanım alanı geniş ve ekonomik değeri yüksek olan daha pek çok tıbbi bitkinin de yetişmesi için uygun bir ekolojiye sahiptir. Bu amaçla Türkiye florasında yetişen *S. yosgadensis, S. virgata, S. ceratophylla, S. sclarea* ve *S. candidissima* türlerinin çimlenme probleminin tohum kabuğundan kaynaklanıp kaynaklanmadığının belirlenmesi amaçlanmıştır.

MATERYAL VE METOD

Materyal

Bu çalışmada *in vitro* şartlarda tohum ve embriyodan çimlenme potansiyelleri değerlendirmek için kullanılan beş *Salvia* türü Türkiye florasından toplanmıştır. Bu türler Tablo 1'de verilmiştir. Çalışmada kullanılan türlerin tohumları, aynı yılda (2018) tohumların olgunlaşma dönemlerinde toplanmıştır.

Tablo 1. *Salvia* türlerinin isimleri

	***Salvi*a taksonları**	**Türkçe adı**
1	*S. sclarea* L.	Paskulak
2	*S. ceratophylla* L.	Tarak şalba
3	*S. virgata* Jacq.	Fatmanaotu
4	*S. yosgadensis* Freyn & Bornm.*	Bozok şalbası
5	*S. candidissima Vahl. occidentalis Hedge*	Akgalabor

* Endemik

Çimlenme Testi

Salvia türlerinin olgun tohumları %20 çamaşır suyu (ACE) ile 15 dk steril edildikten sonra 3 kez steril saf su ile durulanmıştır (Yaman 2017). Tohumlar ve tohum kabuğu çıkartılmış tohum taslakları sadece agar içeren ortamda kültüre alınmıştır. Ortam saf su içerisinde %0.64 g agar ile hazırlanmış ve otoklavda 121 °C'de steril edilmiştir. Tohum ve tohum taslakları her bir petride 25 adet ve 4 tekerrürlü olarak kurulmuş, 8 saat karanlık, 22±2°C ve 16 saat ışıklı ortamda 27±2°C'de iklim dolabında tutularak çimlenmeye bırakılmıştır. Kültürden iki hafta sonra sayımlar yapılarak, ortalama çimlenme oranı belirlenmiştir. Kökçük (radikul)'ün 2 mm'lik çıkışı çimlendirme kriteri olarak ele alınmış ve çimlenme oranları tespit edilmiştir. Çimlendirme testleri sonucuna göre, çimlenen tohum oranı % olarak ifade edilmiştir.

İstatistik Analiz

Çimlenme verileri, varyans homojenliğini sağlamak için yüzde değerler istatistiki analizden önce açı değerlerine dönüştürülerek (Snedecor ve Cochran 1967) varyans ve Duncan analizine (p<0.05) tabi tutulmuştur. Her bir türün tohumdan ve embriyodan çimlenme yüzdesi arasındaki fark ANOVA ile analiz edilmiş ve p değerleri ile kıyaslanmıştır. Analizler IBM SPSS Statistics 20 bilgisayar programında yapılmıştır (Düzgüneş ve ark.,1983).

BULGULAR ve TARTIŞMA

Salvia Türlerin Çimlenmesi

Bu çalışmada Türkiye florasında yayılış gösteren ve üzerinde çok az araştırma bulunan *S. yosgadensis*, *S. virgata*, *S. ceratophylla*, *S. sclarea* ve *S. candidissima* türlerin *in vitro* şartlarda tohumdan ve embriyodan çimlenme potansiyelleri değerlendirilmiştir. Önceki araştırmacılar *Salvia* cinsine ait birçok türün *in vivo* ve *in vitro* çimlenme yeteneklerini incelemiştir (Musarurwa ve ark., 2010; de Paiva ve ark., 2016). Fakat *Salvia* türleri ile ilgili önceki çimlendirme çalışmaları çoğunlukla tuz, asit, bitki büyüme düzenleyici ve sıcaklık gibi stres etkilerinin değerlendirmesiyle sınırlıdır (Gorai ve ark., 2011; de Paiva ve ark., 2016; Javaid ve ark., 2018). *Salvia* türlerinde tohum kabuğunun çimlenmeye etkisini ve embriyonun dormansi isteğini ortaya çıkarmak için çok az çalışma yapılmıştır. Bu çalışma bazı *Salvia* türlerinin çimlenmesi üzerine tohum kabuklarının etkisini ortaya koymuştur.

Tablo 2. *Salvia* türlerinin tohum ve embriyolarından *in vitro* şartlarda çimlenme yüzdesi

Türler	Tohum		Embriyo		*P*	Tohum kabuğunun çimlenmeye olası engeli (%)
S. yosgadensis	20.0±13.1	ab	100.0	a	0.034*	80.0
S. virgata	36.7±5.5	a	40.0	c	0.714	8.3
S. ceratophylla	0.0	b	100.0	a	0.000**	100.0
S. sclarea	20.0±11.6	ab	100.0	a	0.028*	80.0
S. candidissima	53.3±5.1	a	93.3±6.14	b	0.073	42.9

Beş tane *Salvia* türünün *in vitro* şartlarda tohum ve embriyodan çimlenmesi en iyi şekilde tanımlanmıştır (Tablo 2). Türlerinin tohumdan ve embriyodan çimlenme yanıtı beş tür arasında farklılık göstermiştir. *Salvia* türlerinin tohumdan çimlenmesi en erken 3 gün sonra başlamış ve *S. candidissima* türünde gözlenmiştir. En yüksek çimlenme oranı %53.3 ile *S. candidissima* türünde tespit edilmiştir. *S. yosgadensis ve S. sclarea* benzer çimlenme oranına (%20.0) sahip olmuştur. Genel olarak, *Salvia* türlerinin tohumdan çimlenme oranlarının düşük olduğu gözlenmiştir. Önceki çalışmalarda da *Salvia* türlerine ait tohumların çimlenme oranlarının düşük olduğu rapor edilmiştir. Tursun (2019) *Salvia verticillata* türünün uygulamalarındaki kontrol grubunda en yüksek çimlenme oranını %43.5 saptamıştır. Sun ve ark. (2018) çalışmalarında *Salvia miltiorrhiza* türünde %43.67, Gorai ve ark. (2011) *Salvia aegyptiaca* türünün farklı konsantrasyondaki tuz ve sıcaklık uygulamalarına rağmen genellikle çimlenme oranını %60'ın altında kaydetmişlerdir. Hatta, Musarurwa ve ark. (2010) *in vitro* çalışmalarında *Salvia stenophylla* türünün tüm uygulamalar sonucunda en yüksek çimlenme oranını %60'ın altında olduğunu vurgulamıştır. Aghilian ve ark. (2014) çalışmalarında *Salvia dorrii* ve *Salvia officinalis* türlerinin çimlenme

oranlarını sırasıyla %55 ve %45 olarak kaydetmiştir. Ancak, de Paiva ve ark. (2016) *Salvia hispanica* türünün yüksek çimlenme oranına sahip olduğunu rapor etmiştir.

Salvia türlerinin embriyodan çimlenme oranlarının tohumdan çimlenme oranlarına göre çok daha yüksek olduğu gözlenmiştir. En düşük çimlenme oranı %40.0 ile *S. virgata* türünde gözlenmesine rağmen, *S. yosgadensi, S. ceratophylla* ve *S. sclarea* türlerinde %100 çimlenme gözlenmiştir. Bu türlerin çimlenmesinde en büyük engelin tohum kabuğundan kaynaklandığı (sırasıyla %80.0, %100.0 ve %80.0) söylenebilir. Tohum kabuğun etrafındaki mumsu yapı (müsilaj), embriyonun su alımı ve gaz alışverişi için büyük bir engel olmaktadır. Tohum kabuğunun yanı sıra bu musilaj yapısıda çimlenme inhibitörü olarak hareket edebilmektedir. *Salvia* türlerinin kabukları genellikle müsilaj benzeri madde ile kaplı olmasından dolayı dormansiye neden olduğu düşünülmektedir (Tursun, 2020). Aghilian ve ark. (2014) tıbbi bitkilerin tohumlarındaki musilaj seviyesinin artmasına bağlı olarak düşük çimlenme oranına neden olma ihtimalinin olduğunu bildirmiştir. Fakat, *S. virgata* türünün tohum ve embriyodan çimlenme oranlarının yakın olduğu ve aralarında istatistiki bir fark olmadığı (P=0.714) gözlenmiştir. *S. virgata* türünün dormansi özelliğinin çok düşük bir oranının tohum kabuğundan kaynaklandığı (%8.3) varsayılabilir. *S. virgata* türündeki dormansi embriyonun genetik yapısından kaynaklanabileceği gibi ışık, sıcaklık, nem gibi abiyotik nedenlerden de kaynaklanabilir. *S. candidissima* türünün de dormansi özelliğinin büyük bir oranı tohum kabuğunun varlığından kaynaklanabileceği (%42.9) gibi daha yüksek bir oranında farklı sebeplerden kaynaklanabileceği tespit edilmiştir.

Salvia Türlerin Kullanım Alanları

Önemli tıbbi bitkiler olan *Salvia* türlerinin yaprak, sürgün uçları, çiçek ve kısmen de sapları kullanılmasına rağmen en fazla faydalanılan kısmı yapraklarıdır. *Salvia* türleri sahip oldukları özelliklerden dolayı geniş bir tüketici grubuna (gıda sanayi, ilaç ve kimya sanayi, perakende ürün olarak satış yapan aktarlar) hitap ettiklerinden, pazar potansiyelleri oldukça yüksektir.

Bazı *Salvia* türleri, Türkiye'de hem doğadan yabani olarak toplanmakta hem de tarım alanlarında kültürü yapılmaktadır. Ticari değeri en yüksek olan türler tıbbi adaçayı (*Salvia officinalis* L.), Anadolu adaçayı (*S. fruticosa* Mill., syn. *S. triloba* L.), elma adaçayı (*S. pomifera* L.), Ispanyol adaçayı (*S. lavandulaefolia* Vahl.) ve misk adaçayı (*S. sclarea* L.)'dır (Sage, 2000). Bunlar içerisinden Avrupa'da tıbbi kullanımı resmen kabul edilmiş *S. officinalis* türü Türkiye florasından doğal yayılış göstermemektedir. Fakat başarılı bir şekilde tarımı yapılmaktadır (Başyiğit ve Baydar, 2016). Ülkemizde yetişen adaçayı türleri içerisinde ise en fazla toplanan ve hem iç tüketimde kullanılıp hem de ihraç edilen ise *S. fruticosa* türüdür. Bununla birlikte, *S. tomentosa* türleri doğadan yoğun olarak toplanmaktadır (Baydar, 2016). Bu türler genellikle çay olarak tüketilmekle birlikte, baharat ve tatlandırıcı olarak kullanıldıkları gibi parfümeride, kozmetikte (Delamare ve ark., 2007) ve halk hekimliğinde mikrobiyal enfeksiyonları, kanseri, sıtmayı, iltihabı tedavi etmek ve hastalıktan sonra evleri dezenfekte etmek için kullanılmaktadır (Kamatou ve ark., 2008). Dünyadaki birçok ülkenin farmakopelerinde belirgin bir şekilde yer almaktadır. Uçucuyağları parfüm yapımında, çiçekleri allık olarak kullanılmıştır (Baytop, 1999). Adaçayı türleri terleme ve ateşe karşı; bir gaz giderici olarak; bir spazmolitik; antiseptik/bakteri yok edici; büzücü; ağız, dil ve boğaz iltihabına karşı gargara; bir yara iyileştirici ajan; cilt ve saç bakımında; ve romatizmaya karşı bir ilaç olarak kullanılmıştır (Kintzios, 2000). Öte yandan, *Salvia* türlerinin Avrupa halk tıbbında hafıza geliştirme amaçlı kullanıldığı bildirilmiştir (Perry ve ark., 2003). Ürdün, Lübnan ve Suriye gibi Orta Doğu ülkelerinde karın ağrısı, baş ağrısı, mide ağrılarını hafifletmek ve kanser, mikrobiyal enfeksiyonlar, astım, öksürük ve diğer pulmoner ve üriner hastalıkları tedavi etmek için *Salvia* türleri kaynamış çay olarak reçete edildiği bildirilmiştir (Cardile ve ark., 2009).

Çalışmadaki türlerin kullanım alanları incelendiğinde, *S. yosgadensis* üzerinde hak sağlığı açısından etkileri hakkında henüz yeterli bir bilgi kaydedilmemiştir. Fakat, Şarer (1988) *S. yosgadensis* türünün yaklaşık %0.3 uçucu yağ içeriğine ve bu yağın % 24.8 monoterpenik hidrokarbon, % 75.2 oksijenli monoterpen ve seskiterpen içerdiği saptamıştır. *S. virgata*, cilt hastalıkları ve yaralarının tedavisinde kullanılmaktadır. Toprak üstü kısımlarından elde edilen herbal çayları, Türkiye'de kan kanserine karşı tüketilmektedir (Baytop, 1999). Akkol ve ark. (2008) *S. virgata* üzerindeki çalışmalarında, ağrılı ve iltihaplı durumlarda *Salvia* türlerinin geleneksel kullanımını desteklemekte olduğunu bildirmişlerdir. *S. ceratophylla* iltihap, mantar ve nosiseptif hastalıklar karşı Orta Doğu'nun geleneksel tıbbında kullanılmaktadır (Shehadeh ve ark., 2014; Kasabri ve ark., 2014; Al-Bakri ve ark., 2010). *S. sclarea* türü aromatik özelliklerinden ötürü büyük ölçüde övülür ve Bulgaristan, Fransa, Rusya ve Fas'ta parfümeri endüstrisi için uçucu yağ üretiminde büyük ölçüde yetiştirilmektedir (Yaseen ve diğerleri, 2014). Bu tür

stres, astım, amenore, dismenore, boğaz ağrısı, boğaz ağrısı, kolikler, depresyon, yorgunluk, sinirlilik, migren, varisli damarlar, hemoroid, yağlı cilt ve saç, spazmodik öksürük, sindirim ve adet sorunları dahil olmak üzere birçok yaygın rahatsızlığın tedavisi için geleneksel tıpta uygulaması bulunmaktadır (Yaseen ve ark., 2014; Kumar ve ark., 2017). Ayrıca, önemli bilimsel çalışmalar *S. sclarea*'nın çoklu farmakolojik potansiyelini antioksidan, nöroprotektif, sindirim bozuklukları, artrit, romatizma, antiinflamatuar ve antimikrobiyal olarak değerlendirmiştir (Kostić ve ark., 2017; Durgha v ve ark., 2016). *S. candidissima*'nın ise ekstreleri antioksidan etkiye sahiptir (Tosun ve ark., 2009).

SONUÇ

Bu çalışmada *S. yosgadensis, S. virgata, S. ceratophylla, S. sclarea, S. candidissima* türlerinin çimlenmesi üzerine tohum kabularının etkisi ve kullanım alanları araştırılmıştır. Tohum kabukları *S. yosgadensis, S. ceratophylla* ve *S. sclarea* çimlenmeleri üzerine dormansi özelliğinin çok yüksek, *S. virgata* türünde ise çok düşük olduğu tespit edilmiştir. Sonuç olarak *Salvia* türlerin çimlenmeleri üzerine tohum kabuğunun etkisinin olduğu tespit edilmiştir. Gelecekte, tohum kabuğu yüksek dormansi gösteren türlerin tohum kabukları çatlatılarak veya kırılarak arazi veya sera koşullarında çimlendirme denemeleri yapılabilir. Dormansi özelliği tohum kabuğundan kaynaklanmayanlar için priming çalışmaları yürütülebilir.

KAYNAKLAR

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➢ *ORAL PRESENTATION*

Su Ürünlerinde Mikrobiyal Fermentasyon ve Önemi

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Özet
Günlük hayatta tüketilen gıdaların önemli bir bölümünü mikrobiyal faaliyetler sonucu üretilen fermente ürünler oluşturmaktadır. Bu tip fermente gıdaların raf ömrü daha uzun olup, fermentasyonda rol oynayan mikroorganizmaların özellikleri ile ilişkili olarak karakteristik bir tat ve aromaya da sahiptirler. Ayrıca fermente gıdaların diğer gıdalara kıyasla insan sağlığı açısından olumlu etkileri olduğu bilindiği için her geçen gün probiyotik mikroorganizmaların bu alanda kullanımı artmaktadır.
Günümüzün temel ihtiyaçlarından biri de, tüketicilerin sağlık ve refahını geliştirmek amacıyla fonksiyonel gıda olarak bilinen yenilikçi gıdaların ve gıda işleme teknolojilerinin geliştirilmesidir. Bu açıdan su ürünleri ile ilgili genel eğilim, bu ürünlerdeki sağlığa olumsuz etkisi olan içerikleri azaltmak (tuz) ya da ürüne sağlığa katma değer sağlayacak içerikleri (probiyotikler) ilave etmek şeklindedir. Böylece koroner kalp damar hastalıkları ile ilişkilendirilen et ürünlerine tüketicilerin bakış açısı da değiştirilebilmektedir.
Günümüzde, probiyotik bakterilerle elde edilen fermente gıdalar, en önemli fonksiyonel ürünler (lakerda, çiroz, kefir, sucuk, yoğurt, turşu, elma sirkesi…) olarak insan tüketiminde yer almaktadır. Probiyotik kullanımı çok çeşitli endüstri ve bilim dallarında hızla yayılmaktadır. Fermente ürünlerde probiyotik mikroorganizmalarının gelişimi birçok faktöre bağlı olmakla birlikte (pH, nem, fermentasyon koşulları vb.) su ürünlerinin fermentasyonu probiyotik mikroorganizmalar açısından ideal bir ortam oluşturmaktadır. Bu mikroorganizmaların su ürünlerine eklenmesinin amaçları; patojen bakterileri inhibe ederek gıda güvenliğini arttırmak, bozulma sebebi bakterileri inhibe ederek raf ömrünü uzatmak, lezzeti arttıran duyusal özellikleri geliştirmek ve insan sağlığı üzerine olumlu etkiler sağlamaktır.
Anahtar Kelimeler: Fermentasyon, Su Ürünleri, Probiyotik Mikroorganizma, Fermente Su Ürünleri

Microbial Fermentation and Its Importance in Seafood

Abstract
Fermented products produced as a result of microbial activities constitute a significant part of the foods consumed in daily life. Such fermented foods shelf life is longer, they possess a characteristic flavor and aroma characteristics associated with the microorganisms involved in the fermentation. In addition, as it is known that fermented foods have positive effects on human health compared to other foods, the use of probiotic microorganisms in this field is increasing day by day.
One of the basic needs of today is the development of innovative foods and food processing technologies known as functional foods in order to improve the health and well-being of consumers. General trends in this respect, aquatic products, to reduce the adverse health effects of content in this product (salt) or to provide added value to the product contents health (probiotics) in the form of the addition. Thus, consumers' perspective on meat products associated with coronary cardiovascular diseases can also be changed.
Today, fermented foods obtained with probiotic bacteria are included in human consumption as the most important functional products (lakerda, çiroz, kefir, sausage, yoghurt, pickles, apple vinegar ...). The use of probiotics is spreading rapidly in a wide variety of industries and sciences. Although the development of probiotic microorganisms in fermented products depends on many factors (pH, humidity, fermentation conditions, etc.), the fermentation of fishery products creates an ideal environment for probiotic microorganisms. The purposes of adding these microorganisms to aquaculture are; To increase food safety by inhibiting pathogenic bacteria, to extend the shelf life by inhibiting the bacteria that cause spoilage, to develop sensory properties that increase taste and to provide positive effects on human health.
Keywords: Fermentation, Seafood, Probiotic Microorganism, Fermented Seafood Products

GİRİŞ

Fermentasyon, gıda aromasını zenginleştirme ve raf ömrünü uzatmadaki avantajları nedeniyle sebze, et ve süt ürünlerinin işlenmesinde yaygın olarak kullanılan gıda koruma ve işleme yöntemlerinden biridir. Bu teknik ile gıdalarda benzersiz tat, doku ve aromalar oluşmaktadır (Giri ve ark., 2010; Majumdar ve ark., 2016; Yang ve ark., 2020). Özellikle mikrobiyal fermentasyon, çabuk bozulan su ürünlerinin işlenmesi ve korunması için etkili bir tekniktir (Xu ve ark., 2019). Fermentasyon esnasında taze balık proteinlerinin mikroorganizma veya enzimlerin aktivitesiyle biyoaktif peptilere oda sıcaklığında parçalanarak, gıdanın biyolojik özelliklerinde ve lezzetinde önemli artışlara sebep olmaktadır (Steinkraus, 2002).

Fermente gıdalar, probiyotik potansiyeli olan yararlı bakteriler içerdiğinden ve aynı zamanda bağırsak bakteri popülasyonunun dengelenmesine faydalı olduğundan dolayı son yıllarda yoğun bir şekilde tüketilmektedir (Singh ve ark., 2018). Son yıllarda ise fermente balıklar, yüksek besin değeri, minerallerinin biyoyararlılığı, kolay sindirilebilirliği, insan sağlığına katkıları ve organoleptik özellikleri nedeniyle dünya çapında gıdalar arasında popüler hale gelmektedir (Das ve ark., 2020).

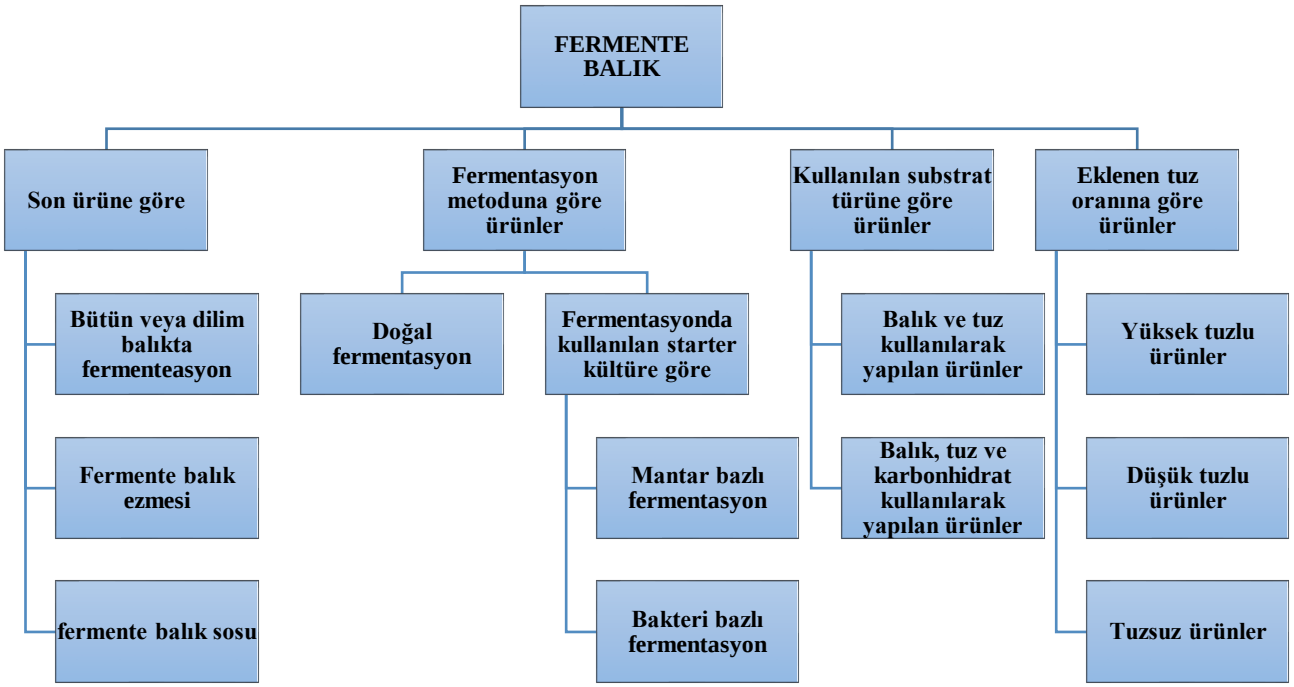
Fermente ürünlerde starter kültürlerinin kullanılması yalnızca fermentasyon süresini kısaltmakla kalmayıp, aynı zamanda bozulma ve patojen bakterilerinin neden olduğu zararlı etkileri de önemli ölçüde azaltmaktadır (Hua ve ark., 2020; Kasankala ve ark., 2011) . Ticari starter kültürler, güçlü fermentasyon performansları, yüksek bakteriyel aktiviteleri ve uzun raf ömrü nedeniyle endüstriyel uygulamada kullanımlarına ilgi her geçen gün artmaktadır. Geçtiğimiz birkaç yıl içinde, et yemekleri (Domínguez ve ark., 2016; Du ve ark., 2019; Van Ba ve ark., 2016), sebze ürünleri (Sun ve ark., 2019), balık ürünleri (Giri ve ark., 2009), süt ürünleri (Güler ve Gursoy-Balci, 2011; Zhou ve ark., 2019) ve şarap (Liu ve ark., 2019) gibi uygulamalarda bu alanda önemli araştırmalar yapılmıştır.

Tablo 1. Ticari Uygulamalarda Probiyotik Seçiminde Kullanılan Kriterler (Vasiljevic ve Shah, 2008)

Kriter	Özellik
Güvenlik kriterleri	Orjin Patojenite ve enfektivite Toksiklik, antibiyotik direnci
Teknolojik kriterler	Genetik stabilitesi olan suşlar Proses ve depolama sırasında yaşama kabiliyeti İyi duyusal özellikler Faj dayanıklılığı Geniş ölçekli üretim
Fonksiyonel kriterler	Gastrik asit ve sulara karşı tolerans Safra tuzlarına tolerans Mukozal yüzeylere yapışabilme Onaylanmış ve rapor edilmiş sağlığa yararlı etkiler
Fizyolojik kriterler	Bağışıklık sistemi modülasyonu Gastrointestinal patojenlere karşı antagonist etki Kolesterol metabolizması Laktoz metabolizması Antimutajen ve antikanserojen özellikler

Fermente Su Ürünleri Çeşitleri

Fermente su ürünlerindeki mikrobiyal flora, ürünün iç ve dış faktörlerine bağlı olarak karakteristik lezzetinin gelişmesini sağlamaktadır. Mikroorganizmalar karbonhidratların, proteolizin, amino asitlerin parçalanmasını sağlayarak, lipolizi ve lipit oksidasyonunu doğrudan ve dolaylı olarak etkilemekte ve bunun sonucu olarak ürünün lezzet özelliklerini geliştirmektedir (Ercoşkun ve Ertaş, 2003).



Şekil 1. Geleneksel fermente balık ürünleri türleri (Zang ve ark., 2020)

Çok çeşitli fermente su ürünleri bulunmaktadır. Şekil 1'de gösterildiği gibi, son ürüne göre fermente edilmiş balıklar üç gruba ayrılır: Fermente edilmiş bütün (Mısır da Feseekh, Orta Doğu da Katheef) veya parçalar (İsveç de Gravlax, Norveç de Rakfisk) halindeki balık mümkün olduğunca orijinal yapısını koruyacak şekildedir. Ayrıca balıkların macun benzeri ürünlere dönüştürüldüğü fermente balık ezmeleri (Tayland da Kapi, Kamboçya da Cambodia) ve balığın sıvı hale dönüştüğü fermente balık sosu (Filipinler de Patis, Vietnam da Nuoc-nam, Tayland da Nam-pla) bulunmaktadır.

Fermente balık ürünlerinde işleme yöntemlerine göre iki gruba ayrılır: doğal yöntemlerle (Kore de Sikhae) ve starter kültür kullanılarak (Filipinler de Balao-balao, Tayland da Kungchao) oluşan fermentasyonlardır. Doğal fermentasyon, halihazırda ham maddeler üzerinde veya ilave edilen katı maddelerindeki mikroorganizmalar aracılığıyla ve üreticinin deneyimleri sayesinde fermentasyon işlemi gerçekleştirilir. Doğrudan hammaddelere eklenen starter kültürleri kullanan fermentasyonlar, fermentasyon işleminin daha iyi kontrol edilmesini sağlar, böylece kalite özellikleri ve lezzeti daha hızlı gelişerek standart hale getirilebilir. Starter kültür olarak bakteri ve mantarlar dahil olmak üzere farklı mikroorganizmalar kullanılarak yapılabilir. Mantar bazlı fermentasyon için, *Aspergillus* ve *Actinomucor* cinsinin türleri kullanılmaktadır. Mantarlar ile gerçekleşen fermentasyonlar, sadece patojenik mikroorganizmaların inhibe etmekle kalmaz, aynı zamanda tat ve aroma indükleyen bileşiklerin üretimini de artırır. Bakteri bazlı fermentasyonlar için, laktik asit bakterileri (LAB) geleneksel olarak birçok fermente balık ürününde kullanılmıştır. Mantarlarla yapılan fermentasyona benzer şekilde, LAB ile fermentasyonun bozulma mikroflorasının gelişmesini baskıladığı ve organoleptik özelliklerini iyileştirdiği bildirilmiştir.

Ek olarak, fermentasyon işlemlerinde kullanılan substratın türüne bağlı olarak, geleneksel fermente edilmiş balık ürünleri iki gruba ayrılır: (1) Balık ve tuz kullanılarak (Kore de Jeotgal) yapılan ürünler (2) Balık, tuz ve karbonhidrat kullanılarak (Çin de Narezushi, Tayland da Plaa-ra ve Som-fak) yapılan ürünlerdir. Karbonhidrat kaynakları genellikle pirinç, mısır, buğday unu hatta şurup veya şekeri içermektedir. Balık ve tuz karışımına karbonhidratların eklenmesi sadece fermentasyonu hızlandırmak için bir enerji kaynağı sağlamakla kalmaz, aynı zamanda eklenen karbonhidratlar fazla nemi emmeye yardımcı olur ve nihai ürüne farklı bir tat vermektedir.

Fermente balıklar, eklenen tuz oranına göre üç sınıfa ayrılır: Yüksek tuzlu (toplam ağırlığın % 20'sinden fazla) (Orta Asya da Awal), düşük tuzlu (% 3-8) (İsveç de Surströmming) ve tuzsuz (İzlanda da Hakarl) ürünlerdir. Tuz eklenmesi su aktivitesinin azalmasına yol açacak ve aynı zamanda antibakteriyel özellikleri nedeniyle bozulma mikroorganizmalarının büyümesini engelleyecektir.

Ülkemizde fermente balık ürünleri olarak kefal havyarı ("Türk havyarı"), çiroz ve lakerda tüketilmektedir. Lakerda; dilimlenmiş palamut ve torik balıklarının kuru tuzlama yöntemiyle tuzlanıp, balıktan çıkan su ile tuzun oluşturduğu derişik tuz çözeltisinde bekletilmesiyle üretilmektedir. Lakerda ülkemizde Karadeniz Bölgesi başta olmak üzere Ege ve Marmara Bölgelerinde üretilmektedir. Ülkemiz dışında İspanya, Yunanistan ve İtalya'da da lakerda bilinmekte ve tüketilmektedir (Kocatepe ve Tırıl, 2015; Turan ve ark., 2006a; Turan ve ark., 2009b). "Türk havyarı" olarak da adlandırılan ve ülkemizde yaygın bir şekilde üretimi yapılan kefal havyarı, geleneksel olarak balmumu ile kaplanmış veya pres yapılarak pelet hale getirilmiş olarak hazırlanmaktadır. Genellikle Karadeniz ve Marmara Bölgelerinde üretilen kefal havyarı diğer havyarlar ile kıyasladığında farklı tat ve görüntüsü ile öne çıkmaktadır (Şengör ve ark., 2002). Aynı zamanda ülkemizde Ege ve Marmara bölgelerinde uskumru ve mersin balığından yapılan geleneksel tuzlu kurutulmuş balık ürünlerine ise çiroz denir. Uskumrular özellikle Marmara Denizi'nde yumurtlayıp Karadeniz'e göç etmeye başlarlarken iri ve yağlı hale gelirler ve bu zamandayken çiroz yapılan balıklar yılın 12 ayı boyunca sevilerek tüketilmektedir.

SONUÇ

Geleneksel fermente balık ürünlerinin üretimi, dünyada uzun süredir devam eden bir endüstridir, ancak yalnızca birkaç ürün ticarileştirilmiştir. Fermente balık ve su ürünleri; gelişmiş duyusal özellikleri, yüksek besleyici değeri ve insan sağlığına yararları olması sebebiyle geniş kitlelerce tüketimi için yeni işlenmiş su ürünleri olarak geliştirilme ihtiyacı vardır. Bu sebeple, hem yerel hem de uluslararası piyasalarda artan talepler değerlendirilerek sektörde ticarileştirilme çalışmalarına geçilmesi gerekmektedir.

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➢ *ORAL PRESENTATION*

Antioxidant Activity, Total Phenolic and Flavonoid Contents of Selected Medicinal Plants

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Abstract
Excessive formation of free radicals produced by living cells in cell metabolism causes some degenerative diseases such as atherosclerosis, ischemic heart disease, aging, diabetes and cancer. In addition, free radicals cause oxidative damage to macromolecules such as body lipids, proteins and nucleic acids. Antioxidants prevent oxidative damage by neutralizing the harmful effects of free radicals Plant phenolics, such as flavonoids, condensed tannins, coumarins and stilbenes have received greater attention since they have antioxidant effects.
The aim of this study is to investigate antioxidant activities of the methanol extracts of seven medicinal plants, namely *Avena sativa, Ginkgo biloba, Lycopodium clavatum, Ocimum basilicium, Peganum harmala, Taraxacum officinale,* and *Valeriana officinalis* with total phenolic and flavonoid contents. The total phenolic and flavonoid contents of the extracts were calculated as microgram gallic acid equivalents (GAEs) and microgram quercetin equivalents (QEs), respectively. The highest total phenolic (132.89±0.66 µg GAEs/mg extract) and flavonoid (42.07±0.01 mg QE/mg extract) contents were found in *O. basilicium* methanol extract. Antioxidant activities of the extracts were tested by using DPPH$^{\bullet}$ scavenging, ABTS$^{\bullet+}$ scavenging and CUPRAC assays. When *A. sativa* methanol extract showed the highest antioxidant activity in DPPH$^{\bullet}$ (IC_{50}: 134.20±0.74 µg/mL) and ABTS$^{\bullet+}$ (IC_{50}: 56.07±0.43 µg/mL) scavenging assays, *O. basilicium* methanol extract ($A_{0.50}$: 51.36±0.41 µg/mL) displayed the highest antioxidant activity in CUPRAC assay. Also, *O. basilicium* methanol extract showed higher antioxidant activity than α-tocopherol ($A_{0.50}$: 66.72±0.81 µg/mL) used as standard in CUPRAC assay.
Keywords: Medicinal plants, extracts, antioxidant activity, total phenolic and flavonoid contents

Seçilmiş Tıbbi Bitkilerin Toplam Fenolik ve Flavonoid Madde İçerikleri ve Antioksidan Aktiviteleri

Öz
Hücre metabolizmasında canlı hücrelerin ürettiği aşırı serbest radikal oluşumu, ateroskleroz, iskemik kalp hastalığı, yaşlanma, diyabet ve kanser gibi bazı dejeneratif hastalıklara neden olmaktadır. Ayrıca, serbest radikaller vücut lipitleri, proteinler ve nükleik asitler gibi makromoleküllerde oksidatif hasara neden olmaktadır. Antioksidanlar, serbest radikallerin bu zararlı etkilerini nötrleştirerek oksidatif hasarı önlemektedir. Flavonoidler, tanenler, kumarinler ve stilbenler gibi bitki fenolikleri, antioksidan etkiye sahip oldukları için son yıllarda daha fazla ilgi görmektedir.
Bu çalışmanın amacı, *Avena sativa, Ginkgo biloba, Lycopodium clavatum, Ocimum basilicium, Peganum harmala, Taraxacum officinale* ve *Valeriana officinalis* bitkilerinden elde edilen metanol ekstrelerinin antioksidan aktivitelerini toplam fenolik ve flavonoid miktarları ile birlikte araştırmaktır. Ekstrelerin toplam fenolik ve flavonoid miktarları sırasıyla gallik asit (GA) ve kersetine (QE) eşdeğer olarak hesaplanmıştır. En yüksek miktarda toplam fenolik (132,89±0,66 µg GAEs/mg ekstre) ve flavonoid (42,07±0,01 mg QE/mg ekstre) miktarı *O. basilicium* metanol ekstresinde belirlenmiştir. Ekstrelerin antioksidan aktiviteleri, DPPH$^{\bullet}$ giderim, ABTS$^{\bullet+}$ giderim ve CUPRAC yöntemi kullanılarak test edilmiştir. *A. Sativa* metanol ekstresi DPPH$^{\bullet}$ (IC_{50}: 134,20±0,74 µg/mL) and ABTS$^{\bullet+}$ (IC_{50}: 56,07±0,43 µg/mL) giderim yöntemlerinde en yüksek aktiviteyi gösterirken, *O. basilicium* metanol ekstresi ($A_{0.50}$: 51,36±0,41 µg/mL) CUPRAC yönteminde en yüksek aktiviteyi göstermiştir. Ayrıca, *O. basilicium* metanol ekstresi CUPRAC yönteminde standart olarak kullanılan α-tokoferole ($A_{0.50}$: 66,72±0,81 µg/mL) göre daha yüksek aktivite göstermiştir.
Anahtar Kelimeler: Tıbbi bitkiler, ekstre, antioksidan aktivite, toplam fenolik ve flavonoid madde içeriği

INTRODUCTION

Antioxidants undergo metabolic neutralization reactions against reactive oxygen species (ROS). ROS are produced at the end of metabolic and physiological processes and can cause harmful oxidative reactions in the organism. ROS have different forms of active oxygen, which contain free radicals such as superoxide ion ($O_2^{\bullet-}$) and hydroxyl ($OH^{\bullet}$) radicals. ROS can cause tissue and DNA damage (Saral et al., 2015). Antioxidant compounds prevent or slow down the damage caused by cancer or radicals (Halliwell, 2000; Halliwell, 2003). The number of antioxidant compounds synthesized by plants, called secondary metabolites, especially phenolics, is estimated to be between 4000 and 6000 (Havsteen, 2002; Peterson and Dwyer 1998; Robards et al., 1999). Phenolic compounds have many biological properties such as antioxidant, anti-inflammatory, anti-aging and anti-cancer (Han et al., 2007). A linear relationship was found between the phenolic content of plants and their antioxidant capacity.

Plants are widely used in the indigenous medical system for therapeutic purposes, and their use as alternatives to synthetic drugs is increasingly popular in modern society. Herbal remedies are defined as botanical medicine or herbal medicine, and they have become more culturally acceptable because they cause fewer side effects than some synthetic medicines and are less expensive, accessible or easily available (Chikezie et al., 2015). Research on drug discovery from medicinal plants consists of a versatile approach that includes botanical, phytochemical, biological and molecular techniques, and as a result of these approaches, it has been revealed that the plants have hypoglycemic, anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-cancer, anti-cholinergic and anti-leprosy activities (Balunas and Kinghorn, 2005; Negi et al. al., 2011). Thus, pure compounds or extracts from medicinal plants provide unlimited opportunities for new drug leaders in terms of these chemical diversity and bioactive properties (Ramawat et al., 2009; Sasidharan et al., 2011).

The interest of different scientific fields in natural compounds for discovering new alternatives to synthetic drugs has increased in recent years. Medicinal plants are considered to be rich sources of biologically active compounds. Therefore, the aim of this study is to investigate antioxidant activities of the methanol extracts of seven medicinal plants, namely *Avena sativa, Ginkgo biloba, Lycopodium clavatum, Ocimum basilicium, Peganum harmala, Taraxacum officinale,* and *Valeriana officinalis* with total phenolic and flavonoid contents.

MATERIAL AND METHODS

Plant Materials

Avena sativa, Ginkgo biloba, Lycopodium clavatum, Ocimum basilicium, Peganum harmala, Taraxacum officinale, and Valeriana officinalis plant species were were purchased from a local shop selling medicinal and aromatic herbs in Konya, Turkey in 2020.

Extraction

The dried plant samples were macerated with methanol at room temperature. After filtration, the solvent was evaporated under vacuum by an evaporator to obtain methanol extracts. All extracts were stored at +4°C for further tests.

Determination of Total phenolic and Flavonoid Contents

The phenolic contents of the methanol extracts were tested based on the method reported by Slinkard and Singleton (1977). Results were given as microgram of gallic acid equivalents (GAEs) using the following equation that was obtained from standard gallic acid graph:

Absorbance=0.0077[gallic acid (µg)] - 0.007, (r^2, 0.9995)

Total flavonoid contents of the methanol extracts were measured by using the aluminum nitrate method (Park et al., 1997). Results were given as microgram quercetin equivalents (QEs) using the following equation that was obtained from standard quercetin acid graph:

Absorbance=0.0154[quercetin (µg)] - 0.0543 (r^2, 0.9998)

Determination of Antioxidant Activity

DPPH free scavenging, ABTS cation radical scavenging and CUPRAC assays were performed for measurement of antioxidant activities of the methanol extract. The graph of the inhibition percentage (%) versus the concentration (µg/mL) was used to calculate the IC_{50} values of the extracts. The sample concentration showing 0.50 absorbance ($A_{0.5}$) was calculated from the CUPRAC absorbance against the sample concentration. The antioxidant activity results were stated as 50 % inhibition concentration (IC_{50}), $A_{0.50}$ which corresponds to the

concentration producing 0.50 absorbance for CUPRAC assay and inhibition percentage (%) at 100, 200, 400 and 800 µg/mL concentrations for all assays.

DPPH free radical scavenging assay

The free radical scavenging activity was determined spectrophotometrically by the DPPH assay described by Çayan et al. (2019). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, its absorption decreases. Briefly, 40 µL sample solutions at different concentrations was added to 160 µL 0.4 mM 40 µL methanol solution of DPPH. Thirty minutes later, absorbance was measured at 517 nm by using a 96-well microplate reader. The capability of scavenging the inhibition activity (I) was calculated using Eq. (I).

$$I\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \text{x}100 \qquad \text{(I)}$$

ABTS cation radical scavenging assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Çayan et al. (2019). Briefly, $ABTS^{\bullet+}$ was produced by the reaction between 7 mM ABTS in H_2O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. The radical cation was stable in this form for more than 2 days when stored in the dark at room temperature. Before usage, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.708±0.025 at 734 nm with ethanol. Then, 160 µL of $ABTS^{\bullet+}$ solution was added to 40 µL of sample solution in ethanol at different concentrations. After 10 min, by using a 96-well microplate reader, the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance (ethanol). The scavenging capability of $ABTS^{\bullet+}$ was calculated using Eq. (I).

Cupric reducing antioxidant capacity (CUPRAC) assay

The cupric reducing antioxidant capacity was determined according to the method of Çayan et al. (2019). To each well, in a 96 well plate, 50 µL 10 mM Cu (II), 50 µL 7.5 mM neocuproine, and 60 µL NH_4Ac buffer (1 M, pH 7.0) solutions were added. Forty microliter extract at different concentrations were added to the initial mixture so as to make the final volume 200 µL. After 1 h, the absorbance at 450 nm was recorded against a reagent blank by using a 96-well microplate reader.

Statistical Analysis

Total phenolic and flavonoid contents and antioxidant activity results were the average of three parallel sample measurements. The data were registered as the mean ± S.E.M. Student's t test was used to determine significant differences between the means and p values <0.05 were accepted as significant.

RESULT AND DISCUSSION

The total phenolic contents of the extracts were tested based on the Folin Ciocalteu method and the results are given as microgram of gallic acid equivalents (GAEs). Total flavonoid contents of the extracts were tested based on the aluminum nitrate method and the results are given as microgram quercetin equivalents (QEs). (Table 1).
According to the obtained results, when the total phenolic contents of the methanol extracts were decreased in the order of *O. basilicium> A. sativa> T. officinale> G. biloba> P. harmala> V. officinalis> L. clavatum;* the total flavonoid contents of the methanol extracts were decreased in the order of *O. basilicium> A. sativa> L. clavatum> V. officinalis> T. officinale> P. harmala> G. biloba.*

Table 1. Total phenolic and flavonoid contents of the methanol extracts[a]

	Total phenolic content (µg GAEs/mg extract)[b]	Total flavonoid contents (µg QEs/mg extract)[c]
A. sativa	118.09±0.86	32.20±0.15
G. biloba	71.20±0.42	13.24±0.35
L. clavatum	20.03±0.83	27.53±0.50
O. basilicium	132.89±0.66	42.07±0.01
P. harmala	63.80±0.13	13.50±0.36
T. officinale	91.33±0.41	14.80±0.18
V. officinalis	41.81±0.12	21.72±0.37

[a] Values expressed are means ± S.E.M. of three parallel measurements.
[b] GAEs, gallic acid equivalents.
[c] QEs, quercetin equivalents.

ABTS cation radical scavenging, DPPH free scavenging and CUPRAC assays were performed for measurement of antioxidant activities of the methanol extracts. Inhibition percentages (%) values at 100, 200, 400 and 800 µg/mL concentrations can be seen in Figure 1 for ABTS cation radical scavenging assay; in Figure 2 for DPPH free scavenging assay and absorbance in Figure 3 at 100, 200, 400 and 800 µg/mL concentrations for CUPRAC assay. IC_{50} values of the methanol extracts are given in Table 2.

Table 2. IC_{50} values of the methanol extracts[a]

		Code	$ABTS^{\bullet+}$ assay IC_{50} (µg/mL)	$DPPH^{\bullet}$ assay IC_{50} (µg/mL)	CUPRAC assay $A_{0.50}$ (µg/mL)
Plant species	***A. sativa***	**ASM**	56.07±0.43	134.20±0.74	55.62±0.21
	G. biloba	**GBM**	173.78±0.78	592.52±1.06	191.77±0.36
	L. clavatum	**LCM**	134.21±0.23	>800	461.53±0.84
	O. basilicium	**OBM**	66.49±0.41	277.75±0.98	51.36 ±0.41
	P. harmala	**PHM**	353.67±1.10	>800	>800
	T. officinale	**TOM**	263.19±0.16	771.90±0.95	276.92±0.13
	V. officinalis	**VOM**	122.14±0.63	479.33±1.45	227.35±0.75
Standards	**α-Tocopherol**	**α-Toc**	38.51±0.54	37.20±0.41	66.72±0.81
	Vitamin C	**Vit C**	5.24±0.18	6.68±0.94	20.67±0.01

[a] IC_{50} values represent the means ± SEM of three parallel measurements.

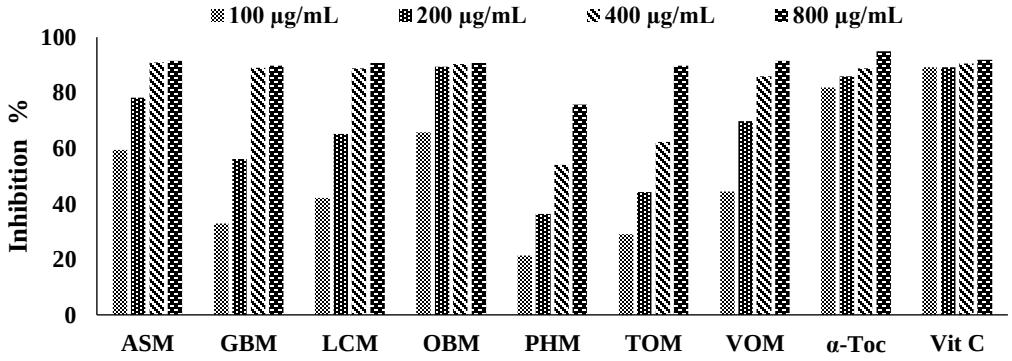

Figure 1. Antioxidant activity of the methanol extracts by $ABTS^{\bullet+}$ assay

$DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals were used to determine the radical scavenging activities of the methanol extracts. *A. sativa* methanol extract showed the highest antioxidant activity in $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays with the IC_{50} values of 134.20±0.74 and 56.07±0.43 µg/mL, respectively. *G. biloba, V. officinalis, L. clavatum, O. basilicium* and *P. harmala* methanol extracts displayed close antioxidant activity to vitamin C used as standard in $ABTS^{\bullet+}$ assays at 400 and 800 µg/mL concentrations. Also, *A. sativa* and *V. officinalis* methanol extracts indicated comparable antioxidant activity with standards in $DPPH^{\bullet}$ assay at 800 µg/mL concentration.

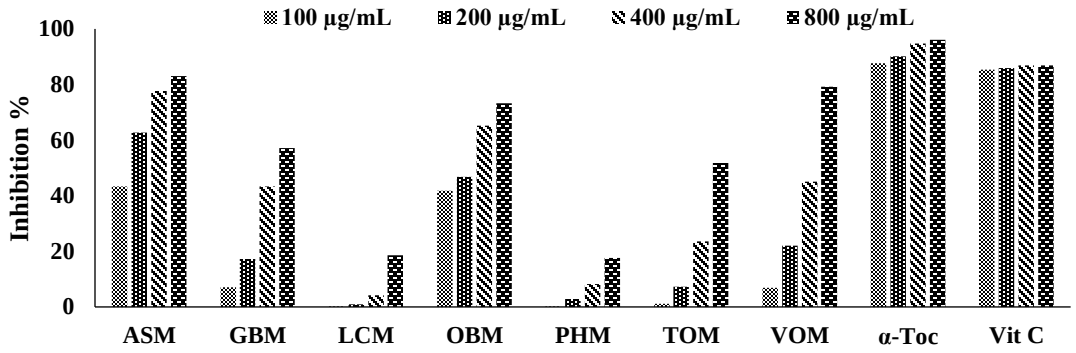

Figure 2. Antioxidant activity of the methanol extracts by DPPH• assay

The reducing power of Cu^{2+} of the methanol extracts was determined by CUPRAC assay. Among the studied extracts, *O. basilicium* methanol extract ($A_{0.50}$: 51.36±0.41 µg/mL) displayed the highest antioxidant activity in CUPRAC assay. Also, *O. basilicium* methanol extract showed higher antioxidant activity than α-tocopherol ($A_{0.50}$: 66.72±0.81 µg/mL) used as standard in CUPRAC assay.

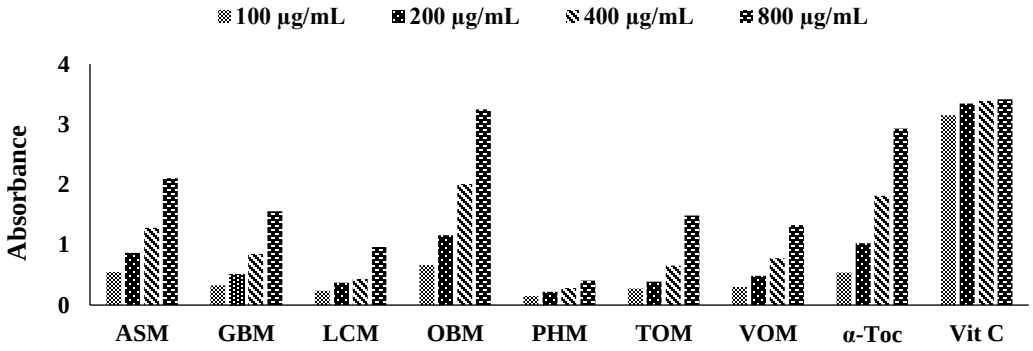

Figure 3. Antioxidant activity of the methanol extracts by CUPRAC assay

According to obtained results, *A. sativa* and *O. basilicium* methanol extracts were found to have the highest antioxidant activity in all activity assays. The highest antioxidant activity is proportional to the highest amount of total phenolic and flavonoid contents in these extracts. In previous literature studies, it has been reported that there is a direct proportion between the amount of total phenolic and flavonoid contents contained in plants and their antioxidant activity (Bardakci et al., 2019; Deveci et al., 2019).

CONCLUSION

In this study, antioxidant activities of the methanol extracts of seven medicinal plants, namely *A. sativa, G. biloba, L. clavatum, O. basilicium, P. harmala, T. officinale,* and *V. officinalis* with the total phenolic and flavonoid contents were investigated. It was determined that *A. sativa* and *O. basilicium* methanol extracts containing the highest concentrations of total phenolic and flavonoid contents had the highest antioxidant activity. The results of this study support the potential use of the studied plant species as natural agents with their antioxidant activities and total phenolic and flavonoid contents.

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➢ *ORAL PRESENTATION*

Citrus myrtifolia meyve ve yaprak metanol ekstrelerinin klinik ve gıda kökenli test mikroorganizmaları üzerine antimikrobiyal aktivitesinin araştırılması

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Özet

Geçmişten günümüze kadar taze narenciye ve türleri insan beslenmesinde çok önemli bir role sahiptir. İçerdiği yüksek lif oranı ve C vitamini nedeniyle ilgileri üzerine toplamayı başaran bir tür olarak karşımıza çıkmaktadır. Çin turuncu meyvesinin en geniş kullanım alanı süs bitkisidir. Esas kullanım alanı İtalyan bir içecek olan *Chinotto*'nun yapımında kullanılmaktadır. Bundan dolayı *Chinotto* adıyla da bilinmektedir. Bu çalışmamızda, Çin turuncunun yaprak ve meyvesinin metenolik ekstresinin gıda ve klinik kökenli (*Bacillus cereus* RSKK 863, *Escherichia coli* ATCC 11229, *Micrococcus luteus* NRRL B-4375, *Staphylococcus aureus* ATCC 25923, *Yersinia enterolitica* ATCC 11175) test mikroorganizmaları üzerine antimikrobiyal aktivitesi disk difüzyon testi, minimal inhibisyon (MİK) ve bakterisidal (MBK) konsantrasyon değerlerinin belirlenmesi ile belirlenmiştir. Disk difüzyon sonuçlarına göre, meyve metanol esktresinde en yüksek inhibisyon zon çapı *Y. enterolitica* ATCC 11175 üzerinde 19.47±0.96 mm olarak kaydedilmiştir. Yaprak metanol ekstresinin en yüksek inhibisyon zon çapı 20.2±0.16 mm olarak *E. coli* ATCC 11229 test mikroorganizması üzerinde tespit edilmiştir. Meyve ve yaprak metanol ekstrelerinin test edilen mikroorganizmalar üzerine MİK ve MBK değerleri 12.5-100 mg/mL arasında belirlenmiştir. Çin turuncu meyve ve yaprak ekstrelerinin doğal antimikrobiyal kaynağı olarak farmasötik ve gıda sanayilerinde alternatif kullanma potansiyeline sahip olduğu tespit edilmiştir.

Anahtar kelimeler: Çin turuncu, antibakteriyel aktivite, ekstre, metanol

Investigation of antimicrobial activity of *citrus myrtifolia* fruit and leaf methanol extract on clinical and food-borne test microorganisms

Abstract

From past to present, fresh citrus fruits and their types have a very important role in human nutrition. Due to the high fiber content and vitamin C it contains, it is a species that manages to attract attention. The most widely used Chinese orange fruit is ornamental plants. Its main use is in the production of Chinotto, an Italian beverage. Therefore, it is also known as Chinotto. In this study, the food and clinical origin (*Bacillus cereus* RSKK 863, *Escherichia coli* ATCC 11229, *Micrococcus luteus* NRRL B-4375, *Staphylococcus aureus* ATCC 25923, *Yersinia enterolitica* ATCC 11175) test microorganisms for antimicrobial activity of the methenolic extract of the leaf and fruit of Chinese orange. was determined by determining the minimal inhibition (MIC) and bactericidal (MBC) concentrations. According to disk diffusion results, the highest inhibition zone diameter in fruit methanol extract was recorded as 19.47 ± 0.96 mm on *Y. enterolitica* ATCC 11175. The highest inhibition zone diameter of the leaf methanol extract was determined as 20.2 ± 0.16 mm on *E. coli* ATCC 11229 test microorganism. The MIC and MBC values of the fruit and leaf methanol extracts on the microorganisms tested were determined between 12.5-100 mg/mL. It has been determined that Chinese orange fruit and leaf extracts have the potential of alternative use in the pharmaceutical and food industries as a natural antimicrobial source.

Keywords:Çin turuncu, antibacterial activity, extract, methanol

GİRİŞ

Çin turuncu (*Citrus myrtifolia)* Chinotto veya mersin yapraklı portakal olarak da bilinen küçük bir turunçgil meyvesidir ve acı portakalın botanik bir çeşididir (*C. aurantium var. myrtifolia*) (Tanaka 1961).Çin turuncu *Rutaceae* familyasına ait olan bir narenciye türüdür. Olgunlaşmamış meyveleri mandalinayı andırırken, olgun meyvelerinin tadı acı ve ekşidir. Geniş yayılımı, süs bitkisi olarak kullanılmasından kaynaklanmaktadır, ancak gıda endüstrisindeki uygulamaları için İtalya ve Güney Fransa'da yetişmektedir (Barreca ve ark., 2011). Birçok ülkede sadece süs amaçlı yetiştirilmekle birlikte, ekşi tatlandırıcı meyvelerinin gıda endüstrisi üzerinde önemli bir etkisi vardır (Barreca ve ark., 2010). Meyve yapısındaki antioksidan bileşenler, vitaminler ve diğer fonksiyonel bileşimleri sayesinde sağlık için faydalı etkileri bulunmaktadır (Çakmakçi ve ark., 2016). Esas olarak *Chinotta* adıyla bilinen gazlı ve alkolsüz içecek yapımında kullanılmaktadır. Meyveleri halk arasında tıbbi amaçlı kullanılmasına rağmen çok fazla ayrıntılı çalışmalar bulunmamaktadır (Su ve ark., 2008). Gıda endüstrisinde ise şekerleme ve reçel yapımında kullanımı mevcuttur. Narenciye türleri ile yapılan birçok çalışmada fitokimyasalar açısından zengin olduğu kanıtlanmıştır. Ayrıca narenciye türlerinin antioksidan, antiviral, antibakteriyal, antienflamatuvar özelliklerinin olduğu literatürde bildirilmiştir (Benavente García ve Castillo, 2008; García-Lafuente ve ark., 2009; Srinivasan ve ark., 2007). Aslında, son on yılda çok sayıda bilimsel araştırma, turunçgillerin farklı bölümlerinde bulunan ayrı ayrı bileşikleri spesifik faydalı etkilere bağlayan böyle bir bağlantının olduğunu öne sürmüştür (Patil ve ark., 2009). Günümüzde bulaşıcı hastalıklar dünyadaki ölümlerin üçte birini oluşturmaktadır; Dünya Sağlık Örgütü, dünya genelinde her gün yaklaşık 50.000 kişinin bulaşıcı hastalıklardan kaynaklı öldüğünü tahmin etmektedir (Chanda ve Rakholiya, 2011). Bakteriyel hastalıklar ile mücadelede antibiyotikler büyük önem kazanmaktadır. Genel olarak mikroorganizmalar, terapötik ajan olarak kullanılan antibiyotik ilaçlara karşı direnç kazanma yeteneğine sahiplerdir (Vaghasiya ve ark., 2008). Antibiyotiklere karşı kazanılan direncin en önemli nedeni olarak kontrolsüz antibiyotik kullanımı gelmektedir. Bilim insanları son yıllarda antibiyotik kullanımını kontrol altına almak ve antibiyotik ilaçlara alternatif olarak doğal ilaç araştırma yoluna girmişlerdir. Günümüzde bitkiler, doğal tedavilere yönelik daha yoğun çalışmalarla insan sağlığını korumak için değerli bir doğal ürün kaynağı olmuştur (Chanda ve Rakholiya, 2011). Kazanılan direncin ve oluşan hastalıkların önüne geçmek için doğal antimikrobiyal ajanlar hızla önem kazanmaktadır. Bu çalışmada Çin turuncu meyve ve yaprak metanol esktrelerinin gıda ve klinik kökenli test mikroorganizmalarına karşı antimikrobiyal aktivitesi araştırılmıştır.

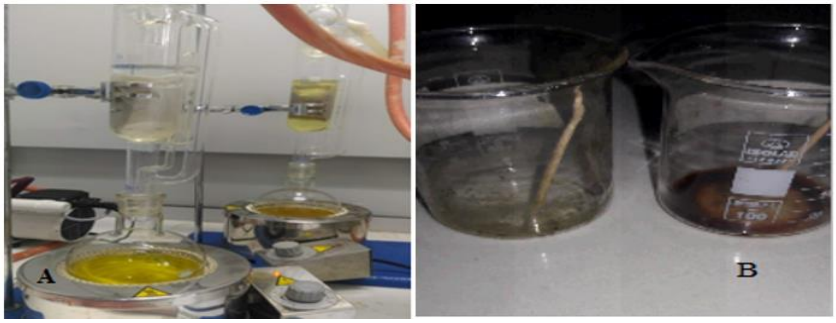
MATERYAL VE METOT

Bitki Materyali

Çin turuncu (*Citrus myrtifolia Raf.*) yaprak ve meyveleri Alata Bahçe Kültürleri Araştırma Enstitüsü'den temin edilmiştir.

Ekstrelerin Hazırlanması

Çin turuncu meyve ve yaprakları yıkanıp kurutulmuş ve Waringblendır ile öğütülmüştür. Öğütülen meyve ve yaprak örnekleri soksilet sistemi ile metanol ile ekstrakte edilmiştir (Şekil 1A). Çözücüler rotaryev aporatör kullanılarak uçurulmuş ve kuru ekstre elde edilmiştir (Şekil 1B). Çin turuncu meyve-yaprak ekstreleri metanolde çözülmüş ve 0.45µm'lik filtre ile steril edilmiştir. Filtre edilen kuru ekstreler +4 °C'de muhafaza edilmiştir.



Şekil 1. Çin turuncu meyve ve yaprak ekstrelerinin hazırlanması
A. Çin turuncu meyve ve yaprak ekstrelerinin soksilet sistemi ile hazırlanması
B. Çözücüleri buharlaştırılmış çin turuncu meyve ve yaprak ekstreleri

Kullanılan mikroorganizmalar

İnsan gıda ve klinik kökenli test mikroorganizmalarının (*Bacillus cereus* RSKK 863, *Escherichia coli* ATCC 11229, *Micrococcus luteus* NRRL B-4375, *Staphylococcus aureus* ATCC 25923, *Yersinia enterolitica* ATCC 11175) 24 saatlik aktif kültürleri kullanılmıştır.

AntimikrobiyalAktivitenin Belirlenmesi

Disk difizyon yöntemi

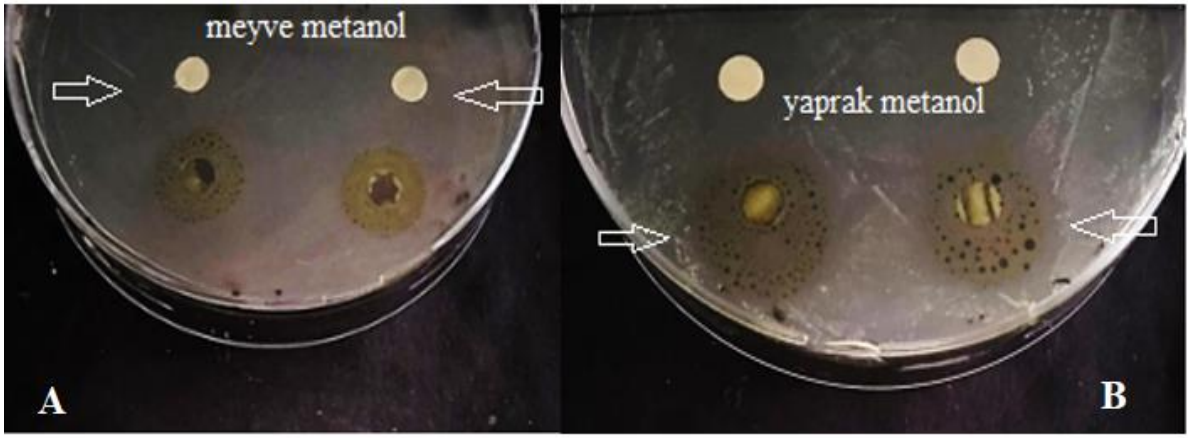
Çin turuncu meyve ve yaprağının metenolik ekstresinin antimikrobiyal aktivitesini belirlemek amacıyla disk difizyon yöntemi kullanılmıştır. Kullanılan 5 adet klinik ve gıda kökenlitest mikroorganizmaları NutrientBroth (NB) besi yerlerine kültürlenmiş ve 37°C de inkübasyona bırakılmıştır.Test mikroorganizmaları, serum fizyolojik ile iki defa yıkandıktan sonra konsantrasyonları 0.5 McFarland'a ayarlanmış ve daha sonrasında katı besi yerine 100 µL inoküle edilmiştir. İnoküle edilenpetrilere steril diskler yerleştirildikten sonra disklere 20 µL (4 mg/disk) çin turuncu meyve ve yaprak ekstreleri damlatılmıştır. Petriler 24 saat 37°C'de inkübe edilmiştir. İnkübasyon süresi sonunda diskler etrafındaki zonlar kumpas ile ölçülerek kaydedilmiştir. Tüm deneyler iki tekrarlı olarak yapılmıştır.

Minimal İnhibisyon (MİK) ve Bakterisidal (MBK) konsantrasyonlarının belirlenmesi

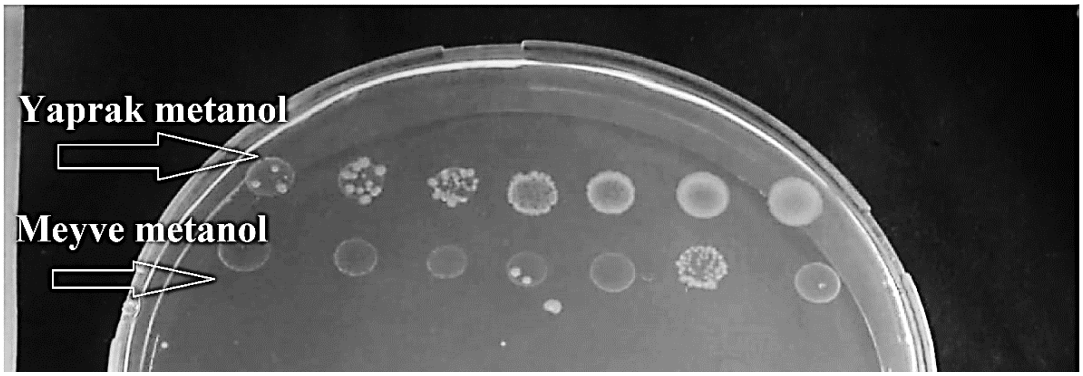
Ekstrelerin MİK ve MBK değerleri insan test mikroorganizmalarına karşı mikrodilüsyon yöntemi ile belirlenmiştir. Ekstre ve besiyeri içeren her tüpe 0.5 McFarland konsantrasyonunda test mikroorganizmaları eklenmiş ve vorteks yapılmıştır. Daha sonra karışımı içeren tüpler 24 saat 37°C'de inkübe edilmiştir. İnkübasyondan sonra, sıvı besi yerinde gelişmenin olmadığı konsantrasyon MİK değerleri olarak kaydedilmiştir. Tüplerden örnekler alınarak spesifik agar besi yerine spot ekimleri yapılmış ve 24 saat 37°C'de inkübe edilmiştir. İnkübasyon süresinin sonunda, katı besi ortamı üzerinde bakterilerin gelişimini önleyen ekstre konsantrasyonları MBC değerleri olarak değerlendirilmiştir.

BULGULAR VE TARTIŞMALAR

Çin turuncu meyve-yaprak metanol ekstrelerinin gıda (*B. cereus* RSKK 863, *M. luteus* NRRL B-4375, *S. aureus* ATCC 25923, *Y. enterolitica* ATCC 11175) ve klinik (*E. coli* ATCC 11229) test mikroorganizmaları üzerine antimikrobiyal aktiviteleri disk difüzyon yöntemi ile belirlenmiştir. Yapılan disk difüzyon deneyinin sonuçlarına göre meyve metanol ekstresinde en yüksek inhibisyon zon çapı *Y. enterolitica* ATCC 11175 üzerinde 19.47±0.96 mm olarak kaydedilmiştir (Şekil 2A). Bu inhibisyon zon çapı, kontrol grubu olarak kullanılan Kanamisin antibiyotiğinin (30 µg) *Y. enterolitica* ATCC 11175 üzerine kaydedilen inhibisyon zon çapından (14.84±0.43 mm) daha yüksek olarak tespit edilmiştir. Çin turuncu meyve metanol ekstresi *Y. enterolitica* ATCC 11175 test mikroorganizmasına kanamisin antibiyotiğinden (30 µg) daha fazla antibakteriyel etki göstermiştir. Yaprak metanol ekstresinde en yüksek inhibisyon zon çapı 20.2±0.16 mm olarak *E. coli* ATCC 11229 test mikroorganizması üzerine tespit edilmiştir (Şekil 2B). Kontrol grubu olarak kullanılan kanamisin antibiyotiğinin *E .coli* ATCC 11229 test mikroorganizması üzerine kaydedilen inhibisyonzon çapı 19.51±0.24 mm olarak tespit edilmiştir. Çin turuncu meyve ve yaprak metanol ekstrelerinin MİK ve MBK değerleri 12.5-100 mg/mL olarak belirlenmiştir (Tablo 1, Şekil 3). En düşük MIK ve MBK değeri 12.5 mg/ml olarak *M. luteus* NRLL 4375 mikroorganizması üzerinde test edilmiştir.



Şekil 2: Çin turuncu meyve ve yaprak ekstrelerinin antibakteriyel aktivitesi
(A) Meyve metanol ekstresinin *Y. enterolitica ATCC* 11175 üzerindeki aktivitesi
(B) Yaprak metanol ekstresinin *E. coli* ATCC 11229 üzerindeki aktivitesi



Şekil 3. Çin turuncu meyve ve yaprak metanol ekstrelerinin *M. Luteus* NRLL 4375'e karşı MBK değerlerinin belirlenmesi

Aşan-Özüsağlam ve Günyaktı (2015)'nın yaptığı bir başka çalışmada ise Çin turuncu meyve ve yaprak metanol ekstrelerinin *Candida albicans* ATCC 10231 ve *Candida glabrata* RSKK 04019 mikroorganizmasına karşı antifungal aktivite gösterdiği bildirilmiştir. Ayrıca gıda ve klinik kökenli bakteriyel test mikroorganizmalarına karşı antibakteriyel aktivitenin araştırıldığı bu çalışmada disk difüzyonu yöntemi verilerine göre, en yüksek inhibisyon zon çapı (12.57 mm) *C. myrtifolia* meyve metanol ekstesinde *S. enteritidis* RSKK 171'e karşı tespit edilmiştir. En düşük inhibisyon zon çapı ise *Pseudomonas aeruginosa* ATCC 27853 üzerinde Çin turuncu yaprak metanol ekstresinde (8.65 mm) belirlenmiştir. Bu çalışmamızda ise aynı miktarda kullanılan Çin turuncu meyve ve yaprak metanol ekstreleri (4 mg/disk) test edilen tüm bakteriler üzerinde daha yüksek inhibisyonu zonu oluşturmuştur.

Tablo 1: Çin turuncu meyve-yaprak metanol ekstresinin antimikrobiyal aktivitesi

Test mikroorganizmaları	**İnhibisyon zon çapı (mm)**		**MİK (mg/mL)**		**MBK (mg/mL)**		**Antibiyotik inhibisyonzon çapı (mm)**
	Meyve	**Yaprak**	**Meyve**	**Yaprak**	**Meyve**	**Yaprak**	**Kanamisin (30 µg)**
***B.cereus* RSKK 863**	15.32 ±0.96	16.25 ±0.13	50	100	100	100	20.13 ±0.64
***E. coli* ATCC 11229**	18.21 ±0.96	20.20 ±0.16	50	100	100	100	19.51 ±0.24
***M. luteus* NRLL B-4375**	16.03 ±0.47	14.55 ±0.91	12.5	50	12.5	100	15.85 ±0.97
***S. aureus* ATCC 25923**	16.14 ±0.47	16.79 ±0.72	50	50	100	100	16.16 ±2.41
***Y. enterolitica* ATCC 11175**	19.47 ±0.96	16.25 ±0.53	100	50	100	100	14.84 ±0.43

SONUÇ

Çin turuncu meyve ve yaprak metanol ekstrelerinin test edilen gıda ve klinik kökenli insan test mikroorganizmalarına karşı antimikrobiyal aktivite gösterdiği tespit edilmiştir. Elde edilen veriler, bu ekstrelerin gıda ve ilaç sanayisinde doğal antimikrobiyal madde katkısı olarak kullanım potansiyelinin olabileceğini göstermiştir. Ayrıca, yaprak ve meyvelerinin yıl boyunca temin edilebilir olması bu bitkilerin önemini artırmaktadır.

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➢ *ORAL PRESENTATION*

Ethidium Bromide Yerine Schiff Bazı ile DNA Görüntüleme

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Özet
Agaroz kullanan DNA jel elektroforezi, moleküler biyoloji laboratuvarlarında DNA fragmanlarının boyuta göre ayrılmasına izin veren yaygın bir araçtır. Ayrıldıktan sonra DNA boyama ile görselleştirilir. Bu yöntemde genellikle etidyum bromür kullanılır, ancak etidyum bromür toksiktir. Bu nedenle etidyum bromür kullanımına dikkat edilmelidir. Bu yüzden DNA moleküllerini görüntülemek için birçok boya veya molekül yapısı geliştirlmektedir. Floresans özellik gösteren Schiff bazı molekülleri bu DNA yapısıyla etkileşebilen kompleks yapılar içermektedir. Schiff bazı molekülleri, kolay sentezlenebilen, yaygın kullanılan ve etidyum bromürden daha az toksik özellik gösteren yapılardır. Bu çalışmada, Schiff bazı moleküllerinin DNA molekülleri yapısını nasıl boyadığı çalışılmıştır. Daha az toksik olan Schiff bazı molekülleri ile DNA molekülleri etkileştirilmiş ve UV ışık altında jelde yürütülen DNA yapısı görselleştirilmiştir.

Anahtar Kelimeler: Schiff bazı, DNA görüntüleme, UV, Floresans

DNA Imaging with Schiff Base Instead of Ethidium Bromide

Abstract
DNA gel electrophoresis using agarose is a common tool in molecular biology laboratories that allows for the separation of DNA fragments by size. After separation, the DNA is visualized by staining. Ethidium bromide usually is used in this method, but ethidium bromide is toxic. Therefore, attention should be paid to the use of ethidium bromide. For this reason, many dyes or molecular structures have been developed to display DNA molecules. Schiff base molecules with fluorescence feature contain complex structures that can interact with DNA structure. Schiff base molecules are easily synthesized, widely used and less toxic than ethidium bromide. In this study, it has been studied how Schiff base molecules stain the structure of DNA molecules. The less toxic Schiff base molecules and DNA molecules were interacted and the DNA structure executed in gel was visualized under UV light.

Keywords: Schiff base, DNA imaging, UV, Fluorescence

GİRİŞ

Agaroz jel elektroforez tekniği, fragmanlarına ayrılmış DNA molekülünün varlığını tespit etmek için yaygın olarak kullanılan bir metoddur. Bu agaroz jel elektroforezi yönteminde DNA yapısını görüntüleyebilmek için çeşitli boyalar kullanılmaktadır. Bu boyalardan en yaygın olarak kullanılanlar propidyum iyodit ve etidyum bromür'dür. Etidyum bromür bir DNA ipliği üzerinde komşu iki baz arasına girebilen interkalatör bir boyadır. Etidyum bromür pozitif yüklü aromatik bir bileşiktir ve baz çiftlerinin arasına girerek DNA'ya bağlanmaktadır.
Propidyum iyodid ise etidyum bromürden farklı olarak 488 nm'de kırmızı floresans yayar ve yine DNA'ya baz çiftleri arasına girerek bağlanan interkalatör bir boyadır. Bu boyalar DNA yapısına baz seçmeden ve/veya çok az seçicilik göstererek 4-5 baza karşılık bir molekül olacak şekilde stokiyometrik bir bağlanma gösterir. Bu durumda DNA ile boya moleküllerinin bağlanması sonucu konformasyonel farklılıklar meydana gelir. Ve bu boya moleküllerinin bağlanma sonucu baz çiftleri arasına yerleşmesi fragmanlarına ayrılmış ya da dsDNA molekülünün transkripsiyona ve replikasyonuna engel olur. Schiff bazı molekülleri de DNA molekülleri ile etkileşime girebilen kompleks yapılardır. Schiff bazı molekülleri, aminlerin aldehit veya ketonlar ile oluşturdukları aldiminlerin yaygın kullanılan adlarıdır ve ilk kez Hugo Schiff tarafından 1864 yılında açıklanmıştır (Malik ve ark., 2018). Kolay

sentezlenebilen bu moleküller, baz çiftleri arasına girerek DNA yapısıyla çapraz bağlanma gösterirler. Aynı zamanda bu bağlanma ile UV ışık altında DNA molekülleri hareketi görselleştirilmektedir.
Bu çalışmada Schiff bazları ile agaroz jel elektroforezinde, DNA yapısının görüntülenmesi amaçlanmaktadır. Çünkü etidyum bromür ve propidyum iyodid boyaları mutajen ve karsinojendir, yaygın olarak kullanılan bu boyaların yanı sıra Schiff bazı molekülleri toksik özellik göstermemektedir.

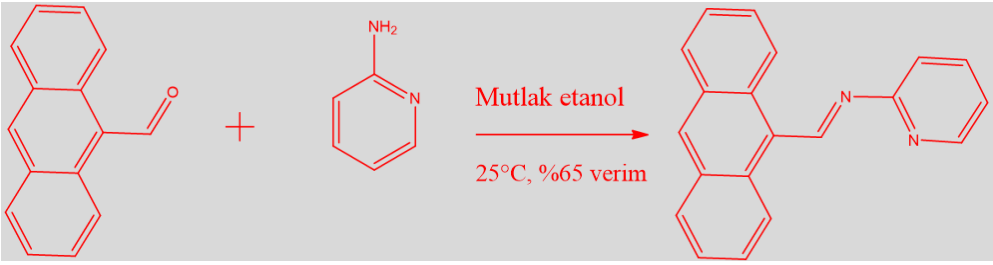
MATERYAL VE METOD

Schiff bazı literatüre uygun şekilde modifiye edilerek sentezlendi (Fathima ve ark., 2019). Sentezi gerçekleştirilen Schiff bazının sentezinde, 9-antrasenaldehit bileşiği ile 2-amino-piridin bileşikleri kullanılmıştır. Elde edilen molekül DNA yapısını görüntülemek için jel elektroforezinde yürütme işlemi gerçekleştirildi ve UV görüntüleri elde edildi.

Jel Elektroforezi, boyut, taşıdığı yük veya konformasyon bakımından çeşitlilik gösteren özellikle proteinleri ve nükleik asitler gibi farklı makromolekülleri ayırmak ve bazen de saflaştırmak için sık sık tercih edilen bir tekniktir (Nimse ve Kim, 2013). Çift zincirli DNA'nın bileşik ile etkileşimi agaroz-jel elektroforezi aracılığıyla izlenebilir. Elektroforez DNA molekülünü (negatif yüklü) pozitif elektrota doğru yürütmek için bir elektrik alan oluşturmaktadır (Cai veark., 2009). Jel elektroforezi, Tris-HC1 (50 mM) / NaCl tamponu (pH 7.2) içerisinde dsDNA ve Schiff bazı içeren örnekler kuyucuklara yüklenecek şeklinde hazırlandı. Hazırlanan tüm örnekler %2'lik agaroz jele yükleme tamponu içersinde yüklendi. Ardından, TBE tamponu (1 mM-EDTA, 50mMTris, 50mM H_3B0_3, pH 7.2) içinde örnekler 80V'da 1 saat boyunca yürütüldü. UV ışığı ile ortaya çıkan bantlar görselleştirilerek fotoğraflandı (Fraňo ve ark., 2016).

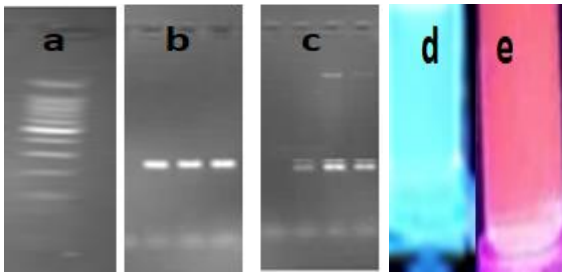
BULGULAR ve TARTIŞMA

Sentezlenen bileşiğin,yapısı şekil 1'de gösterilmiştir ve ^{1}H-NMR ve FT-IR analizleri ile karakterize edilmştir.
1-(antrasen-9-il)-N-(piridin-2-il)metanimin sentezi: Açık sarı katı, verim: 0.74 g (%66), E.N.: 288-290 °C, FT-IR (ATR, cm^{-1}): 1664 (CH=N). ^{1}H NMR (600 MHz DMSO-d_6): δ 8.90-8.79 (m, 3H, Ar-H ve CH=N), 7.97-7.73 (m, 5H, Ar-H), 7.50 (m, 1H, Ar-H), 7.43-7.20 (m, 5H, Ar-H). $C_{20}H_{14}N_2$ için hesaplanan; C, 85.08; H, 5.00; N, 9.92. Bulunan: C, 85.17; H, 4.95; N, 9.89.



Şekil 1. 1-(antrasen-9-il)-N-(piridin-2-il)metanimin sentezi

Sentezi gerçekleştirilen Schiff bazı bileşiği agaroz jelde, çift veya tek zincirli DNA molekülü ve RNA molekülü yapısını görüntülemek için kullanılmıştır. DNA ya da RNA molekülüne bağlandığında floresans yayan bu bileşiğin floresans emisyonu 350 nm civarında vermektedir. Schiff bazı molekülük karsinojenik değildir. Bu bileşik DNA ve RNA moleküllerini poliakrilamid jel ve agaroz jelde görüntülemek için kullanılmış kolay sentezlenebilen hassas bir moleküldür. Etidyum bromür ile karşılatırdığımızda DNA veya RNA yapılarına bağlanma durumları şekil 2'de gösterilmektedir.



Şekil 2. DNA marker (a) Etidyum bromür ile DNA etkileşimi (b) Schiff bazı ile DNA etkileşimi (c) UV ışık altında Schiff bazı (d) UV ışık altında Etidyum bromür (e)

Etidyum bromür ile boyanan DNA molekülü şekil 2b'de görülmektedir, Schiff bazı molekülü ile şekil 2c'de gösterilmektedir. Görüldüğü üzere sentezlenen bileşik DNA molekülü ile etkileşerek boyanmıştır ve UV ışık ile agaroz jelde görselleştirilmiştir. UV ışık altında etidyum bromür ve Schiff bazı moleküllerinin görüntüsü şekil 2d ve 2e'de görüntülenmiştir. Gözlemlerimiz, Schiff bazı ile DNA molekülü arasında bir etkileşimin gerçekleştiğini UV ışık altında yapılan gözlemle ortaya çıkarılmıştır. Ve bileşik ile baz çiftleri arsında çapraz bağlanma ortaya çıktığını düşünülmektedir (O'Neil ve ark., 2019).
Agaroz jel elektroforezi, DNA ve RNA moleküllerinin yapısını görüntülemek içi hızlı, hassas ve ucuz bir test tekniğidir. Yöntemin uygulanmasında yaygın olarak etidyum bromür kullanılmaktadır, ancak toksik olduğundan yenibirçok boyar madde sentezlenmiştir. Yapılan literatür taramalarında birçok boyar maddeye rastlanılmaktadır. Schiff bazları hem kolay sentezlenen hem de toksik özellik taşımayan yapılar olması nedeni ile etidyum bromür yerine tercih edilebilir.

SONUÇ

UV ışık altında floresans özellik gösteren Schiff bazı molekülü sentezlendi. Sentezlenen bileşik DNA molekülleri arasına yerleşerek agaroz jelde yürütülen DNA fragmanlarını görüntülemeyi sağladı. Schiff bazı bileşiğietidyum bromürden daha az toksik olması ve kolay sentezlenebilir bir molekül olması DNA görüntüleme işlemlerinde daha çok tercih edilebilir.

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➢ *ORAL PRESENTATION*

Adsorption of Brilliant Blue from aqueous solutions by Amberlyst - A21 /*Agaricus Campestris* biocompozite material.

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Abstract
In this paper, the adsorption of Brilliant Blue was performed from synthetic wastewater using biocompozite material that was occurred Amberlyst-A21 resin and Agaricus Campestris culture treatment. Adsorptions models were used to describe our experimental system. Due to the shape of the isotherms, the sorption data were calculated according to Freundlich, Langmuir, Temkin and Harkin's Jura Ishoterms. Harkins Jura isotherm shows better fit than other isotherms. In addition, the characterization of post and pre adsorption process was confirmed by Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). The results showed that our biocompozite adsorbent material was an appropriate and effectively adsorbent for the removal of Brilliant Blue dye from aqueous solutions in some areas.
Keywords: *A. Campestris,* Adsorption, Amberlyst-A21, Brilliant Blue, Removal, Wastewater

INTRODUCTION

In parallel with rapid population growth and industrialization, water resources are increasingly contaminated with many pollutants such as dyestuffs, pesticides and pharmaceutical compounds (Vakili et al., 2019). Dyes are non-aesthetic pollutants in aquatic environments that also cause many health problems for living life (Keskin et al., 2015). The removal and disposal of the relevant dyes are considered important by scientists and various studies are carried out. There are one hundred thousand dyes and their pigments. 7x105 tones of them are produced in the world at the moment (McKay et al., 1985). Dyestuffs are common in the fields of plastics, paper, leather and food, textiles and cosmetics (Mitrovic et al., 2014). Brilliant Blue G (BBG), a anionic compound, is used in many industrial areas. The discharge of BBG containing wastewater to receiving environments causes undesirable environmental conditions (Khambhaty et al., 2012).

Amberlyst A21 resin was chosen as a sorbent due to its specific chemical and physical structure. The anion exchanger Amberlyst A21 is a weakly basic macroporous dimethyl amine group polystyrene resin of NR2 functional groups with an exchange capacity greater than 4.6 eq / kg (Hubicki and Wolowicz, 2009). In recent years, there has been increasing interest in using Amberlyst 21 as an adsorbent due to its highly selective properties. There are not many studies on the use of Amberlyst A21 for paint removal. Amberlyst A21 resin has been used for removal various contaminants such as chromium (VI) (Karekar and Divekar 2017), palladium (Hubicki and Wolowicz, 2009), sulfate (Guimarães and Leão, 2014), acetic acid (Han et al., 2006), phenol (Chen et al., 2017). Removal process is critical to pay low costly and ergonomically for human. Chemical and biological techniques such as adsorption (Yonten et al, 2016; Ozturk et al., 2017) coagulation (Chenna et al., 2016), ozonlama (Turhan et al., 2012), ultra filtration (Liu et al., 2018), oxidation (Arslan et al., 2000), biosorption (Yönten et al., 2020) are used to remove dyes from wastewater. Here, the use of the adsorption process is preferred over other methods. Because it is easily producing and is low cost. Among these methods, adsorption is considered promising due to its easy operation and not creating any by-products. Today, the adsorption process is widely used in wastewater treatment due to its superiority in removing many recalcitrant pollutants in biological systems (Kyzas et al., 2014).

The aim of this study is to assess the adsorption capacity of an Amberlyst A21 weakly basic ion exchanger for the removal of BBG from wastewater and determination of adsorption isotherms. Characterization of Amberlyst - A21 /*Agaricus Campestris (*biocomposite) was performed using F-TIR and SEM. The adsorption mechanism of

the BBG on the biocomposite was determined by the isotherm models of Langmuir, Freundlich, Temkin and Harkins-Jura.

MATERIAL METHOD

Chemical Materials

The ion exchange resins (Amberlyst - A21) were purchased from Merck (Germany). Fungi (*A. Campestris*) was chosen as the biological material and was collected in the Van, Turkey region,
Brilliant Blue G-250 was chosen as the pollutant dyestuff and supplied by Fisher Scientific (USA). The pH of synthetic dye solutions was adjusted with 0.1 M HCl and 0.1 M NaOH respectively. They were analytically purchased from Merck (Germany).

Biocomposite material

Fungi was washed several times with distilled water to remove pollutants. It was then dried at 343 K in an oven for 24 h to ensure the complete death of the dried cells, and the powdered cells were stored in desiccators until further use. Then, preparation and immobilization of A. campestris on Amberlite resin were performed as follows: 0.1 g of fungus powder was mixed with 1 g of Amberlite resin. (Yönten et al., 2020)

Adsorption experiments

Adsorption experiments were carried out in Erlenmeyer (100 mL) bottles. The different BBG concentration were prepared in bottles, and their pH were measured with Hanna HI 2211 (China). 0.1 M HCl and 0.1 M NaOH were used for pH adjustments during experiments. Volumes of 25 and 50 mL. After the bottles were taken to Wise Shake SHO-2D (England) orbital shaker at 250 rpm. The supernatants taken into a centrifuge. Then these solutions were placed into a WTW 6100 UV spectrometer (Germany) device for dye analyses.

RESULT AND DISCUSSION

Characterization of adsorbent

The adsorption capacity of an biocomposite depends on the functional groups on the biocomposite surface (Kumar et al., 2014). F-TIR of the biocomposite before and after the adsorption of the BBG is shown in Figure 1. As shown in Figure 1, the peaks observed in the range of 3273–2812 cm^{-1} show that there are C-H stress vibrations in the pre-adsorption structure of the biocomposite. Also, the band at 1361 cm^{-1} shows the C-N stretch vibration of the amine. There are also bands corresponding to C = C and = C-H aromatic stretching vibrations at 1666-1418 and 1018-668 cm-1, respectively (Nagireddi et al., 2018). Comparing the spectra of the biocomposite before and after the adsorption of the BBG, a significant shift in the spectral peaks was observed at 2324-1983 cm^{-1}. Also, as seen from Figure 1, the band was shown at 3634 cm^{-1} before adsorption, but after adsorption this band shifted to 3948 cm^{-1}. The appearance of new peaks in the FTIR spectrum indicates that the BBG is adsorbed on the biocomposite. These vibrations are caused by the binding of BBG ions to the functional groups of the biocomposite after adsorption. After adsorption, new peaks of 1900 cm^{-1} were observed. Therefore, the variation of the wave number of the peaks confirms the adsorption of the BBG on the surface of the biocomposite. SEM images (15,000 × magnification) of the BBG on biocomposite before and after adsorption are shown in Figure 2. Figure 2a shows that the surface of the composite particles before adsorption is homogeneous, smooth and contains less residue. In Figure 2b, the BBG adsorbed by the adsorbent surface can be clearly seen. The post-adsorption images shown in Figure 2 (b) are more intense and confirmed that the irregular pores on the biocomposite surface and the deposits formed were adsorbed to the particle surface of the BBG. (Zazycki et al., 2018). The results observed from the SEM analysis show that the surface of the biocomposite has a large surface area and a rough porous structure. Similar effects have been reported for some adsorbents in the literature. (Hameed and El-Khaiary 2008, Santhi et al., 2010)

Table 1 Parameters in the adsorption isotherms models of BBG onto Amberlyst - A21 /Agaricus Campestris biocompozite material.

Ishoterms	**Langmuir**			**Freundlich**				**Temkin**			**Harkins Jura**		
Constant	q_m	K_L	R^2	R_L	K_F	n^{-1}	R^2	B_T	K_T	R^2	A_{HJ}	B_{HJ}	R^2
	3,64	13.7	29	51.07	12.8	0.65	62	28	0.25	91	0.35	0.17	19

Table 2. Comparison of the Brilliant Blue adsorption capacities of the various adsorbent.

Adsorbent	**Dye**	**Adsorption capacity, mg g^{-1}**	**References**
P. eryngii/Amberlite XAD-4	Remazol brilliant blue R	1.92	Yonten et al., 2016
Rhizopus nigricans	*Reactive Blue*	83	Kumari and Abraham 2007
Agaricus bisporus + *Thuja orientalis (*mixed)	Reactive Blue 49	72.86	Akar et al., 2009
Aspergillus wentii	Brilliant Blue G	312	Khambhaty et al., 2012
Aspergillus niger	*Basic Blue 9*	0.37	Fu and Viraraghavan, 2002
Amberlyst - A21 /*Agaricus Campestris*	**Brilliant Blue G**	**3.64**	**This study**

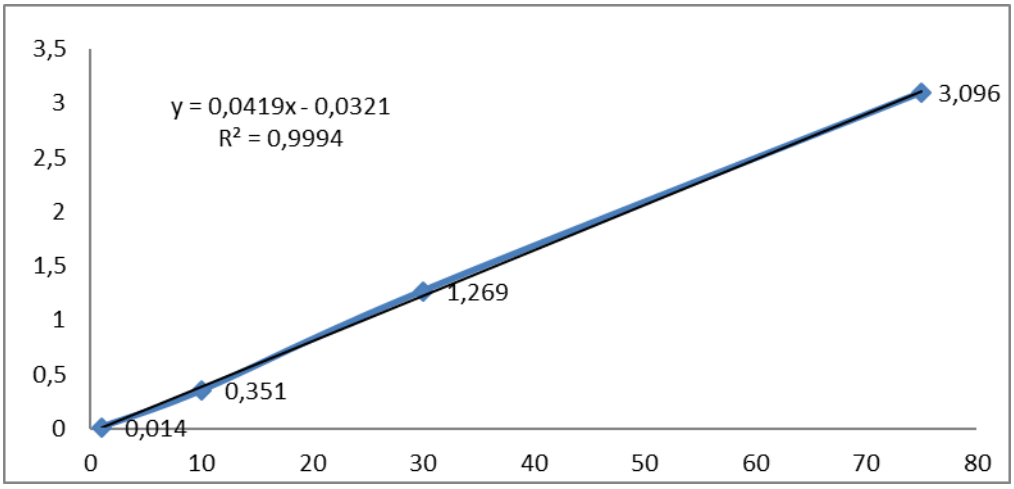

Figure 1. The calibration graphic of Brilliant Blue G.

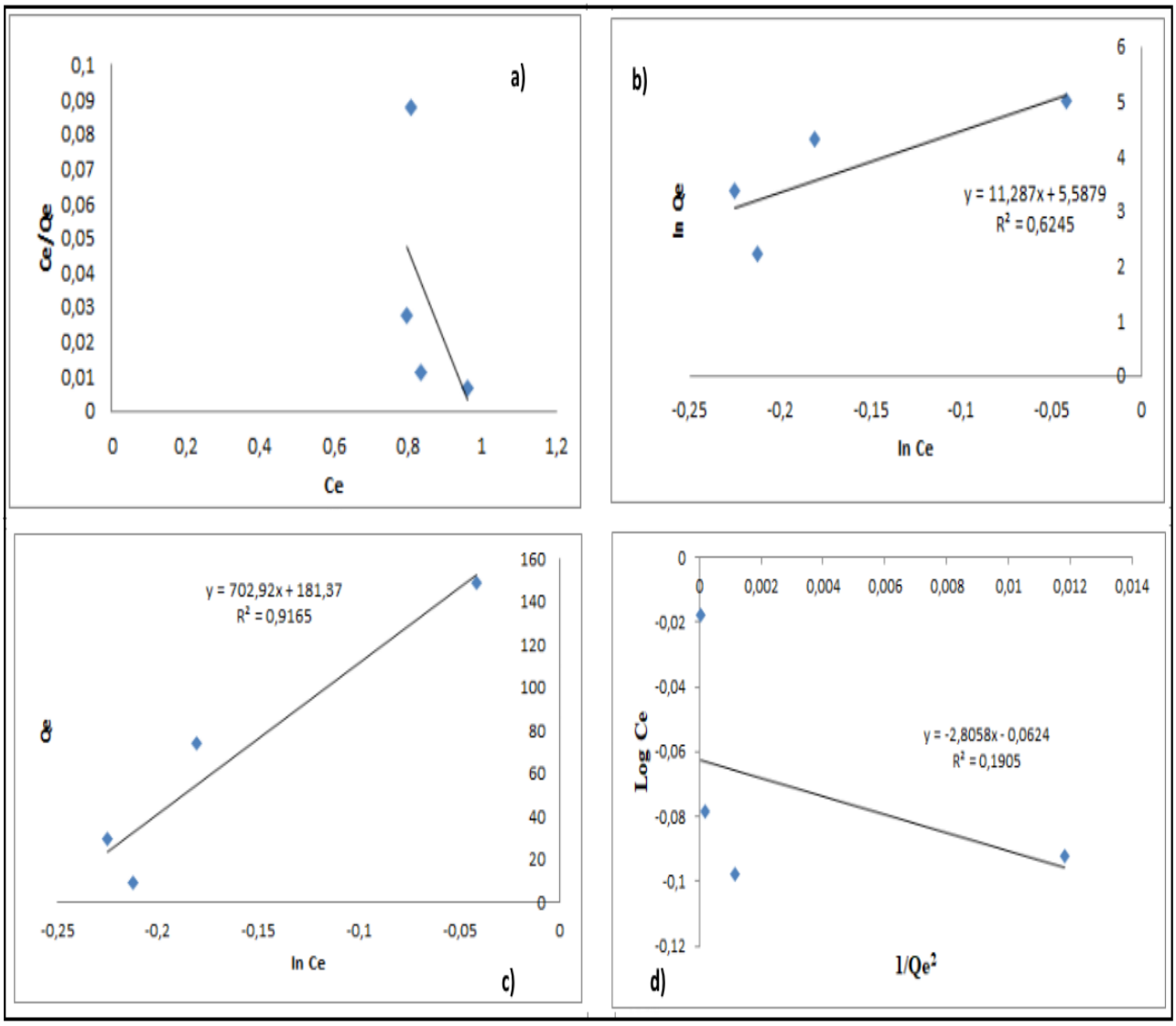

Figure 2. Some Adsorption Ishoterms a) Langmuir b) Freudlich c) Temkin d) Harkins- Jura of process.

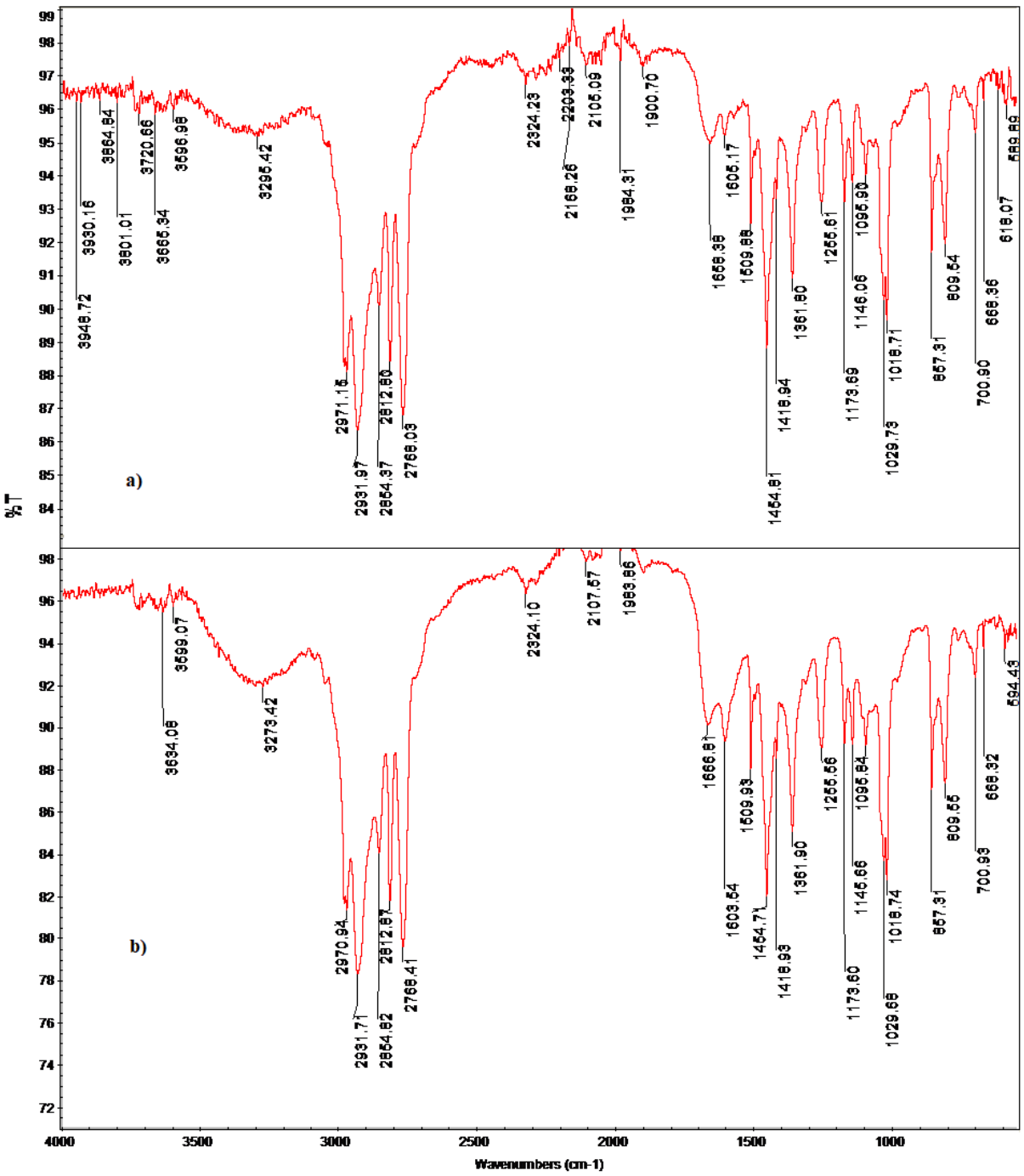

Figure 3. FTIR diagram of pre a) and post adsorption b)

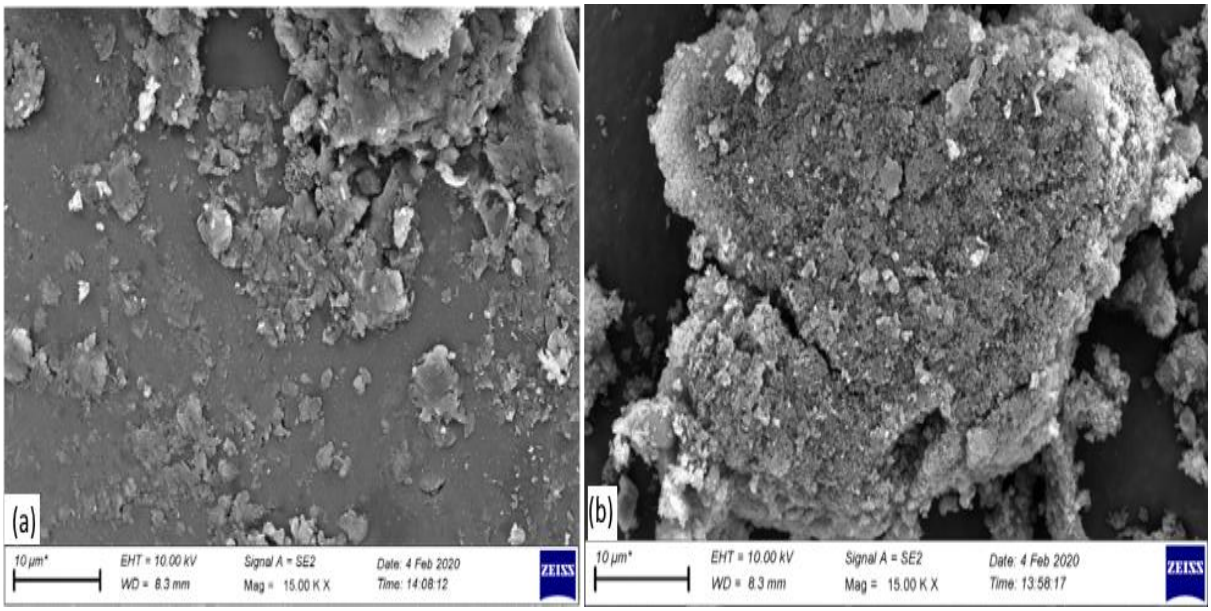

Figure 4. The SEM image of pre a) and post adsorption b)

Adsorption Ishoterms

Adsorbate–adsorbent interaction can be defined for the adsorption process by calculating some isotherms. The equations for some important adsorption isotherms used to describe the system are given by the following equations (1, 2, 3, 4) (Freundlich 1906; Langmuir 1918; Temkin and Pyzhev 1940; Harkins and Jura 1946).

$$log q_\epsilon = log k_F \times log\, C_\epsilon^{1/n} \qquad (1)$$

$$\frac{Ce}{qe} = \frac{1}{KLxqm} \times \frac{1}{qm\, Ce} \qquad (2)$$

$$q_\epsilon = (RT/b_T)\, lnA_T + (RT/b_T) lnCe \qquad (3)$$

$$\frac{1}{{q_e}^2} = \frac{B_{HJ}}{A_{HJ}} - \left(\frac{1}{A_{HJ}}\right) log C_e \qquad (4)$$

where qe (mmol/g) is the experimental dye amount adsorbed at equilibrium, Ce is the equilibrium concentration of dye. K_F (mmol/g) and n are Freundlich constants and the slope of 1/n ranging between 0 and 1 is a measure of adsorption intensity or surface heterogeneity, becoming more heterogeneous as its value gets closer to zero. A value for 1/n below one indicates a normal Langmuir isotherm, while 1/n above one is indicative of cooperative adsorption (Fytianos et al., 2000)
K_L (L g^{-1}) is Langmuir constant and qm (mgg^{-1}) is the maximum monolayer adsorption capacity. The dimensionless separation factor, R_L, is essential characteristic of the Langmuir isotherm,

which is defined as follows:

$$R_L = \frac{1}{(1+KL\, Co)} \qquad (5)$$

b is the Langmuir constant and C_0 is the highest initial dye concentration in the equation 5. The value of R_L indicates the type of the isotherm to be either favorable ($0 < R_L < 1$), unfavorable ($R_L > 1$), linear ($R_L = 1$) or

irreversible ($R_L = 0$) (Baskaralingam et al.,2006) Temkin Adsorption Isotherm was given in Equation 3. Here, when In Ce versus qe is plotted, RT/ b_T is obtained from the slope, and InA_T is obtained from the shift (RT / b_T). Here A_T expresses the isothermal bond coefficient as L g^{-1}. b_T is the Temkin adsorption coefficient. Harkins-Jurassic Isotherm was given in Equation 4. Here, when 1/ q_e^2 versus log Ce is plotted, -1 / A_{HJ} is obtained from the slope, and B_{HJ} / A_{HJ} is obtained from the slope. B_{HJ} and A_{HJ} are Harkins-Jurassic Isotherm coefficients. All Ishoterms graphics were given at Figure 2.

As indicated in Table 1, for Langmuir model R_L is ($R_L > 1$) so the process is unfavorable and the Harkins Jura models yields a somewhat better than other models on adsorption of dye on biocomposite as reflected with correlation coefficients (R^2).

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➢ *ORAL PRESENTATION*

Potential role of salivary irisin as an inflammatory biomarker in recurrent apthous stomatitis patients

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Abstract
Recurrent Aphthous Stomatitis (RAS) is the most common inflammatory condition of the oral mucosa characterised by recurrent onset of single or multiple painful ulcers mainly affecting the nonkeratinized oral mucosa. RAS mostly occurs in healthy individuals with no associated systemic diseases. Irisin is a newly identified adipomyokine and research has revealed that it has anti-inflammatory effects.
Objective: The aim of this study was to investigate the significance of salivary irisin levels in patients with recurrent apthous stomatitis (RAS).
Methods: In this study, 80 individuals were evaluated. The patient group included 30 patients diagnosed with RAS and each control groups consisted of 25 smoker and non-smoker healthy individuals. Saliva samples were collected and salivary irisin and interleukin-2 (IL-2) were determined using enzyme linked immunosorbent assay (ELISA).
Results: IL-2 levels in RAS patients were significantly higher than control smoker and non-smoker groups (p=0.000). Irisin level was higher in RAS patients than smoker controls and non-smoker controls. The level of irisin was found as sensitive and specific as IL-2, The salivary levels of pro-inflammatory cytokine IL-2 and irisin were higher in RAS group compared to controls.
Conclusion: It is the first report evaluating the irisin an adipo-miyokine as an inflammatory biomarker in RAS.
Keywords: Irisin, RAS, inflammation

Introduction

Myokines are mainly secreted in skeletal muscle and adipokines are in adipose tissue. The myokines described in the literature that are additionally known to be secreted by adipocytes are termed as adipo-myokines (Raschke ve Eckel, 2013).There are two types of adipose tissue in mammals: white and brown which is mainly white in humans. The white adipose tissue (WAT) stores excess energy in the form of triglycerides. Conversely, the brown adipose tissue (BAT) is specialized in energy expenditure and responsible for non-shivering thermogenesis called adaptive thermogenesis (Cannon ve Nedergaard, 2004). Adipokines are secreted both from adipocytes and other cells of the adipose tissue such as endothelial cells and macrophages (Fantuzzi, 2005). Obesity, especially visceral adiposity is characterized by a state of low grade systemic inflammation. Proinflammatory adipokines and molecules secreted from adipose tissue are implicated as the cause of increased cardiovascular disease risk, development of insulin resistance and so-called metabolic syndrome associated with obesity.

Since its first report in 2012 by Boström in Harvard University, irisin; a novel myokine, has been one of the most studied peptides in recent years. It is a 112 amino acid cleavage product of fibronectin type III domain-containing protein 5 (FDNC5), which is in turn stimulated by peroxisome proliferator-activated receptor-ɣ co-activator 1-α (PGC-1α). Originally it was described as a myokine secreted in response to physical excercise from skeletal muscle, but later it was found to be also secreted from adipose tissue (Roca-Rivada ve ark., 2013). Therefore, irisin has been included in the adipomyokine family. Although secreted primarily by muscle, especially cardiac muscle and adipose tissue, pancreas, sebaceous glands, liver, lung, testes, kidney, salivary glands, rectum, stomach, tongue, neuronal cells and sweat glands have shown strong immunoreactivity to irisin and its precursor

FNDC5 mRNA indicating irisin synthesis in these tissues (Aydın ve ark., 2013; Aydın, 2014).Serum irisin levels have previously been investigated in obesity, chronic kidney disease, type 2 diabetes mellitus, metabolic syndrome, inflammation and various types of cancer (Zhang ve ark., 2013; Wen ve ark., 2013; Stengel ve ark., 2013; Huh ve ark., 2012; Liu ve ark.,2013; Us Altay ve ark., 2016; Kuloğlu ve ark., 2016; Provatopoulou ve ark.,2015; Aydın ve ark., 2016; Gaggini ve ark.,2016; Shao ve ark.,2017)

Recurrent Aphthous Stomatitis (RAS) is the most common inflammatory condition of the oral mucosa characterised by recurrent onset of single or multiple painful ulcers mainly affecting the nonkeratinized oral mucosa (Chui ve ark., 2016). RAS mostly occurs in healthy individuals with no associated systemic diseases. The prevalence of RAS shows great variation between 2% and 50% in the general population; depending on the population studied, environmental factors and diagnostic criteria (Ship ve ark., 2000) . RAS is more prevalent in adult women, school children, non-smokers, and those with high socioeconomic status (Rivera-Hidalgo ve ark., 2004; Crivelli ve ark., 1998).Onset of the disease mostly occurs in childhood and usually the severity and frequency of episodes decreases with age (Chavan ve ark., 2012).Despite marked research and clinical attention, the etiology and pathogenesis of RAS remain unclear. Several factors such as genetic predisposition, viral and bacterial infections, psychological stress, food allergies, local trauma contribute to development of the aphtous ulcers. Deficiencies of hemoglobin, iron, vitamin B12, Vitamin D and folic acid and abnormally high blood homocysteine level were found to be associated with RAS (Sun ve ark., 2015; Compilato ve ark., 2010)

It is suggested that in genetically predisposed subjects, triggering factors initiates a disrupted immunologic respose and inflammation with subsequent apthous ulcer formation in RAS. Enhanced immunologic reaction occurs through inappropriately iniated cascade of cytokines. In many reports, cytokine profile in RAS patients have been studied. Increased production of Type 1 pro-inflammatory cytokines IL-2, IFN-γ and TNF-α as well as IL-5, IL-6 and IL-8 and decreased production of anti-inflammatory cytokines IL-10 and TGF-β were found in peripheral blood of RAS patients compared to healthy individuals (Lewkowicz ve ark., 2005). Elevated levels of IL-2, IFN-γ and TNF-α mRNAs and lower resting levels of IL-10 mRNA were detected in oral mucosa of RAS patients compared to healthy controls (Buño ve ark., 1998). There are epidemiologic studies indicating lower prevalence of RAS in smokers compared to non-smokers with an increase in the incidence of RAS following smoking cessation (Marakoğlu ve ark., 2007). Since the etiology of RAS cannot be determined precisely, there is no effective treatment and protection. The main goals in treatment are avoidance of local traumatic factors, suppressing local immune response, controlling pain, shortening ulcer time / accelerating healing and preventing relapses and secondary infection. Treatments are usually palliative, but none provide permanent remission (Scully ve Porter, 2008).

Saliva is one of the best known easy collect and non-invasive biological materials. It comprises 99.5 % water and the remaining 0.5 % contains antibacterial compounds such as secretory IgA and lysozyme, electrolytes, mucus, glycoproteins, enzymes and various peptide hormones. Saliva generally reflects blood peptid concentration Saliva plays a key role in the oral health so that it can preserve the integrity of the oral mucosal membrane through liquefaction and amelioration of the soft tissue (Aydin ve ark., 2014) Patient acceptance of saliva sample collection is much higher when compared to obtaining serum or biopsy samples (Bilskive ark.,2015)

Based on the objective of investigating inflammatory biomarkers in RAS we investigated the irisin, an adipomyokine that has not been previously studied in RAS. We compared irisin level with IL-2 level which have been previously investigated in RAS patients. In addition we also aimed to determine the irisin levels in both smoking and non-smoking controls to elucidate the relationship between smoking and salivary irisin which is not previously studied. As mention above, RAS is diagnosed from a history of recurrent ulcers together with an oral examination, Salivary molecules (such as EGF, IL-2, etc.,) play a pivotal role in maintaining the oral health and ameliorating the oral ulcers that irisin secrection from salivary glands and relation with inflammation, caused to wonder it can be a marker in RAS patient.This research aimed to gain new information to the literature by determining whether irisin can be used as an inflammatory biomarker of RAS.

Materials And Methods

The study protocol was approved by Ordu University (ODU) Clinical Research Ethics Committee (Decision No: 2018/16). This study involved 30 patients previously diagnosed with RAS, 25 healthy non-smokers and 25 healthy smokers. The patients were recruited from Otolaryngology Clinics of University Research Hospital. All subjects

provided written consent. Patients over the age of 18, having a diagnosis of RAS (at least 3 spontaneous aphtous ulcer occurrence per year) were included in the study. Exclusion criteria included presence of autoimmune disease, diagnosis of cancer and pregnancy. Exclusion criteria was also applied for age and sex-matched healhty control subjects. All of the RAS patients were free of active aphtous ulcer at the time of saliva collection.

Our study included the following steps: Collection of saliva samples, determination of Irisin, IL-2 levels in saliva samples by ELISA method, and statistical analysis. The methods and procedures used in these steps are detailed below.

Collection of saliva samples

Saliva has a circadian periodicity such that all saliva samples of patients and controls were taken at 10:00 am in the morning. At the same time of the day is important to collection

All subjects were seated on the examination chair and cotton swabs were given to the subjects (Salivette, Sarstedt AG &Co, Nümbrecht). Subjects placed the swabs in the mouth and chewed them for about 60 seconds to stimulate salivation. Then swabs that absorbed saliva were placed in the tubes and cover closed, centrifuged for 2 minutes at 1000 x g. The bottom part of the salivette that contained the clear saliva was removed and saliva transfered into 1.5 ml eppendorf tubes and stored in -80 degrees deep freezer until working time.

Determination of Irisin, IL-2, levels in saliva samples by ELISA method

Human irisin salivary levels were determined using an enzyme linked immunosorbent assay (ELISA) kit (USCN, Life Science Inc., Catalog No: 201-12-5328, Sunred biological technology, Shanghai, China) in line with the manufacturer's instructions. Absorbance of samples was measured at 450nm using a BioTek Instrument EL800 Microplate Reader. Results were expressed as ng/mL.

Human IL-2 salivary levels were determined using an enzyme linked immunosorbent assay (ELISA) kit (USCN, Life Science Inc., Catalog No: 201-12-0095, Sunred biological technology, Shanghai, China) in line with the manufacturer's instructions. Absorbance of samples was measured at 450nm using a BioTek Instrument EL800 Microplate Reader. Results were expressed as ng/L.

Statistical analysis

The test results were analyzed on SPSS (Statistical Package for the Social Sciences) 13.0.1 (license number: 9069727) statistical software. Data were shown as mean±standard deviation for normal distributed and median (interquartile range) for non-normal distributed variables. The distribution of irisin, IL-2 levels in each group were calculated by Kolmogorov-Smirnov test. Comparisons of the groups were done by ANOVA for normal distribution and by Kruskal Wallis test for non-normal distribution. The Mann Whitney U test was used to compare nonparametric two-way parameters for İrisin and IL-2. Spearman correlation analysis was used to assess the relationships among the parameters considering the skewness of data distribution. Receiver operating characteristic (ROC) curves were analyzed on Medcalc software version 11.5.1.0 (Medcalc software BVBA, Belgium). Statistical significance was accepted as $p<0.05$.

Results

Thirty RAS patients, 25 control smoker and 25 non-smoker subjects were enrolled in the study. RAS patients with a median age of 27.5 (range 20 to 40). The healthy control smoker and non-smoker group with median age of 21 (range 20 to 30) and 23 (19 to 37), respectively. There was no significant difference between the groups in terms of age (year). None of the RAS patients were smokers. Distribution of biochemical parameters, and age were shown in Table 1. Comparison of two groups revealed significantly elevated irisin and IL-2 levels in the patients with RAS ($p=0.0001$, $p=0.0001$, respectively). As expected, the levels of IL-2 was high in RAS patients. The level of irisin was studied for the first time in this study in RAS patients and found high as IL-2. Spearman correlation analysis results of irisin, IL-2 in RAS patient was shown in Figure-1. There is a positive and strong correlation between irisin and IL-2 ($p=0,0001$, $R=0,780$).

Table 1. Irisin, IL-2concentrations, age of RAS, smoker and non-smoker control groups.

Parameters	RAS group (n=30)	Control smoker group (n=25)	Control non-smoker group (n=25)	p value
Irisin(ng/mL)	19,3(15,4-32,9)[a]	12,1 (10,0-14,8)	15,6 (10,9-21,3)	0,0001
IL-2 (ng/L)	151,9 (114,1-166,8) [a,b]	48,9 (35,1-62,3)	38,3 (27,9-54,3)	0,0001
Age (year)	27,5(20,0-44,0)	21,0 (20,0-30,0)	23(19,0-37,0)	0,172

Data were expressed as: mean ± SD for parametric tests, median (inter quarter range for 25-75%) for nonparametric tests.
P; according to Kruskall Wallis for irisin and IL-2,age.
The Mann Whitney U test was used to compare nonparametric two-way parameters for irisin, age and IL-2
a, significantly different from the control smoker group.
b, values differ significantly from the control non-smoker group

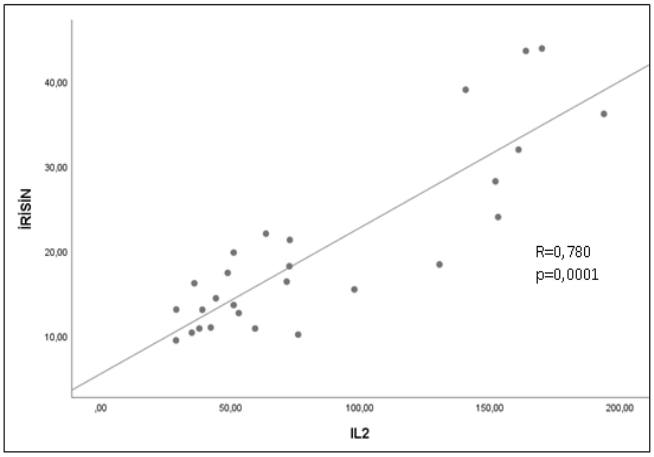

Figure 1. Irisin and IL-2 correlation in RAS patients

Discussion

. Irisin is known as adipo-myokine and until this time serum irisin levels have previously been investigated in obesity, chronic kidney disease, type 2 diabetes mellitus, metabolic syndrome, inflammation and various types of cancer (Zhang ve ark., 2013; Wen ve ark., 2013; Stengel ve ark., 2013; Huh ve ark., 2012; Liu ve ark.,2013; Us Altay ve ark., 2016; Kuloğlu ve ark., 2016; Provatopoulou ve ark.,2015; Aydın ve ark., 2016; Gaggini ve ark.,2016; Shao ve ark.,2017).Researchers reported that salivary and serum irisin levels were correlated and they found significantly higher irisin levels in saliva than the serum levels in both obese and normal weight subjects (Aydın ve ark .,2013). It was found that addition of recombinant irisin decreased the expression of inflammatory markers and stimulated the phenotypic switching of adipose tissue macrophages from M1 (pro-inflammatory) to M2 (anti-inflammatory) state in adipose tissue(Dong ve ark., 2016). RAS is the most common inflammatory condition of the oral mucosa characterised by recurrent onset of single or multiple painful ulcers with unknown etiology. Irisin has been associated with inflammation, but has never been studied in RAS patients before. In our study we demonstrated elevated irisin levels in RAS patients accompanied by elevated IL-2 levels and stronger correlation of irisin with IL-2. Besides, irisin was as much sensitive and specific as IL-2 in our RAS patients. In an experimental study conducted in colitis induced rats, It has been shown that exercise prior to induction of colitis diminished the severity of colonic damage, associated with marked increase in plasma irisin levels confirming that irisin could be involved in the mechanism linked to the mucosal healing of colitis (Subramanyam ve ark.,2011).We think that elevated irisin levels in RAS can be explained by anti-inflammatory role of irisin in compensatory process to overcome ongoing inflammation in RAS.

Conclusion

This study is the first research to show the relationship between inflammation and irisin in saliva samples of RAS patients. We demonstrated elevated level of irisin in RAS patients. When we analyze the results, irisin is as sensitive and specific as IL-2 for RAS. Irisin is decreased and pro-inflammatory cytokine IL-2 isɣ increased in salivary samples of smoking healthy subjects.

The major limitation of our study is the relatively small number of patients and controls involved. Irisin and other inflammatory parameters were studied only in saliva samples, and the results could not be evaluated in serum / plasma samples due to budget limitation. Also salivary samples were taken from the RAS patients during their ulcer free period and this can be a drawback. During active ulcer phase in which the inflammation is expected to be more pronounced, differences would be more significant. In our opinion, irisin is involved in inflammation and whether it has proanti-inflammatory role needs to be elucidated.

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Ethics committee approval
Ethics committee approval was received for this study from the Ordu University the Local Ethical Committee by under reference no: 2018-16.

Conflict of interest
All authors of original research article declare that they have no conflict of interest.

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➢ *ORAL PRESENTATION*

Farklı bitki ekstre ve krem karışımlarının güneşten koruma faktörlerinin in vitro olarak belirlenmesi

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Özet

Güneş ışığına maruz kalmak, güneş yanığından döküntülere ve cilt kanserlerine kadar çeşitli biyolojik reaksiyonlara neden olmaktadır. Birkaç sentetik güneş kremi mevcut olmasına rağmen, insanlarda potansiyel toksisiteleri ve sadece seçilmiş karsinojenez yollarına müdahale etme kabiliyetleri nedeniyle kozmetikte sınırlı uygulamaları bulunmaktadır. Kimyasalların bu zararlı etkilerinden dolayı bitkiler kozmetik endüstrisinde iyi bir alternatif olarak karşımıza çıkmaktadır. Türkiye'nin bazı yerlerinde yetişmekte olan, *Chamaerops humilis* (palmiye), *Brassica oleracea gongylodes* (alabaş) ve *Opuntia ficus indica (L.) miller* (dikenli incir) ekstreleri soksilet sistemi kullanılarak etanol, metanol ve su çözücüleriyle ekstrakte edilmiştir. Ekstre ve krem karışımlarının ticari olarak satılan bir el kreminin güneşten koruma faktörü üzerine etkileri test edilmiştir. Çalışmanın sonuçlarına göre, test edilen ekstreler arsında en yüksek güneşten koruma aktivitesi 10.988 değeri ile alabaş etanol ekstresinde 10 ml konsantrasyonda tespit edilmiştir. En düşük güneşten koruma etkisi ise 0.103 değeri 2.5 ml konsantrasyonu palmiye meyve etanol ekstresinde ölçülmüştür. Çalışma sonuçları, test edilen tüm ekstrelerin ticari bir el kreminin güneşten koruma etkisini arttırdığı göstermiştir.

Anahtar Kelimeler: SPF, *Chamaerops humilis* ,*Opuntia ficus indica (L.) miller, Brassica oleracea gongylodes,* soksilet

In vitro determination of sun protection factors of different plant extracts and cream mixtures

Abstract

Exposure to sunlight causes a variety of biological reactions, from sunburn to rashes and skin cancers. Although few synthetic sunscreens are available, they have limited applications in cosmetics due to their potential toxicity in humans and their ability to interfere with only selected carcinogenesis pathways. Because of these harmful effects of chemicals, plants emerge as a good alternative in the cosmetics industry. Being grown in Turkey in some places, *Chamaerops humilis* (palm), *Brassica oleracea gongylodes* (kohlrabi) and *Opuntia ficus indica* (L.) *miller* (barbed figs) ethanol using extracts soxilet system, it was extracted with methanol and aqua solvent. The effects of the extract and cream mixtures on the sun protection factor of a commercially available hand cream were tested. According to the results of the study, the highest sun protection activity among the tested extracts was detected in kohlrabi ethanol extract at 10 ml concentration with a value of 10.988. The lowest sun protection effect was measured in palm fruit ethanol extract with a concentration of 0.103 and 2.5 ml. The study results showed that all the extracts tested enhanced the sun protection effect of a commercial hand cream.

Keywords: SPF, *Chamaerops humilis*, *Opuntia ficus indica (L.) miller, Brassica oleracea gongylodes,* soxilet

GİRİŞ

Chamaerops humilis (palmiye) Palmae/Aracaceae ailesine ait, genelde tropik ve subtropik iklim bölgelerinde yaşamakta olup (Çatıkkaş, 2019), Türkiye'de Akdeniz ikliminin olduğu yerlerde yetiştirilmekte ve genellikle süs ağacı olarak park ve bahçelere dikilmektedir (Anonim, 2015). Opuntia cinsine ait birçok kaktüs yenilebilir ve

oldukça aromalı meyve üretir. Dikenli incir (*Opuntia ficus indica (L.) miller)* Opuntia cinsinin en verimli türüdür ve meyveleri oldukça lezzetli bir aromaya sahiptir (Barbera ve ark. 1992, Belviranlı 2016). Anavatanı batı yarıkürede Amerika kıtasıdır ve buradan Avrupa, Kuzey Afrika, Akdeniz ülkeleri, Orta Doğu gibi birçok ülkeye yayılmıştır (Yılmaz 2013).

Brassica oleracea var. brassicaceae ailesinin bir üyesi olan *Brassica oleracea gongylodes* (Alabaş), hemen hemen her yerde yetişen ve hem yiyecek hem de geleneksel ilaç olarak kullanılan bir lahana çeşididir. Yapay kimyasal bileşiklerin tüketiminden kaynaklanan istenmeyen sorunlar ve yan etkiler nedeniyle, başta yenilebilir ve tıbbi olanlar olmak üzere çeşitli bitki türlerinden elde edilen ekstraktlar, oldukça ilgi görmüştür. Ayrıca alabaş bitkisinin antimikrobiyal ve antioksidan özellik gösterdiği de çalışmalarla kanıtlanmıştır (Akagün 2009, Asan-Ozusaglam ve Karakoca, 2013).

Ultraviyole radyasyon (UVR) ultraviyole A (UV-A, 315-400 nm), ultraviyole B (UV-B, 280-315 nm) ve ultraviyole C (UV-C, 100-280 nm) olmak üzere üç ayrı bölgeye ayrılır (Polonini ve ark., 2011). Cilde toplam etkiyle ulaşan UVR, morfolojik ve kimyasal reaksiyonlarla ilişkili karmaşık bir sürece neden olur. DNA, UVR'yi absorbe eden önemli bir makromoleküldür. İlerleyen zamanlarda UVR mutasyona neden olabilmekte ve cilt kanserlerine yol açabilmektedir (Balogh ve ark., 2011). Güneşten koruma faktörü değeri hem in-vivo hem de in-vitro yöntemlerle saptanabilir. Daha basit ve ucuz olması sebebiyle genellikle in-vitro yöntemler tercih edilmektedir (Fageon ve ark, 2009). Güneş ışığına maruz kalmak, güneş yanığından döküntülere ve cilt kanserlerine kadar çeşitli biyolojik reaksiyonlara neden olabilir. Piyasadaki sentetik güneş kremleri çeşitli yan etkilere neden olmaktadır. Bu nedenle, bitkisel güneş koruyucu kremlerin formüle edilmesi ve güneş koruyucu aktivitesinin değerlendirilmesi kozmetik endüstrisinde büyük önem taşımaktadır (Patil ve ark, 2015). Güneş kremleri, güneşten gelen zararlı UV ışınlarına karşı vücudun doğal savunmasına yardımcı olmak için kullanılmaktadır (Mbanga ve ark, 2014a). SPF (Solar Protection Factor) değerlerinin ölçülmesi, güneş koruyucu formülasyonların etkinliğini belirlemenin nihai yoludur ve güneş koruyucu ürünlerin etkinliğini ölçmek için dünya çapında standart haline gelmiştir. Güneş ışınları tarafından yanmadan güneşte ne kadar süre kalınabileceği konusunda fikir verir. SPF değeri ne kadar yüksekse, güneş koruyucu krem UV ışığına karşı koruması o kadar fazladır (Mbanga ve ark, 2014b). Birkaç sentetik güneş kremi mevcut olmasına rağmen, insanlarda potansiyel toksisiteleri ve sadece seçilmiş karsinojenez yollarına müdahale etme kabiliyetleri nedeniyle kozmetikte sınırlı uygulamaları vardır (Malsawmtluangi ve ark, 2013). Günümüzde doğal bileşikler içeren ürünlerin faydaları, bu ürünlerin kullanıcılar tarafından kabulü, sistemik absorbsiyon potansiyeli ve UV ışığını absorbe edebilen doğal ürünlerin kullanımı büyük ilgi görmektedir. Bitkilerden ekstrakte edilen doğal maddeler, UV bölgesindeki UV emilimi ve antioksidan aktiviteleri nedeniyle son zamanlarda potansiyel bir güneş koruyucu kaynağı olarak kabul edilmektedir (Bambal ve ark, 2011). Bu çalışmada ise farklı çözücüler (etanol, metanol, su) kullanılarak hazırlanan palmiye, alabaş ve dikenli incir ekstrelerinin, ticari olarak satılan bir el kreminin güneşten koruma faktörü üzerine etkisi test edilmiştir.

MATERYAL VE METOD

Ekstrelerin Hazırlanması

Chamaerops humilis (palmiye) Mersin Alata'dan, *Opuntia ficus indica (L.) miller* (dikenli incir) Antalya'dan ve *Brassica oleracea gongylodes* (alabaş) ise Aksaray'da bulunan bir pazardan temin edilmiştir. Bitkiler yıkandıktan sonra kurutularak toz haline getirilmiştir. Daha sonra toz halindeki bitkiler ayrı ayrı soksilet sistemi kullanılarak farklı çözücüler (etanol, metanol, su) ile ekstrakte edilmiştir. Büyük partiküllerden ayırmak için Whatman kağıdı ile süzülen örnekler rotary evaporatör yardımıyla çözücüsünden uzaklaştırılmıştır (Şekil 1) Çözücüsü uçan ekstreler kullanılıncaya kadar kuru koşullarda 4°C'de saklanmıştır.

Şekil 1. Ekstrelerin hazırlanması

Ekstre ve Krem Karışımlarının Güneşten Koruma Faktörlerinin Belirlenmesi

Ekstre ve krem karışımlarının güneşten koruma faktörü üzerine etkilerinin belirlenmesinde Imam ve arkadaşlarının (2015) ve Bambai ve arkadaşları (2011)'nın metodları derlenip modifiye edilerek kullanılmıştır.

Her ekstre için ayrı ayrı 1 gr krem tartılmıştır. Bu kremin içerisine ayrı ayrı her ekstreden 0.5 gr tartılarak eklenmiştir. Karışım saf su ile 10 gr'a tamamlanmıştır. Hazırlanmış olan bu karışımdan 0.1 gr başka bir tüpe alınarak etanol (%40) ile 10 ml hacme tamamlanmıştır. Daha sonra 5 dakika sonike edilmiştir. Bu karışım No:1 Whatman filtre kağıdından süzüldükten sonra 0.5 ml başka bir tüpe alınmıştır ve etanol ile 5 ml hacme tamamlanmıştır. Daha 5 ml olan karışımdan da 0.5 ml alınarak 2.5 ml hacme tamamlanmıştır. 2.5 ml,5 ml ve 10 ml olarak ayarlanan karışımlar 3'er tekerrür olarak 290 nm-320 nm dalga boyu aralığında 5'er nm aralıklarda spektrofotometrede (Beckman Coulter) ölçülmüştür. Çıkan değerler Mansur denklemi (1) kullanılarak hesaplanmıştır.

Mansur denklemi;

$$\textbf{Güneşten koruma faktörü (SPF) spektrofotometrik} = \textbf{CF x } \sum_{290}^{320} \boldsymbol{EE}(\boldsymbol{\lambda}) \text{x } \boldsymbol{I}(\boldsymbol{\lambda}) \text{x } \boldsymbol{Abs}\ (\boldsymbol{\lambda}) \quad (1)$$

CF = Düzeltme faktörü (= 10); **EE(λ)** = Eritemetojenik etki radyasyon dalga boyu (λ);
I (λ) = Güneş ışığının dalga boyundaki yoğunluğu (λ);**abs (λ)** = Ekstrelerin dalga boyundaki absorbansı (λ).

Şekil 2. Ekstre ve krem karışımının hazırlanması

A. Ekstre ve krem karışımı

1. Alabaş metanol ekstresi ve krem karışımı
2. Alabaş etanol ekstresi ve krem karışımı
3. Dikenli incir su ekstresi ve krem karışımı

B. Etanol eklenen karışımın sonikasyonu

C. Farklı konsantrasyonlardaki ekstre ve krem karışımları

BULGULAR VE TARTIŞMA

Soksilet sistemi ve farklı çözücüler (etanol, metanol, su) kullanılarak elde edilen bitki ekstrelerinin güneşten koruma faktörleri test edilmiştir. Elde edilen değişik ekstrelerin güneş koruma faktörleri Mansur ve ark. (1986)'na göre hesaplanmış ve Tablo 3-7'de sunulmuştur. Kontrol grubuna kıyasla en yüksek güneşten koruma faktörünü 10.988 ile alabaş etanol ekstresi 10 ml konsantrasyonunda tespit edilmiştir. En düşük güneşten koruma faktörü ise 0.103 olarak palmiye meyve etanol ekstresi 2.5 ml konsantrasyonunda ölçülmüştür. Her bir dalga boyundaki radyasyon şiddeti ve eritemetojenik etki arasındaki ilişki Tablo 2'de verilmiştir. Elde ettiğimiz SPF değerlerini Tablo 1'de (Imam ve ark., 2015) verilen değerlerle karşılaştırdığımızda palmiye meyve metanol ekstresi en yüksek yaklaşık %50, palmiye meyve etanol ekstresi en yüksek yaklaşık %48, alabaş metanol ekstresi en yüksek yaklaşık %89, alabaş etanol ekstresi en yüksek yaklaşık %91 ve dikenli incir yaşlı yaprak su ekstresi en yüksek yaklaşık %81 oranında UV'yi engelleyebildiği belirlenmiştir.

Tablo 1. SPF değerine göre engellenen UV yüzdesi

SPF	Engellenen UV Yüzdesi
2	50
4	75
5	80
10	90
15	93
25	96

Tablo 2. Her bir dalga boyundaki radyasyon şiddeti ve eritemetojenik etki arasındaki ilişki

λ (nm)	**EE (λ)x I(λ)**
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Toplam	**1.0000**

Tablo 3. Palmiye meyve metanol ekstresi ve krem karışımının güneşten koruma faktörü

λ (nm)	**EE (λ)x I(λ)**	**Palmiye Meyve Metanol Ekstresi**					
		Güneşten Koruma Faktörü					
		Ekstre±Krem			**Krem**		
		2.5 ml	**5 ml**	**10 ml**	**2.5 ml**	**5 ml**	**10 ml**
290	0.0150	0.009	0.013	0.068	0.002	0.007	0.021
295	0.0817	0.028	0.038	0.230	0.019	0.043	0.115
300	0.2874	0.114	0.150	0.729	0.047	0.137	0.376
305	0.3278	0.127	0.161	0.776	0.052	0.152	0.417
310	0.1864	0.067	0.087	0.418	0.027	0.084	0.236
315	0.0839	0.028	0.037	0.178	0.010	0.034	0.098
320	0.0180	0.005	0.006	0.035	0.001	0.006	0.019
SPF±standart sapma		**0.378±0.02**	**0.492±0.01**	**2.434±0.01**	**0.158±0.01**	**0.463±0.01**	**1.282±0.01**

Tablo 4. Palmiye meyve etanol ekstresi ve krem karışımının güneşten koruma faktörü

λ (nm)	**EE (λ)x I(λ)**	**Palmiye Meyve Etanol Ekstresi**					
		Güneşten Koruma Faktörü					
		Ekstre±Krem			**Krem**		
		2.5 ml	**5 ml**	**10 ml**	**2.5 ml**	**5 ml**	**10 ml**
290	0.0150	0.001	0.004	0.052	0.002	0.007	0.021
295	0.0817	0.002	0.008	0.163	0.019	0.043	0.115
300	0.2874	0.028	0.060	0.545	0.047	0.137	0.376
305	0.3278	0.037	0.073	0.583	0.052	0.152	0.417
310	0.1864	0.023	0.042	0.307	0.027	0.084	0.236
315	0.0839	0.011	0.018	0.127	0.010	0.034	0.098
320	0.0180	0.001	0.002	0.024	0.001	0.006	0.019
SPF±standart sapma		**0.103± 0**	**0.207± 0.01**	**1.801± 0.00**	**0.158± 0.01**	**0.463± 0.01**	**1.282± 0.01**

Tablo 5. Alabaş metanol ekstresi ve krem karışımının güneşten koruma faktörü

λ (nm)	EE (λ)x I(λ)	Alabaş Metanol Ekstresi Güneşten Koruma Faktörü					
		Ekstre±Krem			Krem		
		2.5 ml	5 ml	10 ml	2.5 ml	5 ml	10 ml
290	0.0150	0.002	0.016	0.169	0.002	0.007	0.021
295	0.0817	0.010	0.081	0.886	0.019	0.043	0.115
300	0.2874	0.058	0.301	2.978	0.047	0.137	0.376
305	0.3278	0.068	0.322	3.146	0.052	0.152	0.417
310	0.1864	0.036	0.167	1.636	0.027	0.084	0.236
315	0.0839	0.014	0.069	0.677	0.010	0.034	0.098
320	0.0180	0.003	0.013	0.136	0.001	0.006	0.019
SPF±standart sapma		**0.191± 0.00**	**0.956± 0.01**	**9.628± 0.02**	**0.158± 0.01**	**0.463± 0.01**	**1.282± 0.01**

Tablo 6. Alabaş etanol ekstresi ve krem karışımının güneşten koruma faktörü

λ (nm)	EE (λ)x I(λ)	Alabaş Etanol Ekstresi Güneşten Koruma Faktörü					
		Ekstre±Krem			Krem		
		2.5 ml	5 ml	10 ml	2.5 ml	5 ml	10 ml
290	0.0150	0.004	0.015	0.209	0.002	0.007	0.021
295	0.0817	0.026	0.075	1.036	0.019	0.043	0.115
300	0.2874	0.127	0.274	3.430	0.047	0.137	0.376
305	0.3278	0.145	0.287	3.568	0.052	0.152	0.417
310	0.1864	0.083	0.148	1.839	0.027	0.084	0.236
315	0.0839	0.036	0.059	0.754	0.010	0.034	0.098
320	0.0180	0.007	0.012	0.152	0.001	0.006	0.019
SPF±standart sapma		**0.428± 0.00**	**0.870± 0.00**	**10.988± 0.02**	**0.158± 0.01**	**0.463± 0.01**	**1.282± 0.01**

Tablo 7. Dikenli incir yaşlı yaprak su ekstresi ve krem karışımının güneşten koruma faktörü

λ (nm)	EE (λ)x I(λ)	Dikenli İncir Yaşlı Yaprak Su Ekstresi Güneşten Koruma Faktörü					
		Ekstre±Krem			Krem		
		2.5 ml	5 ml	10 ml	2.5 ml	5 ml	10 ml
290	0.0150	0.001	0.011	0.120	0.002	0.007	0.021
295	0.0817	0.012	0.056	0.600	0.019	0.043	0.115
300	0.2874	0.049	0.200	2.020	0.047	0.137	0.376
305	0.3278	0.056	0.219	2.206	0.052	0.152	0.417
310	0.1864	0.030	0.118	1.199	0.027	0.084	0.236
315	0.0839	0.013	0.050	0.517	0.010	0.034	0.098
320	0.0180	0.002	0.010	0.107	0.001	0.006	0.019
SPF±standart sapma		**0.163± 0.01**	**0.664± 0.00**	**6.769± 0.01**	**0.158± 0.01**	**0.463± 0.01**	**1.282± 0.01**

Bambal ve ark.(2011) yaptığı çalışmada *Nyctanthes arbortristis* ve *Tagetes erecta* bitki ekstrelerini ayrı ayrı ekledikleri kremin güneşten koruma faktörünü *N. arbortristis* için 10.21±2.18 ve *T. erecta* için 8.67±1.35 olarak ölçülmüştür. Başka bir çalışmada ise *Musa accumınata (L.), Psıdıum gujava (L.)* ve *Pyrus communıs (L.)* bitki ekstre karışımıyla hazırlanmış krem formülasyonunun güneşten koruma faktörü araştırılmıştır. Yapılan araştırma sonucunda ticari olarak alınan kremin güneşten koruma faktörü 12.25 olarak ölçülürken, ekstreler ile hazırlanmış krem formülasyonunun güneşten koruma faktörü 3.90 olarak ölçülmüştür (Imam ve ark., 2015).

SONUÇ

Yaptığımız çalışmada, soksilet sistemi kullanarak farklı çözücülerle ekstrakte ettiğimiz bitkilerin (palmiye, alabaş, dikenli incir yaşlı yaprak) ticari el kremin güneşten koruma faktörü (SPF) değerini arttırdığı gözlemlenmiştir. Sonuçlar, kozmetik endüstrisinde palmiye, alabaş ve dikenli incir yaşlı yaprak bitki ekstrelerinin uygun konsantrasyonlarda kremlere karıştırılarak kremlerin güneşten koruma etkinliğinin arttırılması sağlanabileceğini ve bitkisel kökenli olası nedeniyle güneşten korunmak amacıyla daha güvenli bir alternatif olabileceğini göstermiştir.

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➢ ***ORAL PRESENTATION***

Determination of lipid peroxidation level in sausages and doners produced from poultry meat

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Abstract

Lipid oxidation is one of the major factors limiting the quality of meat and meat products. The susceptibility of the poultry meat to oxidative deterioration increases due to the fact that poultry meat contains too much polyunsaturated fatty acids. For this reason, oxidative deterioration of products obtained from poultry meat is the most important factor determining the quality. In this study, the oxidative deterioration levels of sausage and 20 chicken doner produced from 40 poultry meat served in Bilecik market were determined using Thiobarbituric Acid (TBA) test. It was determined that the TBA level of the sausage samples changed between 0.070-3.221 µgMA/g and the mean TBA number was 0.756±0.684 µgMA/g, while the TBA level of the chicken doner samples varied between 0.086-2.480 µgMA/g and the mean TBA number was found as 0.624±0.566 µgMA/g. According to the obtained results, it was determined that 27.50% of the sausage samples and 10.00% of the chicken samples were above the lipid oxidation level determined by the Turkish Standards Institute.

Keywords: Chicken Doner, Sausage, Lipid Oxidation

1. INTRODUCTION

Lipid oxidation is the most common form of chemical degradation in meat and meat products (Kanner, 1994). This lipid oxidation begins immediately after the animal is slaughtered and continues to increase until it is brought to the customer (Gray et al., 1996). Particularly poultry meat and products are highly susceptible to oxidation due to polyunsaturated fatty acids in their structure (Cengiz, 2018; Gatellier et al., 2007). Hydroperoxides, which form in the chain phase of lipid oxidation and are not stable compounds, cause oxidation of pigments and vitamins and therefore they form polydispersed dark organic polymers. In particular, they take various metabolic forms such as aldehydes, ketones, alkanes, alkenes, alcohols, carboxylic acids and polymerisation products by undergoing structural degradation particularly in degradation process of chain (Comporti, 1993; Gray, 1994; Meriç and Demir, 2011; Morissey et al., 1998; Pie et al., 1991; Wu and Brewer, 1994;). Aldehydes which are among these metabolic products occurred as the result of autocatalytic reaction of free radicals are considered as the main cause of bad odor and loss of taste and cause decrease in shelf life and toxic compounds are formed in advanced oxidation formation (Mercier et al., 1998; Nawar, 1996; Renerre et al., 1999; Sklan et al., 1983). Moreover, free radicals cause decrease in nutritive value of the foods by reacting with vitamins (Gordoni, 2001). Products such as malondialdehyde, which are the result of this reaction, are the substances that determine the severity of oxidation and show mutagenic and carcinogenic effects. Malondialdehyde is a product which forms by the degradation of particularly three or more double-linked fatty acids. This substance forms a lysine-malondialdehyde compound by reacting with the ε-amino groups of the lysine residues occurring in the nutrients during protein breakdown. Meanwhile, malondialdehyde forms compounds with amino acids such as serine, guanine, etonolamineas well as lysine(Comporti, 1993; Philips, 1988; Şahin, 1991; Wills, 1987). These products have shown to cause cardiovascular diseases by inhibiting enzymes, increasing levels of cholesterol and peroxide in the blood (Ames, 1983; Frankel, 1991; Yıldız et al., 2018). For this reason, many physical (polarography, infared spectroscopy, refractometry, fluorescence and conjugated dienes) and chemical (peroxide values, kreis testing, detection of total and volatile carbonyl compounds and thiobarbituric acid test) analytical methods have been developed to determine the lipid oxidation (Fernandez, 1997). Thiobarbituric acid reaction is often used in many studies since the determination of malondialdehyde level is the most preferred way of determining oxidation (Demirkaya, 2014;

Ceylan et al., 2007; Gomes et al., 2003; Oruç et al., 2005; Şahin, 1991). Thyobarbituric acid (TBA) test is widely used to determine lipid oxidation levels in red and poultry meat as it is fast, convenient and sensitive (Gomes et al., 2003). The reaction between thiobarbituric acid and malonaldehyde produces yellow, orange and red chromogenic colors. The TBA test is based on the photometric measurement of this reaction (GuilleSans and Guzman Chozas, 1998). This study was carried out to determine lipid oxidation levels of sausages and chicken doner produced from poultry meat consumed in Bilecik market by TBA test.

2. MATERIALS AND METHODS

In the study, sausage and 20 chicken doner samples produced from 40 poultry meats and sold in markets in Bilecik city were supplied according to random sampling method without intervention to the routine sales procedure and packaging material and brought to the laboratory under cold chain and kept under refrigerator conditions (4°C) until the analyses were completed. 10 g of sausage and chicken doner sample were subjected to maceration for 2 minutes with 50 ml of distilled water, and then transferred to a distillation flask by being washed with 47.5 ml of water 2.5 ml of 4 M HCI was added to the distillation setup and was distilled in a way to collect 50 ml of distillate within 10 minutes. 5 ml of distillation was transferred to a vial and 5 ml of TBA solution (in 90 % glacial acetic acid) was added and kept in the boiling water bath for 35 minutes. At the end of this period, the tubes were cooled and the absorbance values were read against the standard solution in the spectrophotometer at a wavelength of 538 nm and the TBA number was calculated as µgMA/g (Tarlagdis et al., 1960).

3. RESULTS AND DISCUSSION

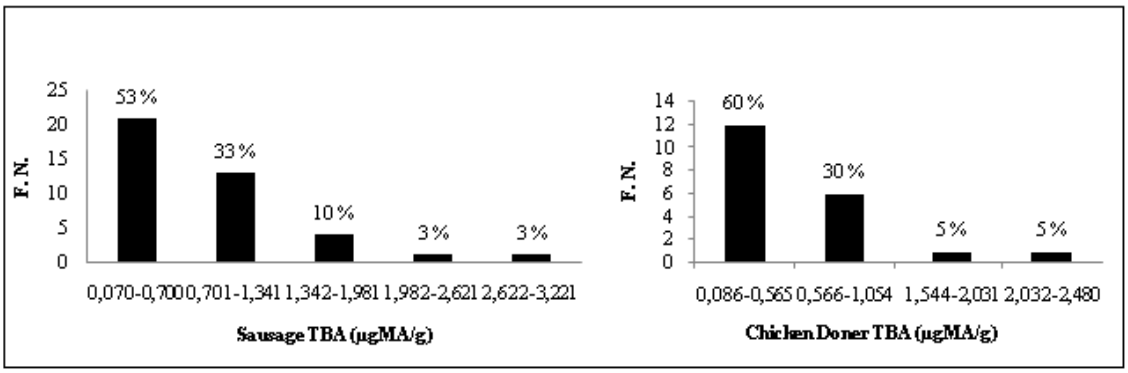
In the study, the mean values (µgMA/g) of TBA numbers of the examined sausage and chicken doner samples are shown in Table 1 and Table 2, and the percentage distribution and frequency numbers are given in Figure 1. The number of TBA in sausage samples was found to range from 0.070 to 2.221 µgMA/g and the mean number of TBA was found as 0.756±0.684 µgMA/g. The number of TBA in chicken doner samples ranged from 0.086-2.480 µgMA/g and the mean number of TBA was found as 0.624±0.566 µgMA/g. The number of TBAs found in the study was determined to be at different rates throughout the samples. The maximum number of TBAs that can be found in meat and meat products was stated as 1 µgMA/g according to Turkish Standards Institute, chicken body meat (carcass) standard, chicken meatless-boneless meat-minced meat standard and meat and meat products minced meat standard (Anonymous, 1995; Anonymous, 1997a; Anonymous, 1997b). According to this; 11 sausage samples and 2 chicken doner samples were found to be above the maximum allowed limit. 29 sausage samples and 18 chicken doner samples were found to be at an acceptable level (<1 µgMA/g).

Table 1. The values of TBA (µgMA/g) numbers found in sausage samples.

	N	Minimum	Maximum	Mean
TBA Number (µgMA/g)	40	0.070	3.221	0.756±0.684

Table 2. The values of TBA (µgMA / g) numbers found in chicken doner samples.

	N	Minimum	Maximum	Mean
TBA Number (µgMA/g)	20	0.086	2.480	0.624±0.566



*F.N.: Frequency Number

Figure1. Percentage distribution and frequency of TBA numbers found in sausage and chicken doner samples.

Lipid oxidation products can easily form in heat-treated chickens, storage conditions, and highly unsaturated fatty acids (Gomes et al., 2003; Rhee et al., 1996; Beltran et al., 2003). Moreover, mechanically separated poultry meat are often used in the formulation of emulsion type meat products such as salami and sausage due to their dense consistency and low cost. However, the main problem with these products is the rapid onset of oxidative degradation (Lee et al., 1975; MacNail et al., 1973). Maxon and Marion (1970) reported in their study conducted on mechanically shredded poultry meat that there was an increase in the amount of TBA during storage at 4°C. It has been noted that the TBA values of chickens mechanically separated into very small particles increase very rapidly in the following processes and this increase is related to the fact that hem pigments forming as a result of separation into very small particles affect lipid oxidation. The obtained values confirm this situation and TBA values of the sausage and chicken doner are found to be higher than the values given by other researchers (Ergönül and Kundakçı, 2006; Ertaş, 1982; Ertaş and Kolsarıcı, 1983; Kayardı et al., 2005). 11 of the sausage samples were found to have ransid taste and mutagenic risk. In the chicken doner samples, only two of them were found to have ransid taste and mutagenic risk. Chen et al. (1996) reported that fluctuations may occur in TBA values and that this is due to the unstable structure of malonaldehyde found in the structure of hydrogen peroxides. Csallany et al. (1984) reported that the formation and accumulation of malonaldehyde in sausages are related to polyunsaturated fatty acids, malondialdehyde without lipid origin, the type of peroxidation catalyst, peroxidation conditions and other biological reactions of malonaldehyde. However, it has been indicated that low TBA values found in the samples below the permissible limit do not indicate the risk of oxidation, as malonaldehyde reacts primarily with proteins and other compounds (Meltoni 1983; Severini et al., 2003).

As a result; the risk of oxidation can be avoided by taking into account the unsuitable containment and hygienic conditions at the beginning of the factors leading to lipid oxidation for prolongation of the shelf life of the product, always standard product production and for public health. It is thought that it is possible to use a good technology in production and it is possible with conscious practices.

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➢ *ORAL PRESENTATION*

Review on microbial fuel cell technologies: Recent developments in electrode materials

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Abstract

Microbial fuel cells have attracted great attention as an alternative energy conversion system used to generate energy in recent years. Microbial fuel cells, which are a bioelectrochemical system provide various benefits such as energy saving and energy conversion, especially wastewater treatment and electricity generation. However, the low power output and expensive electrode materials inhibit the of MFCs wide-spread and large-scale application. One of the most important issues which can potentially be explored in order to optimise power output from MFCs are the electrode metarials. Therefore, electrodes are major components of a MFC. Electrode metarials must be economical and exhibit useful electrochemical properties. Furthermore, electrodes have to have a large surface area, being mechanically stable and provide high current densities. In this study, it is aimed to give information about the general features and recent developments of electrode materials which have a very important issue in studies on MFC.

Keywords: Energy, wastewater treatment, microbial fuel cell, electrodes

1. INTRODUCTION

Global energy demand is increasing every year. Petroleum products meet the vast majority of the increasing global energy demand. However, the ever-increasing energy demand and environmental problems caused by petroleum products create the need for alternative renewable energy technologies research (Davis and Higson, 2007). Microbial fuel cells (MFCs) are an important potential pathway that needs to be explored as a partial solution to reducing fossil resource use (Slate et al., 2019).

MFC technology is a bioelectrochemical system that uses microorganisms to catalyze oxidative reactions of organic or inorganic substances and generate electricity. This system is considered as a promising technology to solve both environmental problems and energy needs (Zheng et al., 2021). Overall, MFC consists of anaerobic anode chamber and aerobic cathode chamber that may or may not be separated by proton selective membrane. Anode chamber generally contains organics which served as substrate for the microorganisms. Bacteria produce electrons while consuming organic compounds. Afterwards these electrons reach the anode electrode directly or indirectly (with the help of mediator) and then they are transferred to the cathode electrode by the external circuit. This flow of electron passing through an external circuit under an external load create bioelectricity (Kumar et al., 2019). Schematic diagram of a typical two-chamber microbial fuel cell as shown in Fig. 1.

The oxidation process takes place at the anode chamber of the MFC. Then, electrons arise from this reaction are transmitted externally to a cathode electrode, where the electrons are used in a reduction reaction. As a result of the oxidation / reduction reaction generates an electric current (Marks et al., 2019). These reactions are shown below in equations 1 and 2 (Li et al., 2018).

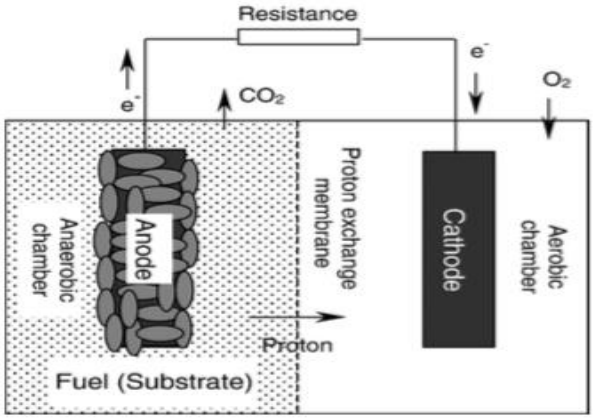

Fig. 1. Schematic diagram of a typical two-chamber microbial fuel cell (Du et al., 2007).

$$\text{Anode: } CH_3COO + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^- \quad (1)$$
$$\text{Cathode: } O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (2)$$

In the anode compartment, the necessary ambiance for the growth of bacteria is provided and electrons produced by the decomposition of organic materials are transmitted. The structure of anode chamber is an important parameter that affects the performance of MFCs. Therefore, the properties that anode electrode should have are listed as good electrical conductivity, biocompatibility, high surface area, non corrosive and low cost (Zeng et al., 2018). The cathode is another key component of an MFC that determines its performance and cost. MFCs could be adapted to a variety of cathodic reactions using different electron acceptors (Chen et al., 2019). Both the low power output and the high cost of anode and cathode electrode materials are hindering the progress of large-scale applications of MFCs (Hu et al., 2019).

In this paper, recent developments in electrode materials and configurations used in the anode and cathode chambers of the MFC are reviewed.

2. ELECTRODE MATERIALS USED IN MFC

The main factors affecting power generation in MFCs systems include reactor configuration (Du et al., 2007), substrates (Pandey et al., 2016), ion exchange membrane (Tsompanas et al., 2019), internal/external resistance of cell (Kim et al., 2020) and electrode materials (Prakash et al., 2018).

Among these factors, it can be said that low cost and high-performance electrode production is the key parameter to improving power output (Cai et al., 2020). Electrode surface modification applications to improve electron transfer and bacterial adhesion have an important part in research on MFC (Wei et al., 2011). Various materials used in an MFC are summarized in the following sections.

2.1. Carbon-based Materials

Carbon based materials such as graphite plates (Rahimnejad et al., 2011), graphite brushes (Logan et al., 2007), graphite foam (Chaudhuri and Lovley, 2003), carbon cloth (Blanchet et al., 2016), carbon paper (Santoro et al., 2014), carbon felt (Mateo et al., 2019), carbon brush (Ma et al., 2016), carbon mesh (Wang et al., 2009) and reticulated vitreous carbon (Chen et al., 2012) are widely used as electrodes in MFCs. These materials have specific surface area, high electrical conductivity, chemical stability, biocompatibility and low cost (Santoro et al., 2017). Different electrode materials used as anodes are shown in Fig. 2.

Carbon cloth is a carbon-based material used very often as electrode material in MFCs. Despite its high cost, the carbon cover provides properties such as flexibility, mechanical strength, high electrical conductivity, high surface area and high surface porosity in 3D composite electrodes (Santoro et al., 2017). Carbon fiber brushes are used as electrode in MFC due to their wide surface area and good scalability (Brunschweiger et al., 2020). Carbon mesh

is a highly suitable electrode material for MFC. Compared to other fabric electrodes, it is both cost-effective and less reducing biofouling that can occur due to its wider network structure (Wang et al., 2009). Granular activated carbon (GAC) may be the most cost-effective electrode material due to its low cost. GAC provides a large surface area for bacterial adhesion. Furthermore, because of GAC increases affinity for anodic substrates, it can be said that its kinetic performance is better than other electrodes (Zhao et al., 2016). Graphite plate (or sheet) is a very simple electrode which low cost and high electrical conductivity. Graphite sheet/plate of a low surface/volume ratio leads to a low power output than other porous or structured materials. This electrode material generally use as support for modified structures due to its high mechanical strength (Santoro et al., 2017). Graphite fiber brush provides a simpler architecture for scaling up the reactor to larger size. Because of this feature, it has been used as an electrode in many studies on MFC (Gadkari et al., 2019). Reticulated vitreous carbon (RVC) provides easy access to the surface of microorganisms and mediators. When an open network structure with high electrolyte permeability is present, electron transfer can take place and a very high efficiency can be obtained (Lepage et al., 2012).

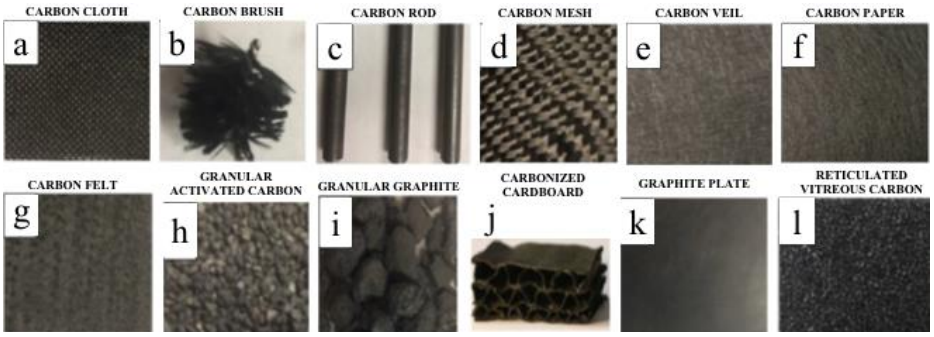

Fig. 2. Digital photographs of carbon based electrode materials (Santoro et al., 2017).

2.2. Metal-based Materials

Metals-based materials such as stainless steel, nickel, aluminium, gold, silver, copper, molybdenum, iron and titanium were used an electrode material (Yaqoob et al., 2020). Different metal-based electrode materials used as electrodes are shown in Fig. 3.

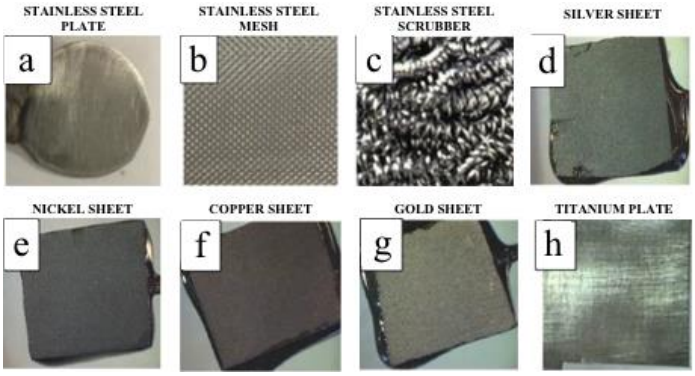

Fig. 3. Digital photographs of metal-based electrodes (Santoro et al., 2017).

Metal materials are more conductive than carbon materials. It is desirable that electrode materials used in MFC are not corrosive. For this reason, metal materials are not preferred widely in MFC applications (Wei et al., 2011). Electrodes such as titanium, platinum and stainless steel are both highly conductive and have good mechanical strength but the smooth surface of these electrodes weakens the adhesion of microorganisms and resulting in insufficient MFC performance (Guo et al., 2015), (Yu et al., 2020). Various studies have been carried out to use copper, gold, silver, titanium and nickel as electrodes. As a result of the researches, it is seen that nickel and copper ions cause toxic effects for microorganisms and negatively affect biofilm formation. Despite this situation, it is seen that these electrodes have high performance properties. As a result of the researches, it is seen that nickel and

copper ions cause toxic effects for microorganisms and negatively affect biofilm formation. Despite to this fact, it was seen that these electrodes have high performance properties (Santoro et al., 2017).

2.3. Composite Materials

Carbon-based and metal-based composite electrodes are designed using conductive polymers to improve the output power of MFC (Niessen et al., 2004). Different types of conductive polymers such as polyaniline (Li et al., 2019) and polytetrafluoroethylene (Rajesh et al., 2018) were used in MFC. 3D carbon-based electrodes are produced by carbonization method (pyrolysis) using synthetic polymer, lignocellulose-containing plant materials and natural cellulose (Kalathil et al., 2017).

Modification or application of nanostructured materials into or on carbon-based electrode, such as 3D carbon black, porous carbon, carbon nanotube, polyaniline and graphene have been identified as useful (Palanisamy et al., 2019).

3. EXTRACELLULAR ELECTRON TRANSFER MECHANİSMS

3.1. Extracellular Electron Transfer Mechanisms Between Microorganisms and Electrodes

Electron production mechanism is discussed in this section in order to reveal the properties that will be needed in the development of electrodes and to assist studies on this subject. Electricity is produced in MFC due to the interaction between microorganism activity (reduction reaction generating electrons and protons) and existing electron acceptors (separated by a membrane) (Rahimnejad et al., 2015). The most important feature of bioelectrochemical systems is the use of electroactive microorganisms that provide electron transfer between cell and electrode by providing extracellular electron transfer through oxidation and reduction reactions (Sun et al., 2017). Electroactive microorganisms are the species that can carry out electron transfer (Slate et al., 2019). These microorganisms provide electron transfer in two ways: direct or indirect electron transfer (Huang et al., 2011). This transfer mechanisms used exoelectrogenic bacterial are shown in Fig. 4.

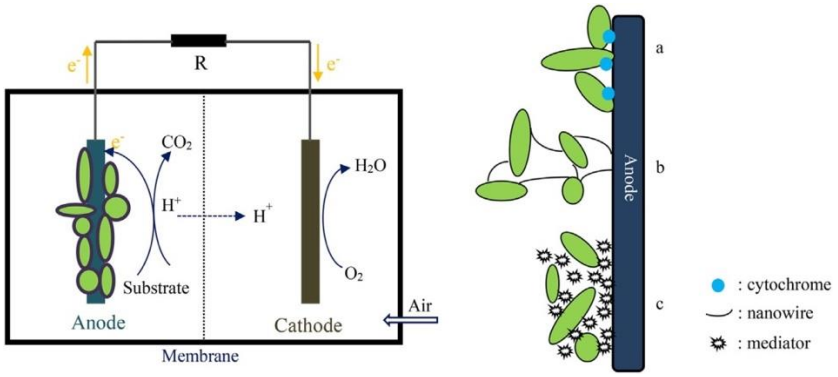

Fig. 4. Figure of MFC and the extracellular electron transfer (a) outer-membrane bound cytochromes, (b) conductive nanowires (pili) and (c) redox mediators (Li et al., 2018).

Direct electron transfer is an electron transfer in which there is a physical connection between the microorganism cell and the electrode. In this type of electron transfer mechanism, nanowires or redox active proteins play an active role in transferring electrons. Indirect electron transfer is an electron transfer in which there is no physical connection. This method is based on electron shuttling molecules (Slate et al., 2019). In indirect electron transfer, electrons inside the cell are transported to the cell surface by redox active proteins and low molecular weight compounds. Then, electrons are passed either cytochromes or outer membrane or potentially shuttles in the periplasm (Velasquez-Orta et al., 2010).

Many types of microorganisms are used for electricity generation in MFC. The use of electroactive bacteria for biomass degradation and electricity generation is of great importance. Approximately 35 pure cultures, such as

Rhodoferax, Shewanella, Geobacter, Pseudomonas sp., Lactococcus lactis and Cupriavidus basilensis, are expressed as electroactive microorganisms in MFC (Kumar et al., 2019).

4. CONCLUSIONS

MFCs are very important systems due to both environmentally friendly energy generation and wastewater treatment features. Continuous energy can be obtained by using cost-effective and highly efficient MFCs. Electrode design is one of the most important factors affecting the cost of the system in MFC systems. Various carbon-based and metal-based materials are being researched for anode and cathode electrode design and various electrode modifications are being developed to improve power output.

The current literature of using carbon-based, metal-based and composite electrode materials on MFC is listed in this review. In addition, the extracellular electron transfer mechanism is also mentioned. The prospects for further research and improve needs on the MFC technologies are expressed.

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➢ *ORAL PRESENTATION*

Role of CSRP2 signal on invasion of breast cancer cells using a developed 3-dimensional breast equivalent model (3D-BEM)

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Abstract

Breast cancer represents the leading cause of death for Iraqi women after cardiovascular disease. Initiation and metastasis of breast cancer still obscure and need intensive investigations. Studies on animal models are valuable, however, they lack the biological matching of human cells. On the other hand, behavior of the cells in the two-dimensional cell culture (2DCC) is quite different from their behavior when they are grown in three-dimensional cell culture (3DCC). Therefore, there is an urgent need to develop a 3D breast model to investigate breast cancer and antitumor drug screening. This study aimed to develop for the first time in Iraq a 3D breast equivalent model (3D-BEM). The model consists of two cellular layers; the lower layer is a sheet of human fibroblasts represent the fibrous tissue of the breast covered by the other cellular layer of MCF-7 breast cancer cells. The methodology included investigations the effect of CSRP2 on the ability of MCF-7 to invade and penetrate the fibrous layer. Results of western blotting and immunofluorescence showed that blocking of CSRP2 enhanced the invasion of MCF-7. Conclusion**:** It could be concluded that the developed 3D-BEM represents a good tool for investigating the invasion of cancer cells and CSRP2 is a promising target for cancer therapy.

Key words: 3D-breast model, breast cancer, CSRP2 signaling, MCF-7 invasion, metastasis

INTRODUCTION

Breast cancer represents the most widely spread cancer. In 2018, there were 270,000 new cases reported in the United States of America, with 2.1 million new cases around the world (Bahcecioglu et al., 2020). In the Eastern Mediterranean Region, breast cancer ranks the first out of five most recorded cancers in women including breast, cervix, ovary, colorectum, and non-Hodgkin lymphoma. In Iraq, breast cancer is the main cancer type in the leading cause of death for Iraqi women after cardiovascular disease (Alwan, 2016). Metastasis is the main threatening step of cancer when tumor cells spread from the original cancerous tissue towards other intact tissues and colonize intact organs (Steeg, 2016). Metastasis of breast cancer still obscure and need intensive investigations (Bahcecioglu et al., 2020). One of the promising approaches as cancer therapy is targeting metastasis cascades to stop or inhibit the ability of cancers cells to migrate and spread from the primary source tissue to other intact organs and tissues in the body (Rankin et al., 2016). It has been reported that members of cystine-glycine rich protein (CSRP) family encode a group of short LIM domain proteins (21 kDa) which are critical regulators of development, proliferation, migration and differentiation (Wang et al., 2017). It has been suggested that cystine-glycine rich protein 2 (CSRP2) upregulates matrix metalloproteinase 13 (MMP13) which plays a crucial role in tumor cell invasion (Hoffmann et al., 2018). Most biological studies on cancer metastasis have either been conducted in standard two-dimensional cell culture (2DCC) or on animal models. Whereas, accumulating evidence has suggested that the behavior of the cells in the 2DCC is quite different from their behavior when they are grown in three-dimensional cell culture (3DCC). Additionally, studies on animal models are of great importance, however, their biological behavior is not mimicking that of human cells (Li *et al.*, 2011). Therefore, *in vitro* cell culture has recently developed from 2DCC to 3DCC, characterized by cultured cells with an environment which significantly mimics the *in vivo* niche by growing the cells as a floating mass (Wozniak and Keely, 2005). To overcome the limitations such as high cost of clinical development and ineffective toxicity during *in vivo* trials, there is an urgent need to establish a new screening step before the *in vivo* screening phase in animal models. The 3D *in vitro* screening represents the most realistic step and mimics the cellular behaviour of cells living in the *in*

vivo niche thereby, providing more predictable data and more accurate results (Breslin and O'Driscoll, 2013; Edmondson *et al.*, 2014).

MATERIALS AND METHODS

Cell Culture

All cell culture was performed in class II cabinets to reduce the risk of infection and contamination. Then, all the cells and 3D-BEM models were incubated at standard culture conditions (SCC) which were provided by humidified incubator adjusted to 37oC, ≈20% O2 and 5% CO2. Unless otherwise stated, all culture conditions set out in this study for cell culture and cell experiments were SCC. The MCF-7 cell line was kindly provided by the Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq. These cell lines were grown at SCC in DMEM-F10, which stands for Dulbecco's Modified Eagle Medium (Sigma / UK) and composed of 500 ml DMEM supplemented with 10% fetal calf serum (FCS) (Sigma / UK), 4% of 200 mM L-Glutamine and 1% of 100 IU/ml penicillin, 100 µg/ml streptomycin (PS) (Sigma / UK). To split cells, old media was aspirated, and cells were rinsed with phosphate buffered saline (PBS), trypsinised with trypsin 1X and incubated for 10 minutes. A quantity of 10 ml of DMEM-F10 was added to prepare the cell suspension and centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded, and cells were counted and a cell number of 5×105 to 1×106 was seeded into a T75 flask. A quantity of 15-20 ml of fresh DMEM were added to the flask and incubated under SCC. Trypan blue stain was used to assess viability of all cells including MCF-7 cell line and primary fibroblasts. A volume of 10 µl trypan blue was mixed with 10 µl of cell suspension. A quantity of 10 µl of this mixture was transferred to a Neubauer chamber and covered with a cover slip prior to counting under the microscope (Sandell and Sakai, 2008). (Equation 1) and (Equation 2) were used to calculate cell count and cell viability.

$$Total\ \boldsymbol{Cell\ Count = No\ of\ cells\ in\ 25\ small\ square \times Dilution\ Fcator \times 10^4} \qquad Equation\ 1$$

$$Cell\ \boldsymbol{Viability} = \frac{\boldsymbol{No\ of\ viable\ cells\ in\ 25\ small\ square}}{\boldsymbol{Total\ cell\ count\ (Live + dead\ cells)}} \times \mathbf{100}\ \% \qquad Equation\ 2$$

Isolation of Human Dermal Fibroblast

Human dermal fibroblast used in this study were obtained with agreement and consent of each donor. Approval for the study was given by the Council of College of Biotechnology / Al-Nahrain University in 2020. A skin biopsy of one square cm (1 cm^2) was obtained from patients suffering from breast cancer and undergone breast removal. As described by (Todd and Reynolds, 1998), a dermal sections were cleared of blood using PBS, chopped into pieces of size 3-5 mm using a scalpel and placed over a tissue wet with 70% ethanol for 2 minutes. Each section was transferred into a well of a 6 well plate, covered with FCS incubated at SCC for overnight. On the next day, a volume of 5 ml of DMEM-F10 was added to each well. The dermal sections were kept in the wells with a regular change of media until fibroblast cells attached on the surface of the culture plate followed by splitting at passage one.

CSRP2 Knockdown: small interfering RNA (siRNA) Transfection

As reported by Hoffmann and colleagues, knockdown of CSRP2 gene could be induced by transfecting 10 nM of both (5′-ACAGTGGCAATTCACGATGAA-3′ and 5′-ACAGGCCTACAACAAATCCAA-3′) directed against human CSRP2 (Hoffmann et al., 2018). Same sequences were predesigned and provided by (ThermoFisher Scientific) and used to block CSRP2 in MCF-7 using DharmaFECT® transfection reagents following the procedure described by the manufacturing company.

Western Blot Assay

Protein extraction was prepared by lysing MCF-7 cell pellet in lysis buffer composed of 30 mM NaCl, 0.5% Triton XP-100, 50 mM Tris with protease inhibitor (PI) (ThermoFisher Scientific). Total protein extracts were incubated

with primary rabbit polyclonal antibody CSRP2 (1:1000) and GAPDH (ThermoFisher Scientific) overnight at 4°C followed by incubation with goat anti-rabbit IgG secondary antibody (1:10000). Primary and secondary antibodies were diluted in antibody solution (5.0 g Skim milk, 1 ml tween, 10 ml TBS and 89 ml distilled water). Bands were detected using developer system and protein levels were quantified using Image J software.

Assessment of Cell Migration in 2-Dimensional Cell Culture (2DCC)

The scratch assay was applied to assess cell migration by measuring the area of the scratch during different periods utilizing the protocol described by (Walter et al., 2010) with minor modifications. Briefly, MCF-7 cells were seeded in 48 well plates at a density of 0.05×10^6 cells per well and incubated at standard culture conditions (SCC in humidified incubator, 37°C and 5 % CO_2). Transfected and non-transfected MCF-7 were seeded at a density of 0.05×10^6 cells per well in 48 well plates and incubated at for 24 hours to allow cell adhesion. On the second day, each confluent monolayer was scratched with a sterile yellow tip making a scratch of ≈ 0.4–0.5 mm in width along the diameter of each well. The medium was removed and replaced with fresh DMEM-F10. The plate was re-incubated at SCC to monitor wound closure by imaging at time zero, followed by imaging every 8 hours. All conditions were achieved in triplicates. The area of each single scratch was measured at the given time points using image J software.

Construction of 3-Dimensional Breast Equivalent Model (3D-BEM)

This study developed a 3D-BEM for the first time in Iraq. The basis of this model is the cytobuilder scaffold that we previously patented. Before seeding cells, cytobuilder was activated with 70 % ethanol for one minute, treated with DMEM-F10 for one minute, fixed in 6 well plates and incubated under SCC until required. To form the fibroblast layer of the model, 2×10^6 fibroblasts were suspended in 100 µl DMEM-F10 and seeded onto the cytobuilder and incubated at SCC for 90 minutes. 9 ml of DMEM-F10 was added to the fibroblast layer and incubated under SCC with a change of DMEM-F10 every two days for 18 days. The breast cancer cellular layer was prepared by seeding 100 µl of DMEM-F10 containing 2×10^6 MCF-7 over the fibroblast layer and incubated under SCC for 3 hours to allow cell adherence. Concurrently, the fibroblast layer was fed from the bottom with 4 ml DMEM-F10. After the incubation time, the whole model was covered with DMEM-F10 for 14 days while feeding the whole model with DMEM-F10. Tissue sections were stained with hematoxylin- eosin (HE) to evaluate the formation of 3D-tissue like structure.

Immunofluorescence Staining of the 3D Breast Equivalent Model (3D-BEM)

Paraffin embedded breast model tissues were always kept on ice during sectioning to ensure effective sectioning. The microtome was adjusted to 4 µm and sections were kept in a water bath adjusted at 40°C for 2-3 minutes before transferring to slides. The slides were then heated in an oven at 60°C overnight and the next day, dehydrated by washing for 5 minutes in each xylene, 100% ethanol, 95% ethanol, 70% ethanol and distilled water. For antigen retrieval, slides were placed in previously heated 10 mM citrate buffer, pH 6.0, in a microwave for 1 minute and then left to cool at room temperature for 30 minutes, and washed in 5 mM Tris buffered saline (TBS) pH 7.6 for 5 minutes. Permeabilisation of the slides was performed in 0.2% Triton-X for 10 minutes at room temperature. All sections were blocked by 10% goat serum (Abcam) in PBS for 10 minutes at room temperature and washed with Tris base solution (TBS) four times to remove the goat serum. The sections were stained with 100 µ of Anti-MUC1 antibody (abcam) at 1:200, and incubated in a moist chamber at 4°C overnight. Control slides were not stained but were incubated with blocking solution instead. The following day the sections were washed four times in TBS for 2 minutes each and incubated with100 µl of Alexa-Fluor 488 conjugated goat anti-rabbit secondary antibody (1:250) mixed with DAPI at a dilution of 1:1000 for 2 hours at 37°C in a moist chamber in the dark. Sections were then washed four times in TBS for 2 minutes, covered with DPX, left in the dark at room temperature overnight, and analysed the next day using fluorescent microscope (Leica).

STATISTICAL ANALYSIS

Data of the research was analysed by using means ± standard error of the mean (SEM) and a Two-way RM ANOVA test to determine significant differences between samples when *P* values were less than 0.05. Multiple comparisons were required to find differences between pairs of means with appropriate adjustment for multiple testing in every single condition during different time points in each separate experiment. Tukey's multiple

comparisons test was used to detect variations, which were considered as significant when the P value was less than 0.05. all types of analyses achieved by Graph Pad prism software.

RESULTS

CSRP2 knockdown in MCF-7 cancer cells

In this study, the effect of CSRP-2 knockdown was assessed on the invasion behavior of MCF-7 breast cancer cells in both 2D cell culture and 3D breast cancer model. As agreed with data reported by Hoffman et al., (2018), CSRP2 was absent or only weakly expressed in T-MCF-7 cells, whereas it was expressed at significant levels in N-MCF-7 cells after 24 hours of transfecting cells. Statistical analysis showed 10 times higher expression of CSRP2 in N-MCF-7 compared to T-MCF-7 (P=0.0016) as shown in (Figure 1 A and B).

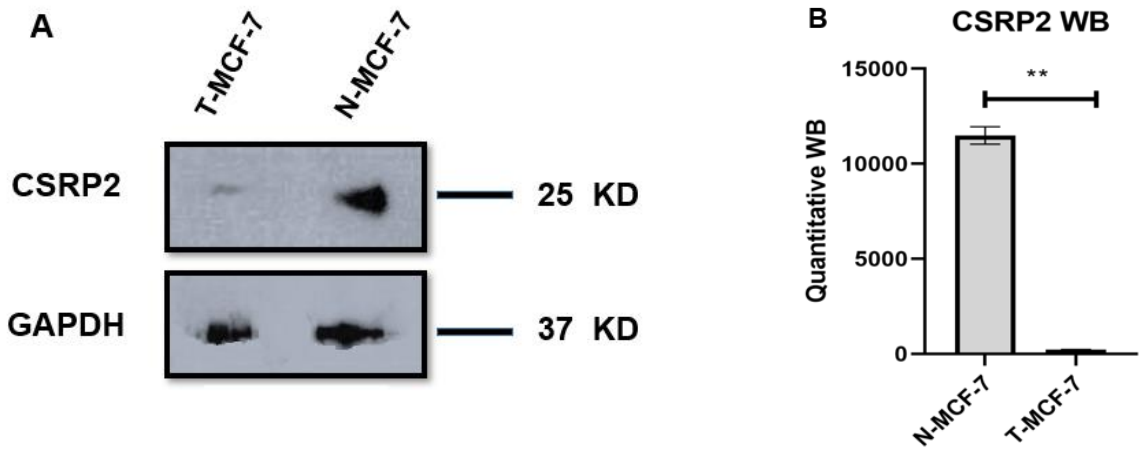

Figure 1. Western blot analysis of CSRP2 protein.
(A) Western blot analysis of CSRP2 protein level in both transfected (T-MCF-7) and non-transfected (N-MCF-7) human breast cancer cells cultured for 24 hours after transfection which shows very weak expression of CSRP2 in T-MCF-7 compared to high stronger expression in N-MCF-7. (B) Protein levels in both groups (T-MCF-7 and N-MCF-7) were quantified after normalization to GAPDH level using Image J software. Tested groups= 3 technical groups, Error bars = SEM, (**= p=0.0016).

CSRP2 Knockdown Enhances Migration of MCF-7 Cancer Cells in 2D Cell Culture (2DCC)

Results of scratch assay in 2D cell culture showed that transfected MCF-7 (T-MCF-7; cells of blocked CSRP2) had faster migration than non-transfected MCF-7 cells (N-MCF-7) leading to close of the scratch area with increased effect within time. After 26 hours of treatment the gap was closed when compared to both controls. Two-way ANOVA showed that blocking of CSRP2 accelerated the migration of transfected MCF-7 cells and enhanced migration within time to completely close the scratch area after 26 hours of starting the test. on the other hand, non-transfected MCF-7 cells failed to close the whole scratch area leaving a gap after the same treatment period (48 hours) (P<0.001) as shown in (Figure 2 A, B and C).

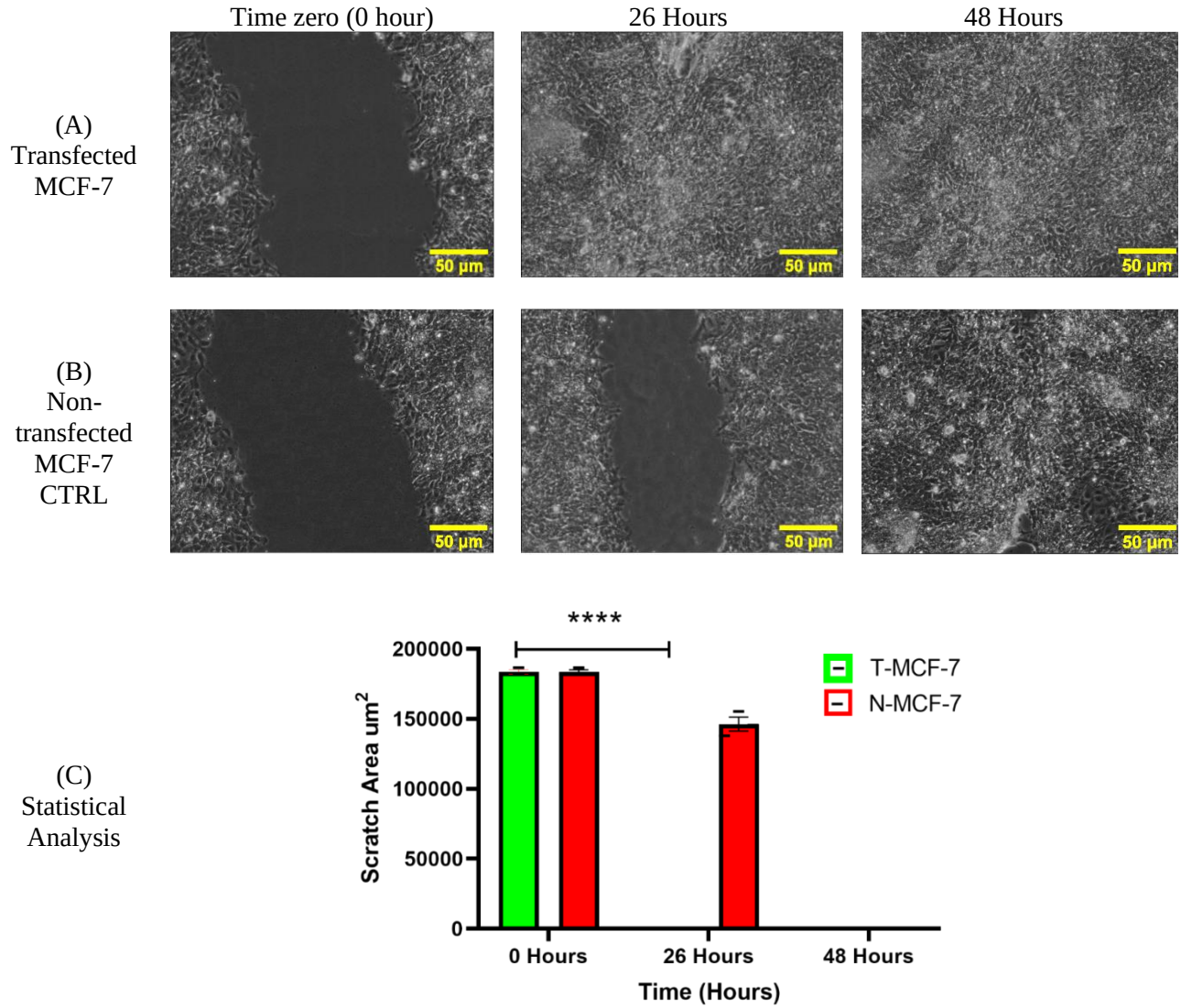

Figure 2. Migration of transfected and non-transfected MCF-7 cells during scratch closure assay.
A monolayer of transfected and non-transfected MCF-7 cells were scratched and incubated at SCC for 48 hours. (A) Representative micrographs show T-MCF-7 has faster migration and closed the scratch area within 26 hours. (B) Representative micrographs show N-MCF-7 has slower migration and closed the scratch area within 48 hours. MCF-7 cells used at passage 6, Scale bar=50 µm. (C) Two-way ANOVA revealed that the transfected M-CF-7 (Green columns significantly reduced the scratch areas within time compared to the control (N-MCF-7) (Red column). Significant variations between the two groups increased within time and reached the highest significance at 26 hours P<0.0001). Columns = mean of scratch areas (N=3 technical replicates), Error bars= standard error of the mean (SEM). (****=P<0.0001).

Evaluation of 3D-Breast Equivalent Model (3D-BEM) and Assessment of Cell Invasion

All models generated in this study (n=3) were prepared as an organotypic culture composed of fibrous and cancerous layers. The fibrous layer was built from fibroblasts which was then encased with the cancerous layer by seeding MCF-7 cells to form a bi-layered breast equivalent model. As shown in (Figure 3A), two distinct layers could be seen represented by upper cancerous cellular layer (MCF-7) covering a lower fibrous layer (fibroblast). After 1 week of constructing the models, the cancerous layer of T-MCF-7 at the top of models invaded and penetrated the fibrous layer and completely spread through the fibrous layer as shown in (Figure 3B). In contrast,

N-MCF-7 had less invasion and only penetrated the top part of the fibrous layer after one week of constructing the model as shown in (Figure 3C).

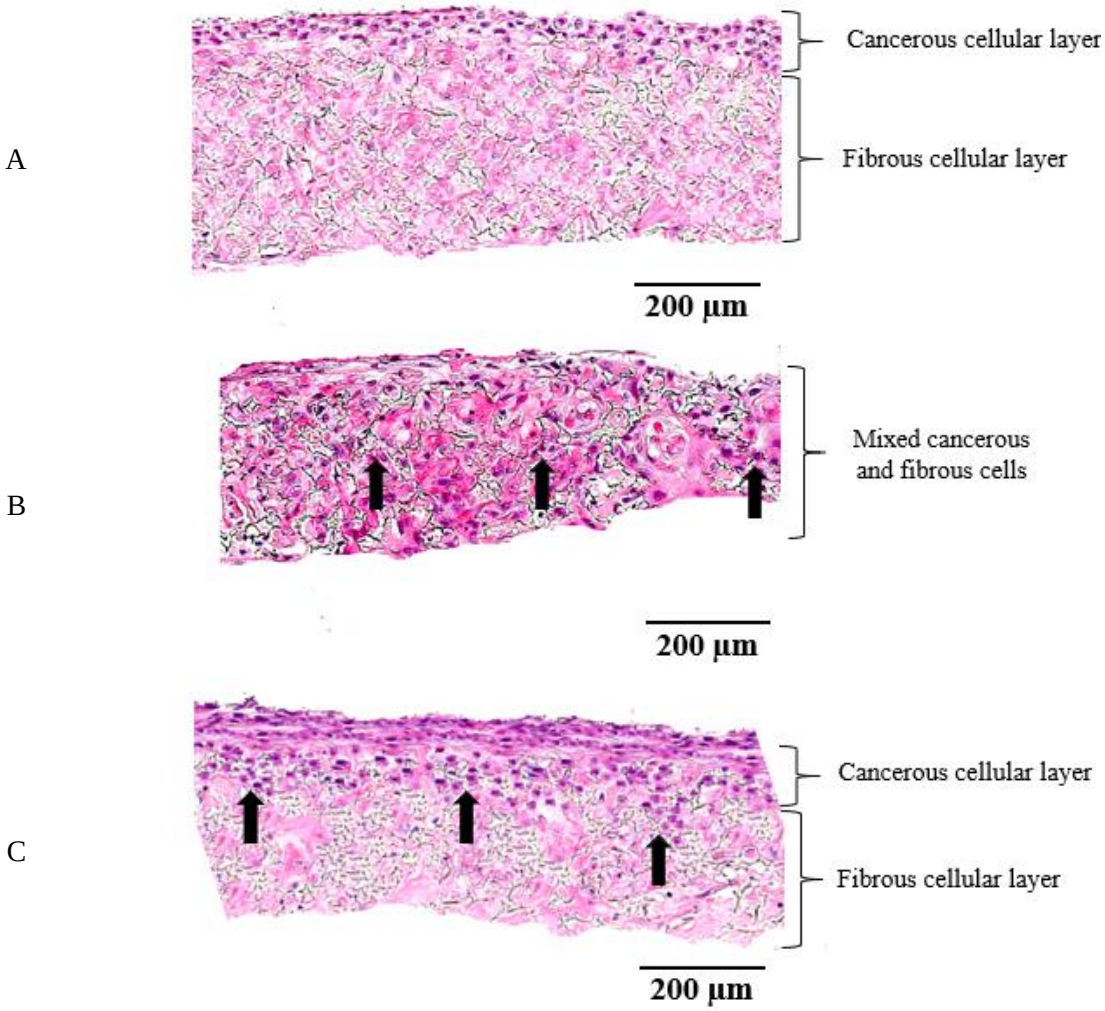

Figure 3. Invasion of Breast Cancer Cell in 3D Breast Model.
(A) 3D Breast cancer model at day one after seeding the MCF-7 cells over the fibrous layer shows formation of two distinct layers of cancerous cellular layer represented by MCF-7 breast cancer cell line at the top of the fibrous layer represented by fibroblast. (B) Micrograph shows invasion of T-MCF-7 in 3D Breast model after 1 week of seeding the cells and reveals that cancer cells spread and deeply penetrate the fibrous layer forming a cancerous tissue (Full spread). (C) Micrograph shows invasion of N-MCF-7 in 3D Breast model after 1 week of seeding the cells and reveals that cancer cells spread and only penetrate the top part of the fibrous layer compared to the T-MCF-7 (Partial spread).

Beyond hematoxylin-eosin evaluation of the invasion and distribution of MCF-7, expression of key biomarker of MCF-7 (mucin antibody) was also evaluated. As demonstrated in (Figure 4A), T-MCF-7 cells stained with anti-mucin antibody invaded the fibrous layer and deeply distributed after 7 days of seeding the cells on the fibroblast layer. On the other hand, N-MCF-7 cells stained with anti-mucin antibody in (Figure 4B) had less invasion and only distributed at the top part of the fibrous layer beyond 7 days of seeding the cells on the fibroblast layer.

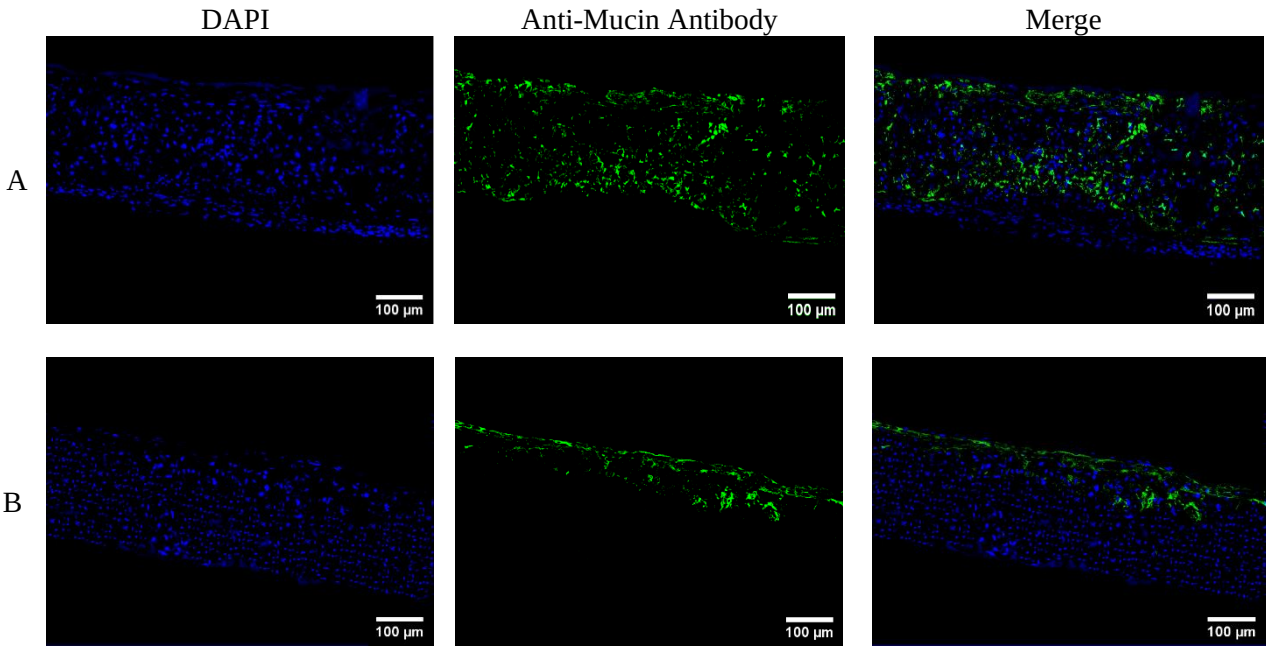

Figure 4. Immunofluorescence Micrographs of Invasion of MCF-7 Cells in 3D Breast Cancer Model. (A) Micrographs show invasion of T-MCF-7 in 3D breast model after 1 week of seeding the cells and reveals that cancer cells stained with anti-mucin antibody (green) spread and deeply penetrate the fibrous layer (Blue DAPI stain). (B) Micrographs show invasion of N-MCF-7 in 3D breast model after 1 week of seeding the cells and reveals that cancer cells (green) spread and penetrate the top part of fibrous layer compared to the T-MCF-7.

DISCUSSION

Clinical and experimental studies have reported that tumor microenvironment is quite complex, however, it could be represented by minimal component that significantly impact tumor progression (Bahcecioglu et al., 2020). Since tumor invasion is the most threating step in tumor progression, this research developed for the first time in Iraq a novel 3D breast model to investigate the invasion of breast cancer cells in 3D microenvironment. The model characterized by its simplicity and ease of establishment. It is composed of two cellular layers; the lower layer is a fibrous layer represented by human dermal fibroblast gown on a 3D scaffold that we previously developed (Al-Shaibani, 2020). The upper layer was presented by the breast cancer cell line (MCF-7). Collectively, the fibrous layer and the cancerous cell layer formed a 3d tissue like structure mimics *in vivo* niche for cancer cell dpraeding when cancer cell invade intact tissues and organs. It has been reported that production of collagen and formation of extracellular matrix (ECM) are key mediators in cancer metastasis since ECM is required to enhance cells-cell contact while degradation of the ECM enhance cell dissociation as a first step in metastasis (Rankin et al., 2016, Semenza, 2016 and Hoffmann et al., 2018). As well established, fibroblasts are collagen producing cells and their presence in the developed model will provide collagenous matrix formation (Vincent and Engler, 2017). The collagenous matrix represents the challenge for cancer cell invasion. This model therefore enables scientists to investigate the effect of various parameters and effector molecules on collagen regulation and / or degradation during metastasis of cancer cells. In this study, migration and invasion of both transfected (T-MCF-7) and non-transfected MCF-7 cells (N-MCF-7) was investigated in both 2D cell culture and 3D breast model. In both experiments, migration of T-MCF-7 was significantly faster than N-MCF-7. A possible explanation is CSRP2 upregulates matrix metalloproteinase 13 (MMP13) which plays a crucial role in tumor cell invasion. Consequently, CSRP2 blocking might upregulated the production of MMP13 which had degraded the ECM secreted by the fibrous layer and facilitated the invasion of T-MCF-7 in contrast to N-MCF-7 when the levels of MMP13 kept at normal level and the ECM kept intact which in turn arrested cell invasion. Additionally, CSRP2 knockdown inhibits formation of invadopodium (protrusions that enable cell migration) via targeting inhibiting hypoxia induction factor (HIF) (Hoffmann et al., 2018). Another possible interpretation is silencing of CSRP2 led to

inhibition of cell proliferation, migration and cell cycle progression (Wang *et al*., 2017). The fibrous layer served as a stable bed for the cancer cells to lie upon and allow the invasion through the fibrous cellular components. Whereas, migration of T-MCF-7 in 2D culture lacks the challenge of invasion in collagenous environment since cells in 2D culture migrate on a flat plastic surface instead of tissue like structure. In another word, cells in 2D cell culture lack the chance to migrate and invade downwards restricting cell migration in even level. Therefore, cells in 2D culture may migrate faster than cells in a real tissue or in 3D breast model. Due to the success of the constructed 3D breast model to visualize the cellular invasion and metastasis during cancer progress, more investigations are required to characterize and validate the model at the molecular level. Further modification however, such as incorporating immune cells would be required to study the impact and contribution of the immune response to cancer invasion. Additionally, the breast model could be modified to investigate invasion of different cancer cells in 3D culture i.e. skin cancer model and lung cancer model. Importantly, the present study reveals that CSRP2 knockdown accelerated cancer invasion indicating the importance of CSRP2 as a target for cancer therapy. However, more research is required to investigate role of confirm this issue.

CONCLUSION

This study is the first study to develop a 3D breast cancer model in Iraq which represents a novel, robust and reproducible means of studying cancer invasion using organotypic breast model that showed cancer metastasis in 3D microenvironment. The developed 3D breast model could be used as a drug screening tool for antitumor drugs and non-chemotherapeutic treatments. Additionally, CSRP2 molecule is a good candidate as nonchemotherapeutic treatment for breast cancer.

CONFLICT OF INTEREST

No conflict of interest.

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➢ *ORAL PRESENTATION*

Manufacturing of a Novel Scaffold to Culture Human and Animal Cells in 3-Dimensional Microenvironment

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Abstract
Accumulating evidences have suggested that the behaviour of the cells in the two-dimensional cell culture (2DCC) is quite different from their behaviour when they are grown in three-dimensional cell culture (3DCC). Human and animal cells including stem cells require strict growth conditions to mimic the *in vivo* niches in order to obtain accurate and more realistic results. Accordingly, local inexpensive materials (cork and acetone) were used to develop a 3D culture scaffold. The developed scaffold was tested for ability to resist chemical and biological solutions. Scanning electronic microscope (SEM) was used to measure its thickness and pore size. Additionally, cytotoxicity analysis was achieved to test suitability of the developed scaffold to grow human fibroblast for 14 days. The result was a thin porous membrane with folds and cavities of a diameter of (50-100 µl) enabled human fibroblast to penetrate and grow through the scaffold. The cells penetrated the scaffold towards different directions and grew as a floating mass in the culture media; thereby, retain their original *in vivo* characteristics. It is concluded that cells grown on this scaffold were able to remain in culture for longer period than in 2D culture, which allow investigation, research, drug screening and therapeutic application with more accurate and reliable results than those obtained from a 2D culture.
Keywords: 3D scaffold, 3D cell culture, human cell culture, fibroblast culture

INTRODUCTION

The majority of biological studies have been conducted in standard two-dimensional cell culture (2DCC), whereas, accumulating evidence has suggested that the behaviour of the cells in the 2DCC is quite different from their behaviour when they are grown in three-dimensional cell culture (3DCC) (Li *et al.*, 2011a). Therefore, *in vitro* cell culture has recently developed from 2DCC to 3DCC, characterized by cultured cells with an environment which significantly mimics the *in vivo* niche by growing the cells as a floating mass (Wozniak and Keely, 2005). The 3DCC design is provided by a collagen matrix and emulates the extracellular matrix (ECM), and mimics a natural environment for cell growth (Wozniak and Keely, 2005). The majority of drug testing assays including drug discovery, cytotoxicity and wound healing assays, rely on 2D culture-based techniques involving the seeding of cells on naked or coated plastic and glass flat surfaces in tissue culture plates. Although, these assays are advantageous in terms of simplicity, quick, and inexpensive when compared to large-scale cell culture, they still have many disadvantages when compared to 3D culture (Edmondson *et al.*, 2014). For instance, cells in 2D culture differ morphologically and physiologically from cells in 3D culture (Baharvand *et al.*, 2006). Also, cells in 2D culture lack the criteria of being surrounded by cellular elements and extracellular matrix as in an *in vivo* environment, whereas 3D culture mimics this habitat and offers the cultured cells, to some extent, an environment mimicking *in vivo* conditions including the growth of cells floating in culture media (Bhadriraju and Chen, 2002). Additionally, 2D culture does not enable adequate exposure of the cells to the tested drug or substance of interest resulting in false, non-predictive and misleading data (Birgersdotter *et al.*, 2005). Moreover, therapeutic studies require testing the ability of the compound to enhance migration and proliferation of cell mass or cell sheets in the damaged area which cannot be performed in 2D culture since the confluent monolayer cells may migrate and proliferate as single cells in two dimensions (Poujade *et al.*, 2007). Given the limitations of 2DCC, and current 3D scaffolds which mainly contain animal materials, the aims of the present study is to further develop a 3D cell culture scaffold free of animal materials for tissue engineering, diagnosis, drug discovery and therapeutic applications.

MATERIALS AND METHODS

Cell Culture

Approval for the study was given by the Council of College of Biotechnology / Al-Nahrain University in 2020. All cell culture was performed in class II cabinets to reduce the risk of infection and contamination. All cells and 3D-tissue like structures (3D-TS) were incubated at standard culture conditions (SCC) which were provided by humidified incubator adjusted to 37oC, ≈20% O2 and 5% CO2. Unless otherwise stated, all culture conditions set out in this study for cell culture and cell experiments were SCC.

Isolation of Human Dermal Fibroblast

Human dermal fibroblast used in this study were obtained with agreement and consent of each donor. A skin biopsy of one square cm (1 cm^2) was obtained from patients suffering from breast cancer and undergone breast removal. As described by (Todd and Reynolds, 1998), a dermal sections were cleared of blood using PBS, chopped into pieces of size 3-5 mm using a scalpel and placed over a tissue wet with 70% ethanol for 2 minutes. Each section was transferred into a well of a 6 well plate, covered with foetal calf serum (FCS) incubated at SCC for overnight. Fibroblasts were grown at SCC in DMEM-F10, which stands for Dulbecco's Modified Eagle Medium (Sigma / UK) and composed of 500 ml DMEM supplemented with 10% FCS (Sigma / UK), 4% of 200 mM L-Glutamine and 1% of 100 IU/ml penicillin, 100 µg/ml streptomycin (PS) (Sigma / UK). On the next day, a volume of 5 ml of DMEM-F10 was added to each well. The dermal sections were kept in the wells with a regular change of media until fibroblast cells attached on the surface of the culture plate followed by splitting at passage one. To split cells, old media was aspirated, and cells were rinsed with phosphate buffered saline (PBS), trypsinised with trypsin 1X and incubated for 10 minutes. A quantity of 10 ml of DMEM-F10 was added to prepare the cell suspension and centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded, and cells were counted and a cell number of 5×10^5 to 1×10^6 was seeded into a T75 flask. A quantity of 15-20 ml of fresh DMEM were added to the flask and incubated under SCC. Trypan blue stain was used to assess viability of all cells.

Preparation and Characterization of the Scaffold (CytoBuilder)

Inexpensive local materials (cork and acetone) were used to prepare the CytoBuilder. These two substances were mixed at a ratio of (1:2) respectively for 30 minutes at room temperature to produce a membranous layer with a diameter of 1 centimetre. The porosity and thickness of the scaffold was determined and measured by scanning electron microscopy. The solubility of the developed scaffold was tested to resist various types of solutions that are used for routine cell culture and the scaffold will be in direct contact with it including distilled water, ethanol, cell culture medium (DMEM), and buffer solutions. The scaffold was treated separately in these solutions for 28 days.

Construction of 3-Dimensional Tissue like Structure (3D-TS)

This study developed a 3D-TS for the first time in Iraq. The basis of this model is the CytoBuilder scaffold. Before seeding cells, CytoBuilder was activated with 70 % ethanol for one minute, treated with DMEM-F10 for one minute, fixed in 6 well plates and incubated under SCC until required. To form the fibroblast layer of the model, 1×10^6 fibroblasts were suspended in 100 µl DMEM-F10 and seeded onto the CytoBuilder scaffold and incubated at SCC for 90 minutes. A volume of 9 ml of DMEM-F10 was added to the fibroblast layer and incubated under SCC with a change of DMEM-F10 every two days for 28 days.

Characterization of the Developed 3D-Tissue like Structure (3D-TS)

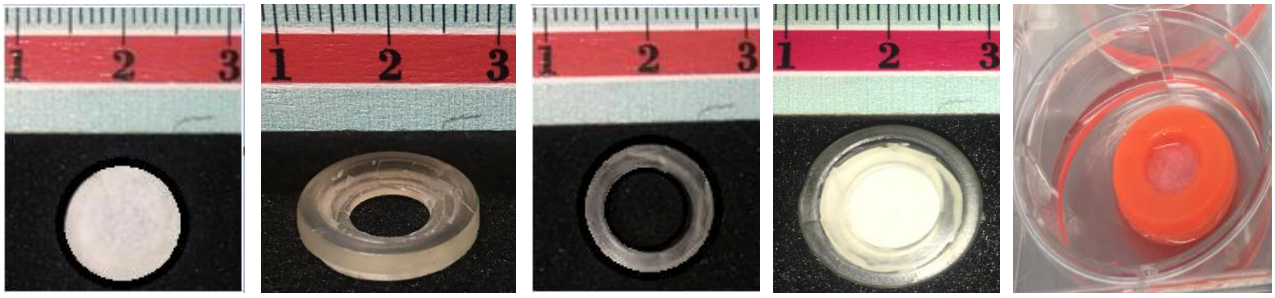
Tissue sections were stained with haematoxylin-eosin (HE) to evaluate the formation of 3D-TS; in addition to staining with DAPI to assess cell penetration through the CytoBuilder scaffold. Paraffin embedded 3D model tissues were always kept on ice during sectioning to ensure effective sectioning. The microtome was adjusted to 4 µm and sections were kept in a water bath adjusted at 40°C for 2-3 minutes before transferring to slides. The slides were then heated in an oven at 60°C overnight and the next day, dehydrated by washing for 5 minutes in each xylene, 100% ethanol, 95% ethanol, 70% ethanol and distilled water. For antigen retrieval, slides were placed in

previously heated 10 mM citrate buffer, pH 6.0, in a microwave for 1 minute and then left to cool at room temperature for 30 minutes, and washed in 5 mM Tris buffered saline (TBS) pH 7.6 for 5 minutes. Permeabilization of the slides was performed in 0.2% Triton-X for 10 minutes at room temperature. All sections were incubated with DAPI at a dilution of 1:1000 for 30 minutes at room temperature in a moist chamber in the dark. Sections were then washed four times in TBS for 2 minutes, covered with DPX, left in the dark at room temperature overnight, and analysed the next day using fluorescent microscope (Leica).

RESULTS

Characteristics of the CytoBuilder Scaffold

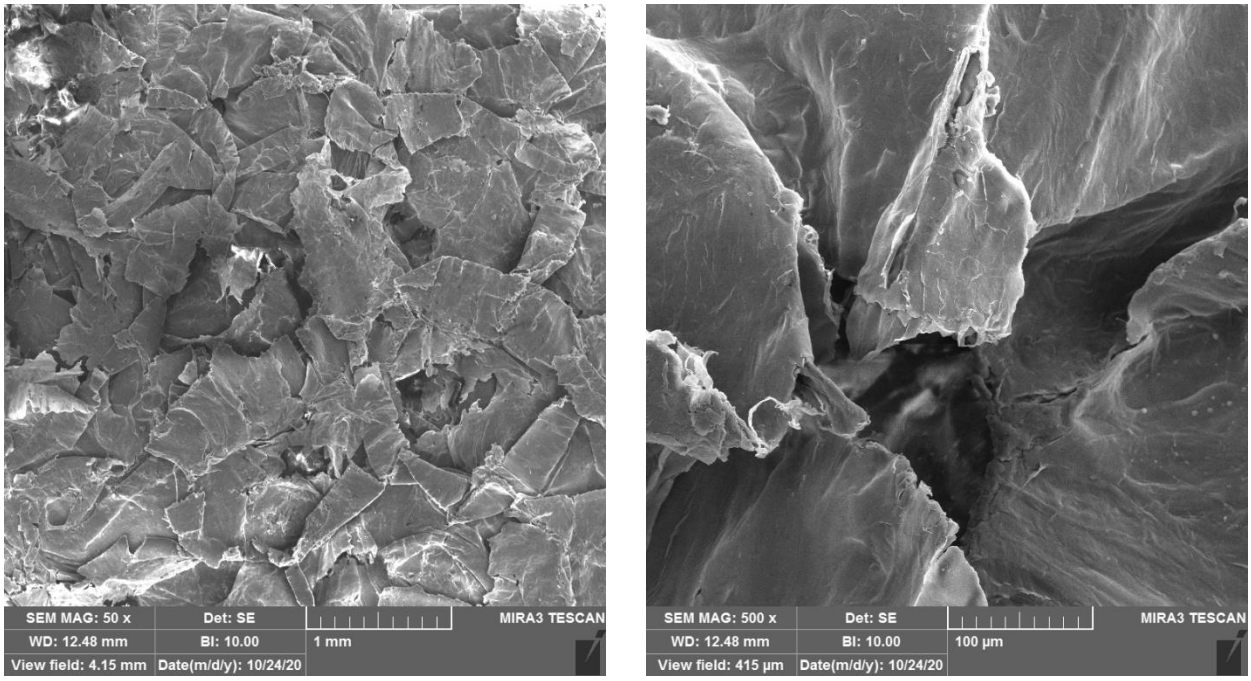
The developed CytoBuilder scaffold composed of three parts as shown in (Figure 1). The first part represented by the developed highly porous membrane which is suitable for the growth of animal cells in a three-dimensional environment (Figure 1A). The other parts are the holder which carry the CytoBuilder scaffold (Figure 1B) and the fixative (Figure 1C) which fix the porous membrane on the holder. These parts collectively form the entire structure of the CytoBuilder scaffold (Figure 1D). This entire structure could be fit inside tissue culture plate (Figure 1E) to grow the cells and form a 3D-TS.



A- Scaffold | B- Holder | C- Fixative | D- Full Structure | E- Scaffold in Tissue Culture Plate

Figure 1. Parts of the developed cytobuilder scaffold.
CytoBuilder scaffold consists of three main parts: (A) the developed scaffold membrane which is used to grow cells on. (B) The holder which is the part that carry the scaffold membrane. (C) The fixative which is the part that fix the scaffold on the holder. (D) Collectively, these the parts compose the full scaffold. (E) CytoBuilder scaffold in the tissue culture plate with fibroblast supplemented with culture media (DMEM).

Results of scanning electronic microscope (SEM) showed that the scaffold contains cavities and grooves that range in diameter (50-100 μm), which enable cells to spread through the scaffold to form a 3D-TS (Figure 2). In addition, the thickness of the scaffold range was (500–700 μm). both porosity and thickness are important parameters to establish a 3D-TS.



(A) Scaffold Surface (B) Porosity of the Scaffold (Cavities and Grooves)

Figure 2. Thickness and Porosity of the Developed CytoBuilder Scaffold.

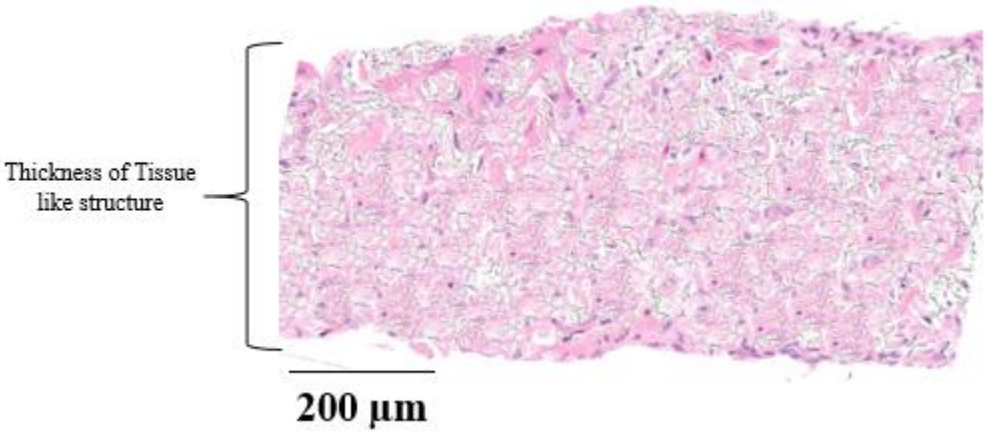
Micrographs picked up by a Scanning Electronic Microscopy (SEM). (A) Scaffold surface shows porosity of the scaffold as grooves and cavities enabled human fibroblast to penetrate and grow through the scaffold. (B) A zoom in micrograph shows the cavities and the grooves of the scaffold.

Moreover, the scaffold was insoluble and had ability to resist solutions that are usually used in tissue culture experiments i.e. distilled water, ethanol, buffer solution and tissue culture media (DMEM) As shown in (Figure 3). Scaffold stability and resistance to these solutions supports the possibility of using it for long periods of time for scientific research purposes that require longer times.

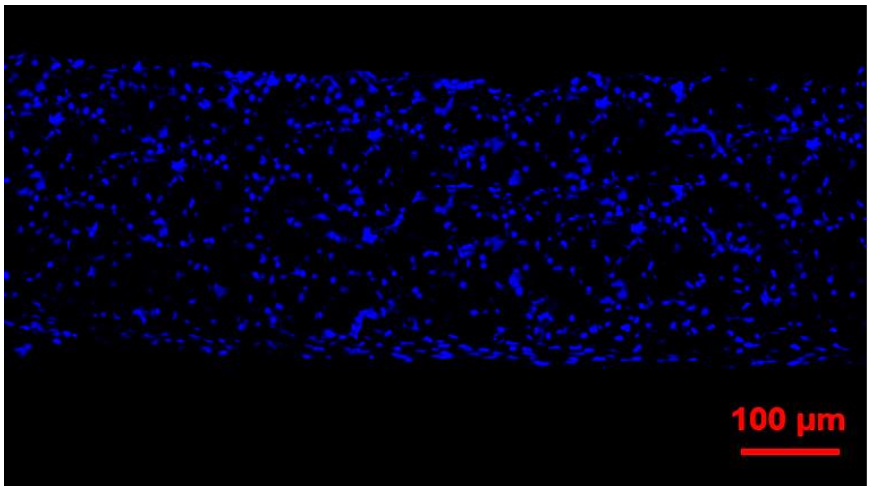
(A) Distilled Wtaer (B) Buffer (C) Ethanol (D) Culture Media

Figure 3. Resistance of the Scaffold (CytoBuilder) to Different Solutions.
The developed CtyoBuilder scaffold was insoluble and showed ability to resist different solutions such as (A) Distilled water, (B) Buffer solution, (C) Ethanol and (D) Culture media. The scaffold was sucked for 28 days in these solutions and showed ability to resist them.

Histological assessment showed formation of a 3D-TS upon staining with Haematoxylin – eosin (HE) staining as shown in (Figure 4A). The cell nuclei are stained with the blue Haematoxylin while the cytoplasm is stained with the pink Eosin. This confirms the formation of a 3D-TS. On the other hand, other slices of the same 3D-TS were stained with the DAPI stain for examining the ability of cells to spread and penetrate into the scaffold as shown in (Figure 4B), which demonstrates formation of 3D-TS with consistent spread of cells inside the scaffold.



(A) Formation of Tissue like Structure (HE staining).



(B) Penetration and Distribution of Cells within the CytoBuilder Scaffold.

Figure 4. Growth, Proliferation and Penetration of Cells on CytoBuilder Scaffold.
Micrographs explain growth of cells on the developed scaffold for 28 days. (A) Micrograph captured by light microscope explains growth and proliferation of cells within the scaffold and formation of a 3D-TS stained by Haematoxylin-Eosin stain. (B) Micrograph captured by fluorescence microscope explains penetration of cells within the scaffold stained by nucleic acid stain "DAPI".

DISCUSSION

Tissue engineering is one of the modern technologies has emerged to the field of biotechnology and represents a promising therapeutic approach. These approaches include construction of 2D human skin model in vitro using a 3D scaffold and re-cultivated on the damaged area caused by burns or wounds. In this case, skin cells can be isolated from a healthy area of the injured person and use these cells to establish skin equivalent in the laboratory and transplant again on the damaged area. Previous attempts to treat burns and wounds included cutting an entire healthy part of the affected person's skin enough to cover the damaged site and re-paste and sew over the damaged area and this causes further damage to the body. This problem can be avoided using 3DCC. Drug discovery studies have been tested on animal models for more than 30 years and the pharmaceutical industry grows, high-throughput

drug screening has become a high cost with the longer time to perform these tests. Alongside this phenomenon, ethical issues arose regarding drug testing on laboratory animals which in turn did not translate well into human applications due to genetic and physiological differences (Edmondson *et al*. 2014). Therefore, the use of 3D scaffolds can contribute to overcome these problems to some extent by providing a human tissue mimics in vivo environment which returns with more positive drug responses. Moreover, cell secretions are considered one of the pillars of scientific research. These secretions contain abundant quantities of cytokines, interleukins, growth factors, hormones, enzymes, cellular signals, and other proteins. Often these secretions are collected in a 2DCC that contains one type of cell and therefore it is not possible to collect secretions from a more than one cell type while this problem can be overcome when using the scaffold where more than one cell type can be cultured which gives secretions from a 3D-TS that mimics secretions by live body. Additionally, experiments of tiny molecules like nucleic acids (DNA, RNA, small RNAs and interfering RNAs) require high amounts of cells in order to obtain sufficient quantities of these molecules. Other experiments also require the extraction of these nucleic acids from tissue instead of cells, and this cannot be accomplished in the 2DCC. The scaffold provides a means to grow cells either single or with another type of cells in a three-dimensional environment for extracting the required molecule. The developed scaffold enables the formation of a 3D-TS consisting of multiple cellular layers similar to the tissue structure in vivo, which allows many tests to be carried out using the developed scaffold instead of experiments on laboratory animals. Examples of this application include the formation of skin tissue that contains the layer of fibroblasts and the layer of keratinocytes using the scaffold and conducting many tests on skin diseases such as skin cancer, burns, and wounds. Also, the breast tissue that consists of a fibrous cellular layer and cancerous cellular layer can be constructed using 3D scaffold. Additionally, a cartilage tissue from the synovial cell and chondrocyte could be formed on the scaffold. These in vitro models can be used instead of using costly laboratory animals. The developed scaffold can be used as a modern laboratory technique to perform many important tests in scientific research such as investigating cell migration, proliferation and differentiation in a 3DCC for the first time in Iraq. Cell migration and proliferation are the most important assays in the follow-up and treatment of many important diseases, including tumours and cancers, as conducting these assays in a 3DCC has a great role to obtain more accurate results than 2DCC. This product is considered inexpensive and reduces the expenses incurred by the researcher. The thickness and permeability of the scaffold are very important factors in the growth, movement and spread of cells during experiments. The thickness of the scaffold increases the surface area on which the cells grow, thereby allowing the largest possible number of cells to grow within time, which allows the experiment to stay as long as possible. On the other hand, the porosity of the scaffold allows cells to spread, move, and penetrate the scaffold. It is very important feature where it is possible to study the migration, proliferation and spread of cells in a 3DCC that mimics the in vivo environment and avoid the problem of dense growth that occurs in the 2DCC. On the other hand, cell proliferation, their growth and penetration into the scaffold within time is an evidence of the non-toxicity of the developed scaffold, which means the validity and suitability of the scaffold for research and laboratory experiments without side effects on the experiment and its results. Experiments in 2DCC may be limited to only three days, when the researcher is obliged to end the experiment within a short period that may give misleading results. In contrast, using the CytoBuilder scaffold, the cells grow on a porous layer that enables the cells to penetrate and spread into the scaffold so that the 3D-TS may continue to grow for longer periods that may exceed 30 days, allowing more time to conduct lengthy experiment that gives more realistic results. Scaffolding can be produced in different sizes, it can be fit in growth plates of different sizes such as (6 well plate 12 well plate, 24 well plate, 48 well plate) and according to the researcher's need and experiment purposes. Sometimes it is impossible to obtain a sufficient number of cells and other experiments require expensive culture media. So, small size scaffolds could be used to reduce the number of required cells and volume of culture medium.

CONCLUSION

This cytobuilder is considered the first three-dimensional (3D scaffold) to be synthesized in Iraq and used for 3D cell culture application. It is synthesized free of animal material (cork and acetone) and characterized by high porosity and resistance to organic solutions. The CytoBuilder scaffold represents a novel and robust technology could be used for 3D cell culture to develop 3D models of different tissues i.e. skin model, breast model and bone model and could be used as drug screening tools to investigate invasion of different cancer cells in 3D culture.

CONFLICT OF INTEREST

No conflict of interest.

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➢ *ORAL PRESENTATION*

Atık Sulardan Makrolid Grubu Antibiyotiklerin Adsorpsiyon Yöntemi ile Giderimi için Uygun Adsorbanların Araştırılması

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Özet

Son yıllarda, antibiyotiklerin kullanımı insan ve hayvan tıbbında oldukça artış göstermiştir. Antibiyotikler, insan ve hayvan atılımları yoluyla metabolize edilmiş veya değişmemiş formda atık suya geçmektedir. Bu yolla, ilaçlar yüzeye ve yer altı sularına ulaşabilir (Carvalho ve Santos, 2016). Bu nedenle, antibiyotik kirliliğinin kaynağı kentsel ve tarımsal atıksuların deşarjı olarak kabul edilir (Halling-Sorensen ve ark., 1998; Perez ve ark., 2008). Antibiyotik kirliliği çevresel bakterilerde antibiyotik direncinin artmasına neden olabilir. Zirai uygulamalarla daha büyük hale getirilen ortamdaki antibiyotik direnç genlerinin rezervuarı, kirli yiyecek veya suyun alınması yoluyla insan sağlığına yönelik tehdit oluşturmaktadır (Ashbolt ve ark., 2013). Bu tür organik kirleticilerin çevresel kaliteye ve insan sağlığına oluşturduğu riskin ortadan kaldırılması için atık sulardan uzaklaştırılması oldukça önem taşımaktadır (Licul-Kucera ve ark., 2019). Antibiyotiklerin atıksu ortamından uzaklaştırılmasında genel arıtma tesisi prosesleri yeterli değildir. Antibiyotiklerin sulu çözelti ortamından uzaklaştırılmasında birçok yöntem kullanılmaktadır. Adsorpsiyon yöntemi, diğer yöntemlere kıyasla uygulamadaki basitlik, yüksek verim ve düşük maliyet gibi avantajları nedeniyle son zamanlarda araştırmacılar tarafından oldukça ilgi görmektedir (Jung ve ark., 2013).

Bu çalışmada, solunum yolu enfeksiyonları ve yumuşak doku enfeksiyonları ile mücadelede yaygın olarak kullanılan son zamanlarda Dünya Sağlık Örgütü (WHO) tarafından insan tıbbı için en önemli antibiyotiklerden biri olarak sınıflandırılmış makrolid grubu antibiyotiklerinden roksitromisin, tilosin ve tilmikosinin atık sulardan adsorpsiyon yöntemi ile uzaklaştırılması araştırılmıştır. Bu üç antibiyotiğin çözelti ortamından giderimi için; çağla kabuğu, yeşil ceviz kabuğu, ceviz dış kabuğu, kalsine yumurta kabuğu, manyetik kestane kabuğu, manyetik polen, kalsine kemik ve kemik tozu adsorban olarak araştırılmıştır. Bu antibiyotiklerin kantitatif tayini yüksek performanslı sıvı kromatografisi (HPLC) kullanılarak gerçekleştirilmiştir.

Anahtar Kelimeler: Antibiyotik, Makrolit, Antibiyotik Giderimi, Atıksu, Adsorpsiyon

Investigation of Suitable Adsorbents for Removal of Macrolide Group Antibiotics in Wastewater by Adsorption Method

Abstract

In recent years, the use of antibiotics has increased considerably in human and veterinary medicine. Antibiotics can enter wastewater in metabolized or unchanged form through human and animal excretions. In this way, drugs can reach the surface and groundwater (Carvalho and Santos, 2016). Therefore, the source of antibiotic pollution is considered to be the discharge of urban and agricultural wastewater (Halling-Sorensen et al., 1998; Perez et al., 2008). Antibiotic pollution can lead to increased antibiotic resistance in environmental bacteria. The reservoir of antibiotic resistance genes in the environment, which is made larger by agricultural applications, poses a threat to human health through the intake of contaminated food or water (Ashbolt et al., 2013). It is very important to remove such organic pollutants from wastewater in order to eliminate the risk of environmental quality and human health (Licul-Kucera et al., 2019). General treatment plant processes are not sufficient to remove antibiotics from the wastewater environment. The adsorption process has attracted a lot of attention recently by researchers due to its advantages such as simplicity in application, high efficiency, and low cost compared to other methods (Jung et al., 2013).

In this study, the removal of roxithromycin, tylosin, and tilmicosin from the wastewater by the adsorption method from macrolide group antibiotics, which has been recently used as a most important antibiotic for human medicine, was investigated by the World Health Organization (WHO). To remove these three antibiotics, cashew shell, green walnut shell, walnut outer shell, calcined eggshell, magnetic chestnut shell, magnetic pollen, calcined bone, and bone powder were examined as adsorbents. High-performance liquid chromatography (HPLC) was used for the quantitative determination of these antibiotics.
Keywords: Antibiotic, Macrolide, Antibiotic Reduction, Wastewater, Adsorption

GİRİŞ

Son yıllarda, antibiyotiklerin kullanımı insan ve hayvan tıbbında oldukça artış göstermiştir Antibiyotikler, vücuda alındıktan sonra % 50-80'i metabolize olmadan, dışkı ve idrar yoluyla atılır (Jia ve ark., 2016). Bu nedenle, antibiyotikler değişmemiş formda belediye atık sularına katılarak veya hayvan gübrelerinin gübre olarak kullanılması yoluyla çevreyi kirletirler. Sulu ortamda antibiyotiklerin ortaya çıkması, antibiyotik direnç genlerindeki artış nedeniyle büyük endişe kaynağı haline gelmekte ve canlılar için risk oluşturmaktadır (Bai ve ark., 2015; Yin ve ark., 2016). Bu tür organik kirleticilerin çevresel kaliteye ve insan sağlığına oluşturduğu riskin ortadan kaldırılması için atık sulardan uzaklaştırılması oldukça önem taşımaktadır. Antibiyotiklerin atıksu ortamından uzaklaştırılmasında genel arıtma tesisi prosesleri yeterli değildir. Adsorpsiyon yöntemi, diğer yöntemlere kıyasla uygulamadaki basitlik, yüksek verim ve düşük maliyet gibi avantajları nedeniyle son zamanlarda araştırmacılar tarafından oldukça ilgi görmektedir (Jung ve ark., 2013). Kullanılan biyokütlelerin toksik etkilerinin olmaması, atık suların geri kazanımında oldukça önemlidir.

Makrolitler, en önde gelen antibiyotik gruplarından biri olarak kabul edilir. Gram pozitif ve bazı gram negatif bakterilere karşı aktif oldukları için insan ve hayvan tıbbında yaygın olarak kullanılırlar. Bu çalışmada makrolid grubu antibiyotiklerinden roksitromisin, tilosin ve tilmikosinin çözelti ortamından giderimi için uygun adsorbanların belirlenmesi amaçlanmıştır. Bu antibiyotiklerin uzaklaştırılması için çağla kabuğu, yeşil ceviz kabuğu, ceviz dış kabuğu, kalsine yumurta kabuğu, manyetik kestane kabuğu, manyetik polen, kalsine kemik ve kemik tozu adsorban olarak incelenmiştir. Bu amaçla asidik ve orjinal çözelti pH'larında çalışılmış ve kantitatif tayinler HPLC yöntemi ile gerçekleştirilmiştir.

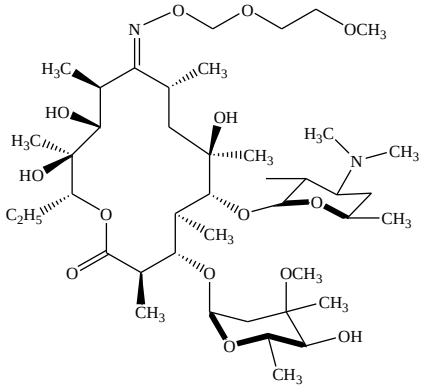
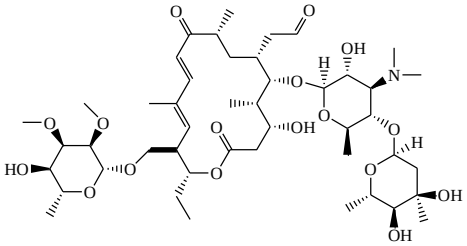
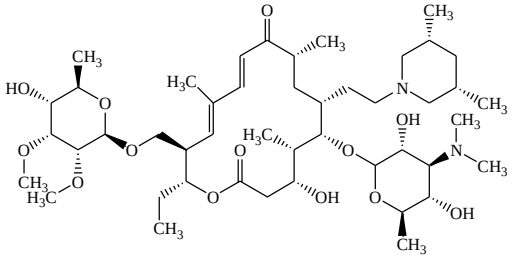
MATERYAL

Kimyasallar ve Cihazlar

Roksitromisin, tilosin ve tilmikosinin 100 mg L^{-1} derişiminde stok çözeltileri hazırlanmış ve çalışmada kullanılacak diğer derişimler bu çözeltilerden ultra saf su seyreltilerek hazırlanmıştır. Çalışmada kullanılan antibiyotikler Sigma–Aldrich firmasından temin edilmiştir. Çalışmada kullanılan antibiyotiklerin molekül yapıları Tablo 1'de verilmiştir.

Çalışmada, kantitatif tayinler Shimadzu (Kyoto, Japan) marka HPLC cihazı ile yapılmıştır. Sistemde, pompa (LC-20 AD), dedektör (SPD-M 20A), kolon fırını (CTO 20 AC) ve gaz giderme birimi (DGU 20 A) bulunmaktadır. Cihaza bağlı olarak Intertsil® ODS-4V (5µm, 4.60 x 250 mm) kolon kullanılmış ve kolon sıcaklığı 35 ^{0}C olarak ayarlanmıştır. Mobil faz olarak %40-60 asetonitril-su karışımı (20 mM orto-fosforik asit) kullanılmış ve pH 2,8'e 1 M NaOH ile tamponlanarak ayarlanmıştır. Akış hızı 1 mL dk^{-1} olarak belirlenmiştir (Angenent ve ark., 2008; Paíga ve ark., 2019; Prats ve ark., 2001).

Tablo 1: Antibiyotiklerin molekül yapıları

Antibiyotik	*Molekül Formülü*
Roksitromisin	
Tilosin	
Tilmikosin	

Adsorbanların Hazırlanması

Çalışmada kullanılan adsorbanlardan çağla kabuğu, yeşil ceviz kabuğu, ceviz dış kabuğu Bafra/Samsun bölgesinden toplanmıştır. Yumurta kabuğu ve kestane kabuğu yerel marketlerden; hayvan kemik tozu ise bir yem fabrikasından temin edilmiştir. Çalışmada kullanılan polen Kastamonu'nun Tosya ilçesinde bulunan çam ağaçlarından toplanmıştır.

Çağla, yeşil ceviz, ceviz dış, yumurta ve kestane kabukları saf su ile yıkandıktan sonra etüv de 50 ^{0}C'da kurutulmuştur. Kuruyan adsorbanlar öğütücü ile öğütülerek 150 µm'lik elekten elenmiştir. Polen ve kestane kabuğu manyetit (Fe_3O_4) ile bir araya getirilerek manyetik özellik kazandırılmıştır (Madrakian ve ark., 2012). Yumurta kabuğu ve kemik ise 900 ^{0}C'da kalsine edilmiştir.

YÖNTEM

Adsorpsiyon, bir katının ya da bir sıvının sınır yüzeyinde derişiminin değişmesi olarak tanımlanmaktadır. Konsantrasyonun artması ile gerçekleşen adsorpsiyona pozitif adsorpsiyon, azalması ile gerçekleşen adsorpsiyona negatif adsorpsiyon denir. Adsorpsiyon işlemi, fazlardan birindeki bir maddenin (çözeltideki molekül) diğer fazdaki maddenin (katı faz) yüzeyine birikerek ayrılması şeklinde olmaktadır.

Bu çalışmada, makrolid grubu antibiyotiklerden roksitromisin, tilosin ve tilmikosinin atıksu ortamından giderimi için 8 farklı adsorban ile adsorpsiyon çalışması gerçekleştirilmiştir. Antibiyotik çözeltisinin orijinal pH'ında ve pH 2,5 da hazırlanan 10 mg L^{-1} derişimindeki çözeltilerden 10 ml alınarak, 0,1 g adsorban ile 1 saat boyunca rotatörde 30 RPM' de temas ettirilmiştir. Daha sonra 3000 devir/dk hızda 3 dakika boyunca santrifüjlenerek katı ve sıvı kısım birbirinden ayrılmıştır. Elde edilen süpernetant HPLC ile kantitatif olarak tayin edilmiştir. Çıkan alan

değeri kalibrasyon denkleminde yerine konarak uzaklaşan madde miktarı hesaplanmıştır. Elde edilen sonuçlar ile adsorpsiyon kapasitesi ve % giderim değerleri hesaplanmıştır.

Tüm çözeltiler için adsorpsiyon kapasitesi ve % giderim verileri eşitlik 1 ve eşitlik 2 ile hesaplanmıştır.

$$q_d = \frac{(C_o - C_d)\,V}{m}\ x100 \qquad (1)$$

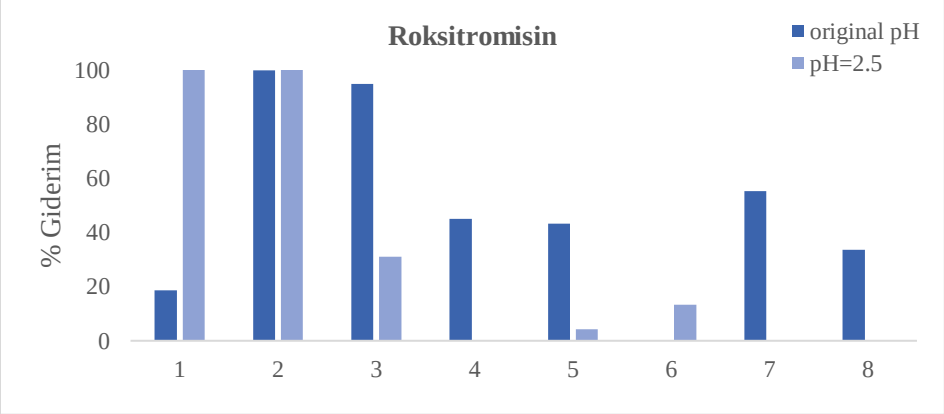
$$\%\ Giderim = \frac{C_0 - C_d}{C_0}\ x100 \qquad (2)$$

Burada,

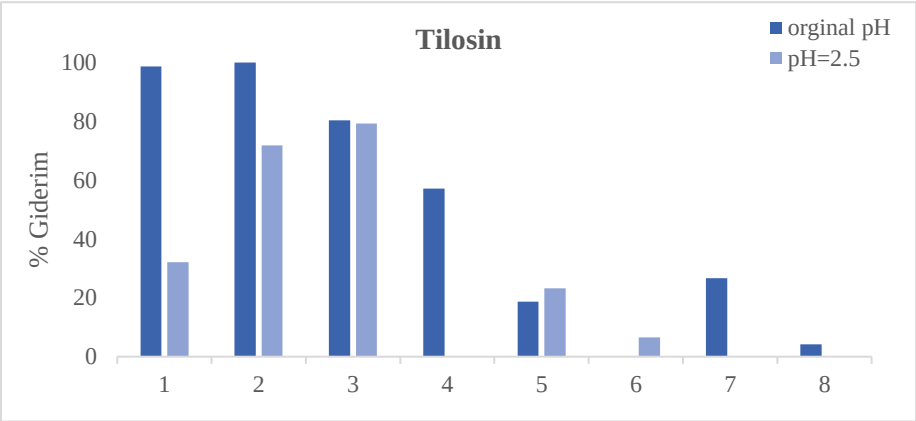
q_d: Dengedeki adsorpsiyon kapasitesi (mg g^{-1})
C_o: Başlangıç adsorbat derişimi (mg L^{-1})
C_d: Dengede adsorplanmadan kalan adsorbat derişimi (mg L^{-1})
V: Kullanılan çözelti hacmi (L)
m: Kullanılan adsorban miktarı (g)

SONUÇLAR ve TARTIŞMA

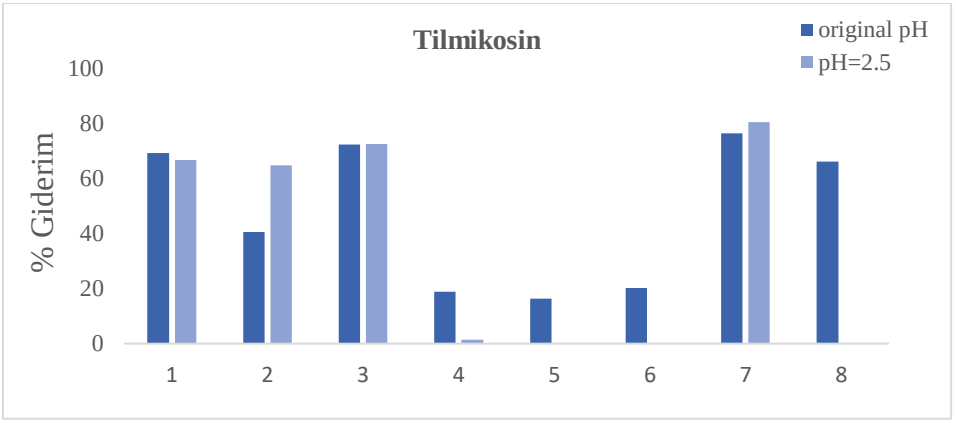
Roksitromisin, tilosin ve tilmikosinin ve çözelti ortamından adsorpsiyon yöntemi ile uzaklaştırılması için çağla kabuğu, yeşil ceviz kabuğu, ceviz kabuğu, kalsine yumurta kabuğu, manyetik kestane kabuğu, manyetik polen, kalsine kemik ve kemik tozunun adsorban olarak kullanılabilirliği araştırılmıştır. Adsorpsiyon çalışmaları asidik pH (pH=2.5) ve antibiyotik çözeltilerinin orjinal pH'larında (roksitromisin pH 6,27, tilosin pH 6,05 ve tilmikosin pH 7,16yapılmıştır. Şekil 1. roksitromisin, Şekil 2. Tilosin ve Şekil 3.'te tilmikosinin adsorpsiyon çalışmalarına ait % giderim verileri ve Tablo 2.'de ise karşılaştırmalı olarak adsorpsiyon kapasitesi değerleri verilmiştir.



Şekil 1. Roksitromisinin 8 farklı adsorban ile orijinal pH ve pH=2.5'da % giderim değerlerine ait grafik. (1: Çağla kabuğu, 2: Yeşil ceviz kabuğu, 3: Ceviz kabuğu, 4: Kalsine yumurta kabuğu, 5: Manyetik kestane kabuğu, 6: Manyetik polen, 7: Kalsine kemik, 8: Kemik tozu)



Şekil 2. Tilosinin 8 farklı adsorban ile orijinal pH ve pH=2.5'da % giderim değerlerine ait grafik. (1: Çağla kabuğu, 2: Yeşil ceviz kabuğu, 3: Ceviz kabuğu, 4: Kalsine yumurta kabuğu, 5: Manyetik kestane kabuğu, 6: Manyetik Polen, 7: Kalsine kemik, 8: Kemik tozu)



Şekil 3. Tilmikosinin 8 farklı adsorban ile orijinal pH ve pH=2.5'da % giderim değerlerine ait grafik. (1: Çağla kabuğu, 2: Yeşil ceviz kabuğu, 3: Ceviz kabuğu, 4: Kalsine yumurta kabuğu, 5: Manyetik kestane kabuğu, 6: Manyetik polen, 7: Kalsine kemik, 8: Kemik tozu)

Şekil 1. ve Tablo 2. de görüldüğü gibi roksitromisinin çözelti ortamından adsorpsiyon yöntemi ile gideriminde yeşil ceviz kabuğu ve ceviz kabuğu, çözeltinin orijinal pH'ında %100'e yakın bir giderim sağlamıştır. Yeşil ceviz kabuğu ile yapılan adsorpsiyon çalışmalarında hem asidik pH'da hem de çözeltinin orijinal pH'ında (pH 6,27) adsorpsiyon verimi %100'e yakındır. Yeşil ceviz kabuğu ile roksitromisinin gideriminde çözelitinin pH'ının adsorpsiyon verimi üzerine etkisinin olmadığı söylenebilir.

Şekil 2'de tilosinin 8 farklı adsorban ile adsorpsiyonuna ait % giderim verileri görülmektedir. Tilosinin adsorpsiyon çalışmalarında yeşil ceviz kabuğu ve ceviz kabuğu dışında asidik pH da adsorpsiyon verimleri oldukça düşük bulunmuştur. Çözeltinin orijinal pH'ında ise çağla kabuğu ve yeşil ceviz kabuğunda %100'e yakın bir giderim; ceviz kabuğu ve kalsine yumurta kabuğunda ise sırasıyla %80 ve %58 giderim elde edilmiştir.

Tilmikosinin (Şekil 3) uzaklatırma çalışmasında ise orijinal pH'da çağla kabuğu ve yeşil ceviz kabuğu oldukça yüksek verimle giderim sağlamıştır. Ceviz kabuğu ve kalsine yumurta kabuğu ile tilmikosin adsorpsiyonunda ise kayda değer bir adsorpsiyon kapasitesi değeri elde edilmiştir.

Tablo 2. Roksitromisin, tilosin ve tilmikosin için adsorpsiyon kapasitesi değerleri

Roksitromisin	**q (mg g^{-1}) pH 2,5**	**q (mg g^{-1}) Orijinal pH (6,27)**
Çağla Kabuğu	11,45	4,57
Yeşil Ceviz Kabuğu	10,10	25,66
Ceviz Kabuğu	0,40	23,16
Kalsine Yumurta Kabuğu	1,53	10,43
Manyetik Kestane Kabuğu	0,42	8,87
Manyetik Polen	1,31	0,08
Kalsine Kemik	3,37	13,51
Kemik Tozu	6,64	8,21
Tilosin	**q (mg g^{-1}) pH 2,5**	**q (mg g^{-1}) Orijinal pH (6,05)**
Çağla Kabuğu	1,14	25,22
Yeşil Ceviz Kabuğu	2,96	26,19
Ceviz Kabuğu	3,63	20,70
Kalsine Yumurta Kabuğu	0,36	12,29
Manyetik Kestane Kabuğu	1,00	4,81
Manyetik Polen	0,30	0,26
Kalsine Kemik	0,39	6,89
Kemik Tozu	0,56	1,10
Tilmikosin	**q (mg g^{-1}) pH 2,5**	**q (mg g-1) Orijinal pH (7,16)**
Çağla Kabuğu	16,80	16,9
Yeşil Ceviz Kabuğu	13,4	9,90
Ceviz Kabuğu	18,4	17,30
Kalsine Yumurta Kabuğu	0,71	6,00
Manyetik Kestane Kabuğu	9,33	3,01
Manyetik Polen	8,03	4,02
Kalsine Kemik	20,41	18,7
Kemik Tozu	3,92	16,1

SONUÇ

Makrolid grubu antibiyotiklerinden roksitromisin, tilosin ve tilmikosinin atıksu ortamından adsorpsiyon yöntemi ile giderimi için uygun adsorbanların belirlenmesi amacıyla çağla kabuğu, yeşil ceviz kabuğu, ceviz dış kabuğu, kalsine yumurta kabuğu, manyetik kestane kabuğu, manyetik polen, kalsine kemik ve kemik tozu adsorban olarak araştırılmıştır. pH 2,5 ve antibiyotik çözeltilerinin orijinal pH'larında adsorpsiyon çalışması yapılmıştır. Roksitromisin için çözeltinin orijinal pH'ında yeşil ceviz ve ceviz dış kabuğu en yüksek adsorspiyon kapasitesine sahiptir. Çağla ve ceviz kabuğu ile Tilosinin gideriminde çağla, yeşil ceviz ve ceviz dış kabuğu ile orijinal çözelti pH'ında %100'e yakın bir giderim elde edilmiştir. Tilmikosinin gideriminde ise, hem asidik pH'da hem de çözeltinin orijinal pH'ında %70'in üzerinde bir giderim sağlanmıştır. Her 3 antibiyotik için de ceviz kabuğunda yüksek %giderim sonuçları elde edilmiştir.

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➢ *ORAL PRESENTATION*

Çikolatada Fonksiyonel Gıda Çalışmaları

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Özet

Son yıllarda tüketicinin gıda beklentileri; sağlık-besin ilişkisinin daha belirgin hale gelmesiyle haz ve temel besin öğelerini karşılama gibi geleneksel taleplerin ötesine taşınmıştır. Bu nedenle Türk Gıda Kodeksine göre besleyici etkilerinin yanı sıra bir ya da daha fazla etkili bileşene bağlı olarak sağlığı koruyucu, düzeltici ve/veya hastalık riskini azaltıcı etkiye sahip olup, bu etkileri bilimsel ve klinik olarak ispatlanmış gıdalar şeklinde ifade edilen fonksiyonel/özel beyanlı gıdalara yönelik talep artış göstermiştir.
Kakao ürünleri ile şekerlerden elde edilen, en az %18 kakao yağı ve en az %14 yağsız kakao kuru maddesi içeren toplam kakao kuru maddesi içeriği en az %35 olan ürün şeklinde tanımlanan çikolata; sütlü, bitter ve beyaz olmak üzere üç ana kategoride incelenmekte, ayrıca sade, çeşnili ve dolgulu olmak üzere üç çeşitte üretilebilmektedir.
Çikolatanın; kardiyovasküler hastalıkları, kanseri ve yaşa bağlı sağlık sorunları gibi bazı kronik rahatsızlıklara iyi geldiği ve/veya engellediği çeşitli araştırmalar sonucu bildirilmiştir. Bu faydalarına karşın, çikolata ve şekerleme ürünlerinin yüksek kalori değeri, yüksek glisemik indeksi, diş sağlığı üzerindeki olumsuz etkileri, obezite ve çeşitli sistemik sağlık problemleri ile interaksiyon potansiyeli gibi sağlık sorunlarını tetiklemesi tüketicileri kaygılandırmaktadır. Lakin dolgulu ve çeşnili çeşitlerinin biyoaktif bileşen ilavesine uygun materyal olmaları bu kaygıları inhibe edecek fonksiyonel gıda niteliğe kavuşturacak yapıdadır.
Konvansiyonel üretimde kullanılan bileşen ve yöntemler dışında; çeşitli meyve, sebze ve baharat ekstraktlarının, posalarının ve çekirdeklerinin ilave edilmesi, kalori düzeyinin kısıtlanması, mono- ve di-sakkarit içeriklerinin modifiye edilmesi, doymuş yağ oranının azaltılması, probiyotik canlılar ve prebiyotik maddelerin kullanılması, çoklu doymamış yağ asitlerinin ilavesi, çeşitli mineral maddelerin düzeyinin geliştirilmesi ile biyoaktif bileşenlerce zengin bileşenler dolayısı ile de sağlık üzerindeki olumlu potansiyel etkileri arttırılmış çikolata geliştirme çalışmaları mevcuttur.
Bu çalışma, çikolatanın bu tür fonksiyonellik çalışmalarını derlemiştir. Bu çalışmadaki amaç ise çikolata üretici ve tüketicileri için bir rehber oluşturmanın yanında bu konuda çalışan araştırmacıların da istifade edecekleri bir literatür kaynak oluşturmaktır.

Anahtar Kelimeler: Çikolata, Fonksiyonel Gıda, Biyoaktif Bileşen

Functional Food Studies on Chocolate

Abstract

Consumer food expectations, in recent years, have moved beyond traditional demands such as meeting pleasure and essential nutrients due to factors such as health awareness, desire to have a quality life, increased life span, and increased treatment costs. The demand for functional/special foods have, therefore, increased which have an effect of protecting health, corrective and/or reducing the risk of disease, and have been proved scientifically and clinically, due to one or more effective components besides its nutritional effects that are defined according to the Turkish Food Codex.

Chocolate, which is obtained from cocoa products and sugars, with a total cocoa dry matter content of no less than 35%, containing at least 18% cocoa butter and at least 14% fat-free cocoa dry solids, is examined in three main categories: milk, bitter and white, and can be produced in three varieties: plain, flavoured and filled.
It has been reported by some studies that chocolate prevent and/or heals some chronic diseases such as cardiovascular diseases, cancer, and age-related health problems. Despite these benefits, chocolate and confectionery products worry the consumers due to their high calorific value, high glycaemic index, negative effects on dental health, obesity, and systemic health problems. However, stuffed and flavoured varieties are suitable materials for the addition of bioactive components, which will inhibit these concerns and provide functional food quality.
In addition to the components and methods used in conventional production; chocolate enhancement studies with the positive potential effects on health due to the components rich in bioactive ingredient exist by adding of various fruit, vegetable and spice extracts, pulp, and kernels, limiting the level of calories, modification of mono- and di-saccharide content, reduction of saturated fat ratio, use of probiotic living and prebiotic substances, the addition of polyunsaturated fatty acids, improvement of the level of various mineral substances,
This study reviews the works made on the functionality of chocolate. The purpose of this study is to create a guide for chocolate manufacturers and consumers. It also aims to create a literature resource for researchers working on this subject.

Keywords: Chocolate, Functional Food, Bioactive Ingredient

GİRİŞ

Dünya genelinde şekerli mamullerle beraber üretimi 11 milyon tonu geçen çikolata, rekabetin had safhada yaşandığı bir sektör durumundadır (Önder, 2016). Bu rekabet koşullarının yanında tüketici talep ve beklentilerinde meydana gelen değişimler, çikolata ve şekerleme üreticileri ile bu alanda çalışan diğer araştırmacıların, yenilikçi ürünler geliştirilmeye yönelik çalışmalarına ivme kazandırmakta, başta çocuklar olmak üzere her kesime hitap edecek cazip renk, şekil, fiyat ve fonksiyona sahip ürünler üretmeye yönlendirmektedir (Erdem, 2014).

Son yıllarda tüketicinin gıda beklentileri; sağlık bilinci, kaliteli yaşam sürme arzusu, yaşam süresinin uzaması ve hastalık tedavi ücretlerinin artması gibi faktörlerden dolayı haz ihtiyacı ve temel besin öğelerini karşılama gibi geleneksel taleplerin ötesine taşınmıştır (Sevilmiş vd., 2017). Bu nedenle Türk Gıda Kodeksine göre besleyici etkilerinin yanı sıra bir ya da daha fazla etkili bileşene bağlı olarak sağlığı koruyucu, düzeltici ve/veya hastalık riskini azaltıcı etkiye sahip olup, bu etkileri bilimsel ve klinik olarak ispatlanmış gıdalar şeklinde ifade edilen fonksiyonel/özel beyanlı gıdalara yönelik talep artış göstermektedir. Çikolata ve şekerleme ürünlerinde, yüksek mono- ve di-sakkarit içerikleri ile doymuş yağ düzeyleri nedeni ile fonksiyonel gıda çalışmaları için

- Yüksek kalori değeri
- Yüksek glisemik indeksi
- Diş sağlığı üzerindeki olumsuz etkileri
- Çeşitli sistemik sağlık problemleri ile potansiyel etkileşimleri
- Obezite

konularındaki tüketici kaygıları paradoks unsuru olmakla birlikte, çikolata tüketiminin;

- Kardiyovasküler hastalıklar,
- Kanser
- Yaşa bağlı sağlık sorunları gibi kronik rahatsızlıklarla ilgili riskleri

azalttığı ve/veya engellediği çeşitli araştırmalar sonucu bildirilmiştir (Beckett, 2009; Adamson vd., 1999; Lee vd., 2003; Cooper vd., 2008; Yiğit vd., 2018; Karğın ve Güneş, 2017). Ayrıca, konvansiyonel üretimde kullanılan bileşen ve yöntemler dışında;

- Çeşitli meyve, sebze ve baharat ekstraktlarının, posalarının ve çekirdeklerinin ilave edilmesi,
- Kalori düzeyinin kısıtlanması,
- Mono- ve di-sakkarit içeriklerinin modifiye edilmesi,
- Doymuş yağ oranının azaltılması,
- Probiyotik canlılar ve prebiyotik maddelerin kullanılması

- Çoklu doymamış yağ asitlerinin ilavesi
- Çeşitli mineral maddelerin düzeyinin geliştirilmesi

ile biyoaktif bileşenlerce zengin bileşenler dolayısı ile de sağlık üzerindeki olumlu potansiyel etkileri arttırılmış çikolata geliştirme çalışmaları da yürütülmektedir (Albak ve Tekin, 2014; Cerit vd., 2016; Muhammad vd., 2018; Oba vd., (2017); Konar vd., 2014a, 2014b, 2015, 2017, 2018; Toker vd., 2016, 2016a, 2017, 2018a; Maillard ve Landuyt, 2008). Gıdalara fonksiyonel özellik kazandıran bu çalışmaların başında; bitkilerden elde edilen fitokimyasal, hayvanlardan elde edilen zookimyasal diye adlandırılan biyoaktif bileşenlerle zenginleştirilmiş ürün üretimi çalışmaları gelmektedir (Jiménez -Kalmenero vd., 2001; Efraim vd., 2011).

Çikolata ürünlerinin fonksiyonelliği incelendiğinde konveksiyonel bileşenlerin fonksiyonelliği ve bu bileşenler dışındaki biyoktif bileşenlerin ilavesi, değişik kaygıları içeren konveksiyonel bileşenlerin ikamesi görülmektedir. Bu anlamda fonksiyonellik konveksiyonel bileşenler bu bileşenlerin ikamesi ve bu bileşenler dışındaki bileşenlerin ilavesi olarak ön plana çıkmaktadır.

ÇİKOLATA BİLEŞENLERİNİN FONKSİYONEL ÖZELLİKLERİ

Çikolata, kakao ürünleri ile şekerlerden elde edilen, en az %18 kakao yağı ve en az %14 yağsız kakao kuru maddesi içeren toplam kakao kuru maddesi içeriği en az %35 olan ürün şeklinde tanımlanan çikolata (Türk Gıda Kodeksi, 2017); sütlü bitter ve beyaz çikolatalar olmak üzere üç ana kategoride incelenmekte, ayrıca sade, çeşnili ve dolgulu olmak üzere üç çeşitte üretilmektedir (Palacıoğlu, 2003). Bu içeriği ile çeşitleri arasında farklılık olmakla birlikte kakao içeriğinden dolayı fonksiyonel niteliktedir. Kakao içeriğinde de Tablo.1 ‘de görüldüğü gibi azımsanmayacak miktarda bulunan fenolik bileşiklerin, biyoaktif bileşikler olarak antioksidan, antiradikal ve antikanserojenik özellikleri birçok çalışmada bildirilmiştir. Buna ilaveden kakao menşei, çikolata çeşitleri ve formülasyon farklılıkları bu kaynaklı biyoaktif bileşen miktarını etkilemektedir.

Tablo 1 Kakaonun kateşin ve prosiyadin içeriği (Gu, L. ve ark. 2006)

	catechins (mg/g)			**procyanidins (mg/g)**					
Ürün	catechin	epicatechin	Sum	monomers	2−3-mers	4−6-mers	7−10-mers	polymers	total
Naturel kakao tozu	0.61-0.90	1.58-2.58	2.36-3.48	3.54-4.49	7.09-8.89	7.36-10.94	4.40-7.45	9.80-16.85	32.19-48.70

Ham kakao çekirdekleri kakao ürünlerine işlenirken yüksek ısıl işlemlere maruz kalarak biyoaktif bileşenlerinde önemli değişimler gerçekleşir. (Güneş ve ark. 2018) derlemede kakao işleme proses koşullarına müdahale edilerek fenoliklerin korunmasına biyoaktif bileşen muhteviyatı üzerine temas edilebileceği belirlenmiştir. Ayrıca, eksikliklerinde insanda bazı hastalıklar görülen bazı minareller bakımından da Tablo 2 ‘da görüldüğü gibi zengindir.

Tablo 2 Naturel kakaonun mineral içeriği (Borchers vb 2000)

Mineral	**Miktar (mg)**
Kalsiyum	169.45
Bakır	4.61
Demir	13.86
Magnezyum	593.64
Manganez	4.73
Fosfor	795.27
Potasyum	2,058.20
Sodyum	8.99
Çinko	7.93

Çikolatanın önemli bileşeni olan kakao azımsanmayacak miktarda protein içerir. Kakaonun protein muhteviyatına bakıldığında fonksiyonellik atfedilecek düzeyde ve hemen hemen hepsini içerecek çeşitlilikte elzem aminoasit içerir. Ayrıca çikolatanın önemli protein kaynağı süt kaynaklı öğelerden gelir. Çikolataya %10-25 arası süttozu ve peyniraltı suyu tozu ilave edilir. Süt kaynaklı proteinler; kardiyovasküler hastalıklar, diyabet ve obezite gibi beslenme kaynaklı hastalıkların görülme riskinin engellenmesinde ve/veya önlenmesinde potansiyel fonksiyonel özelliği ile ilgi görmektedirler (Adeyeye vd, 2010).

Tablo 3 Amino asit profili (mg/g ham protein) fermentasyon öncesi ve sonrası kakao nibs (Adeyeye vd. 2010)

Amino asit	Fermantasyon öncesi kakao nibs'i	Fermantasyon sonrası kakao nibs'i
Lys*	42.0 ± 0.02	52.6 ± 0.02
His*	20.0 ± 0.00	23.3 ± 0.02
Arg*	43.6 ± 0.01	51.4 ± 0.20
Asp	100 ± 0.10	82.5 ± 0.11
Thr*	29.9 ± 0.03	23.3 ± 0.10
Ser	23.7 ± 0.01	32.6 ± 0.03
Glu	128 ± 0.20	153 ± 0.40
Pro	12.5 ± 0.02	12.5 ± 0.03
Gly	20.5 ± 0.01	32.0 ± 0.02
Ala	29.8 ± 0.20	40.1 ± 0.03
Cys	7.8 ± 0.01	6.9 ± 0.02
Val*	32.1 ± 0.10	35.1 ± 0.02
Met*	9.9 ± 0.01	8.0 ± 0.00
Ile*	21.4 ± 0.02	29.3 ± 0.20
Leu*	72.2 ± 0.30	62.4 ± 0.20
Tyr	18.6 ± 0.02	27.0 ± 0.01
Phe*	28.6 ± 0.01	36.3 ± 0.02
Try*	–a	–a
Ham protein (g/100 g)	13.6 ± 0.30	15.2 ± 0.21

* Elzem amino asit.
a belirlenememiştir

ÇİKOLATA ÜRÜNLERİNDE FONKSİYONEL GIDA ÇALIŞMALARI

Çeşitli meyve, sebze ve baharat ekstraktlarının, posalarının ve çekirdeklerinin ilave edilmesi,

Çikolata fonksiyellik çalışmalarda biyoaktif bileşenlerin yoğunlaştığı grup antioksidanlar olduğu görülmektedir. Serbest radikallerin neden olabileceği çeşitli kanser türleri, kalp damar hastalıkları ve yaşlanma gibi sağlık sorunlarının antioksidanca zengin gıdaların tüketimiyle geciktirilmesi veya önlenmesinin mümkün olacağı düşüncesi tüketiciler tarafından rağbet görmesi bu çalışmaları zorunlu kılmıştır (Yiğit vd, 2018). Ayrıca antioksidan içeren gıdaların içermeyenlere oranla daha uzun raf ömrüne sahip olması (Bölükbaşı ve Erhan, 2006) bildirilmektedir. Piyasada acı biberli çikolatanın raflarda olduğu görülmektedir. Bunun dışında antioksidant kaynağı olarak; polen, meyveler, sebze ve baharat bazlı katkıların ilave edildiği görülmektedir (Özgen, 2010; Komes vd., 2013; Muhammad vd., 2018; Wang vd., 1996; Cerit vd., 2016). Ayrıca Fitosteroidler ve fenolik içeriği yüksek çeşitli bitkisel ekstraktların direkt ve enkapsüle ilavesi ile zenginleştirilme imkanlarının olduğu

belirtilmiştir (Belscak-Cvitanovic vd., 2015a; Botelho vd. 2014; Lončarević, vd., 2018). Ayrıca antioksidant kaynakların *in vitro* koşullarda biyoerişebilirlikleri de incelenmiştir (Cervellati vd. 2008).

Kalori düzeyinin kısıtlanması, Mono- ve di-sakkarit içeriklerinin modifiye edilmesi,

Son yıllarda insanların yaşam tarzının daha düşük aktiviteyi gerektiren bir yönelime evirilmesi obesite ve obesiteye bağlı kardiyovasküler, diyabet gibi hastalıklarla mücadele kapsamında enerji değeri düşük ve/veya enerji içermeyen ikame ve dolgu gıda bileşenlerine yönlendirmiştir. Çikolata; yüksek şeker, yağ ve protein içeriği ile enerji değeri yüksek gıdalar içerinde yer almaktadır. Çikolatayı yüksek sakaroz içeriği diyabet hastaları içinde uygun bir materyal olmamaktadır. Bu gibi uygunsuzluklar gidermek için çikolata içeriğindeki sakarozun besleyici değeri olan veya olmayan tatlandırıcılar ile kısmi ikamesini mümkün olmaktadır (Aidoo vd., 2013) Düşük higroskopik özelliğinden maltitol sakaroz ile yapılan çikolata üretimine çikolatanın kendine özgü tat ve aroması geliştirilmektedir (Olinger, 1994; Sökmen ve Güneş, 2006). Ksilitol serinletici etkisinden tüketicilerin daha az tercih ettikleri bildirilmiştir (Kato ve Moskowitz, 2001; Olinger ve Pepper, 2001; Wijers ve Strater, 2001). (Özat, 2018) Yulaf, arpa ve ekmek mayası (*Saccharomyces cerevisiae*) olarak 3 farklı kaynaktan elde edilmiş β-glukan konsantratlarının sütlü, bitter, beyaz çikolata çeşitleri ve sürülebilir kakaolu fındık kreması bileşiminde %5, %10 ve %15 oranlarında şeker ikamesi olarak kullanarak fonksiyonel ürün geliştirme çalışmaları yürütmüştür.

Ayrıca prebiyotik gıda niteliğinde olan oligosakkaritlerin sakkaroz ikamesi ve dolgu maddesi olarak kullanılması kalori değerini düşürmekte ve karbonhidrat içeriğini modifiye edilebilmektedir (Aidoo vd., 2015; Nebesny vd., 2007; Azevedo vd., 2017).

Doymuş yağ oranının azaltılması ve çoklu doymamış yağ asitlerinin ilavesi,

Yağlar içerdikleri yağ asit çeşidi göre beslenme ve sağlık üzerinde önemli etkileri vardır. İnsan sağlığında lipitler; yağ asidi kimyasal yapısı, miktarı, omega yağ asitleri, trans-cis formları ve konjuge linoleik asit gibi muhteviyat önem taşımaktadır. Konveksiyonel çikolata ve kokolinin ortalama yağ asit kompozisyonu mevcuttur. Fonksiyonel çikolata üretiminde doymamış yağ asitleri EPA (eikosapentaenoik asit) ve DHA (dokosaheksaenoik asit) gibi insan beslenmesinde önemli olan omega-3 yağ asitleri (Toker vd., 2018a) ilavesi ve palm ve kakao yağlarının tereyağ, zeytin yağ ve benzeri bitkisel yağların ikamesi şeklinde öne çıkmaktadır (Yıldırım, 2017; Shıehzadeh, 2019).

Probiyotik ve Prebiyotiklerin kullanımı,

Bağırsak mikrobiyal florasını düzenleyen canlı mikrooganizmalar olarak tanımlanan bakteriyel ve viral ishaller ile atopik hastalıklardan enflamatuar barsak hastaklıklarına kadar birçok gastrointesinal sistem hastalığının tedavisi veya korumada etkili olmaktadırlar. Probiyotikler ise kolon bakterilerinin sayı ve aktivitelerinin probiyotiklerin etkisinin artıran, sindirilmeyen karbonhidratlar olarak tanımlanarak immün sistemi uyarıcı ve kolonda karsinogenezisi inhibe edici etkileri belirlenmiştir (İnanç vd., 2005). Bu etkileri ile gıdaya probiyotik canlı organizma ve prebiyotik katkı ilaveleri ile gıdayı fonksiyonelleştirme çalışmaları hız kazanmıştır.

Çikolata, probiyotikleiriçin iyi bir taşıyıcıdır Maillard ve Landuyt (2008) nitekim probiyotikler ve prebiyotiklerin direkt ve enkapsüle olarak kullanımı imkanlara sahiptir (Konar vd., 2016a; Erdem vd. 2011), Silva vd. (2017) tarafından probiyotiklerin in vitro stimule edilmiş sindirim sistemi koşullarındaki canlılıkları da araştırılmış ve kayda değer canlılık tespit edilmiştir. Probiyotik inokülasyonu çikolata materyalide duyusal ve teknolojik özellikler üzerinde etkisi incelenmiş ve kabul edilebilir bir ürün elde edilebileceği belirtilmiştir (Laličić-Petronijević vd. 2014). Ayrıca çikolataya probiyotiklerin farklı bileşenler vasıtasıyla ilave edildiği çalışmalar ile alternatif kullanım önerileri geliştirilmiştir Chetana vd. (2013).

Çikolataya prebiyotik, özellik kazandırmak amacıyla yapılan çalışmalar da mevcuttur. Prebiyotik özellik gösteren karbonhidratların çoğunluğu probiyotikleri beslediği gibi enerji değeri düşük ve glisemik indeksi düşük

karbonhidratlar olmakla ekstra fonksiyonelliği ile önem kazanmaktadır. Çikolataya, polidekstroz ve maltitol karışımı, maltitol ve dirençli nişasta karışımı ve maltitol katkılarının kullanımı denenmiştir Beards vd. (2010).

Protein ve aminoasit kullanımı,

Çikolatada mevzuata göre en az %14 oranında kakao tozu bulunması gerekmektedir. Kakao tozu ile aynı muhteviyata sahip kakao nibs'in amino asit ve ham protein içeriği Tablo 3' de verilmiştir. Tablo 3' te görüldüğü gibi kakao elzem amino asit içeriği bakımında fonksiyonel denecek miktarda içeriğe sahip üründür.

Çikolatada formülasyonuna göre %10-25 oranında süttozu ve peynir altı suyu tozu mevcuttur. Süt kaynaklı proteinlerin peptitleri opioid reseptörlere bağlanma, angiotensin Idönüştürücü enzimin (ACE) inhibisyonu, antimikrobiyal, antihipertansiyon, antioksidatif, antitrombotik etki, immün sistemin düzenlenmesi ve mineral bağlayıcılık gibi farklı biyokimyasal ve fizyolojik etkilere sahiptirler (Kınık ve Gürsoy, 2002).

Deney hayvanları üzerinde yapılan in vivo çalışmalar peynir altı suyu proteinlerinin kolon kanserini geciktirdiği, belirli miktarda peynir altı suyu proteinini tüketen deney hayvanların tüketmeyen diğer deney hayvanlara kıyasla kolon kanserinin geciktirilmesinde etkili olduğu bulunmuştur (McIntosha, 1998).

Çeşitli mineral ve vitamin maddelerin düzeyinin geliştirilmesi,

Yeterli enerji alan bireylerde dahi gizli açlık diye isimlendirilen vitamin, mineral ve başka öğelerinin eksikliği görülebilmektedir. Genellikle belli grup ve yörelerde belli başlı vitamin ve mineral eksikliği görülmesi dikkat çekmektedir. Spesifik grup ve yöreler için beslenme açısından ihtiyaç duydukları vitamin ve minerallerin ilavesi, işleme esnasında korunması ve söz konusu öğelerin zenginleştirilmesi fonksiyonel gıda çalışmaları kapsamaktadır. Çikolata çeşitleri vitamin ve mineral zenginleştirme konusunda farklı birçok öğe içermesi açısından elverişli bir materyaldir. E vitaminlerin sütlü çikolata ilavesi ile biyoaktif özellikleri artırdığı belirlenmiştir (Marsanasco, 2015).

Tablo 2' de görüldüğü gibi kakao tozu zengin mineral içeriğine sahiptir. Çikolata formülasyonunda önemli bir bileşen olan süt tozu ve peyniraltı tozu miktarsal olarak önemli mineral kaynağıdır. Peyniraltısuyu tozu ve süttozu muhteviyatı reformülizasyonu fonksiyonel çikolata kapsamında değerlendirilebilmektedir. Ayrıca bitkisel kaynaklı öğelerin ilavesi vitamin ve mineral zenginleştirmeye katkısı incelenmelidir.

Değişik biyoaktif bileşenlerin ilavesi,

Çikolata çeşitlerindeki fonksiyonel ürün çalışmaları bu başlıklardan ibaret değildir. Ayrıca kandaki kolesterol miktarını düşürerek kardiyovasküler hastalık ve antikanserojen özelliği ile de bazı kanserlerin riskini azaltma nitelikleri ile biyoaktif bileşen olarak bilinen fitostreoidlerin çikolata materyaline katılması söz konusudur (Tetik, 207; Botelho vd., 2014).

SONUÇ

Çikolata geniş çeşitlilikte bileşen içermektedir. Kakao, kakao yağı, kakao kitlesi, süt tozu ve peyniraltı suyu tozu gibi mineral, vitamin, fenolik, ve yağ asidi içeriği ile fonksiyonel ürün denilecek düzeyde biyoaktif bileşenler içermektedir. Ticari kaygıların geri planda bırakılarak daha yüksek fonksiyonelliğe sahip yeni formülasyonlarla elde edilmekte edilebilmektedir. Ayrıca çikolata prosesindeki modifikasyon ile biyoaktif bileşenler daha fazla koruna bilmektedir. Ayrıca çikolata yoğun aroması ve koyu renginden dolayı harici biyoaktif bileşenlerin ilavesini tat, lezzet ve renk değişimlerini tolere etmektedir. Bu açıdan çikolata çeşitleri; bitkisel, hayvansal ve mikrobiyal ilavelere uygun bir materyaldir. Nitekim, fonksiyonel çikolata çalışmaları ile antioksidant, vitamin, mineral ve fitosteroid zenginleştirilmiş, probiyotik ve/veya prebiyotik özellik kazandırılmış, besin değeri düşürülmüş, lifçe zenginleştirilmiş, karbonhidrat ve yağ kompozisyonları modifiye edilmiş ürünler geliştirilmiştir.

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➢ *ORAL PRESENTATION*

Determination of the stability of antioxidant and total phenolic compounds in cream cheeses produced with black carrot, blackberry and beetroot juice concentrates

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Abstract

There is growing interest on natural antioxidant resources, due to the toxic and carcinogenic effects of synthetic antioxidants. Phenolic compounds originating naturally from fruits and vegetables show high antioxidant effects. For food industry, these fruits and vegetables are more available as in concentrated forms. The objective of this study was to investigate the usage of vegetable concentrates with high antioxidant and phenolic compound capacities (black carrot, blackberry and beetroot) in cream cheese, to provide functional properties. Concentrates were added in 5% and 10% dilutions. Cream cheese samples were stored in transparent polystyrene packages for 2 weeks at the conditions of 4°C, 25°C and 25°C under fluorescent lamb. Total antioxidant capacities and total phenolic contents were analysed in days 0., 7. and 14. Results showed that, storage time and temperature are factors for total antioxidant and phenolic compound decrease. However, fluorescent light is not a source that affects data. Cream cheeses with black carrot, blackberry and beetroot concentrates can be alternative ingredients to fruity cream cheeses for their valuable functional products.

Keywords: black carrot, blackberry, beetroot, antioxidant capacity, total phenolic content, cream cheese

INTRODUCTION

Cream Cheese is a soft, spreadable, unripened and rindless cheese, and mixes readily with other foods (Anonymous, 2007). It can be seasoned with vegetables such as onions and peppers, as well as aromatized with meat products, spices, fruits and can adapt to any type of food. Cream cheese is a good daily intake source in terms of some minerals (especially calcium and magnesium) and vitamins (especially vitamin D and A).

Phenolic compounds such as phenolic acids, anthocyanins, flavanols, and flavan-3-ol are of great interest for their functional properties such as antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial effects (Friedman, 2007). Anthocyanins, well-known natural alternatives to the synthetic dyes, are the pigments of many flowers, fruits, and vegetables responsible for their red, blue, and purple colours (Gizir et al., 2008). The growing interest of consumers to nutrition has increased the demand for natural colorants. Black carrot is known to contain high amounts of anthocyanins and other polyphenolics (Kammerer et al., 2004; Kırca et al., 2007). Blackberry contain antioxidants and phenolic acids, especially anthocyanins which are also responsible for the colour of the fruit (Koca and Karadeniz, 2009). Beetroot also exhibits high levels of antioxidant activity. It inhibits lipid peroxidation and prevents the rise of total cholesterol, inhibits heme decomposition, participates in the scavenging of free radicals and by the way prevents cancers and cardiovascular diseases (Kanner et al., 2001; Netzel et al., 2005; Wroblewska et al., 2011). Despite these positive effects of phenolics, anthocyanins and antioxidants exposure to environmental changes such as light, humidity and temperature may lead to their degradation of its. Many studies have emphasized that storage factors such as light and storage temperature affect the stability of the phenolic compounds and antioxidant activity (Kotsiou and Tasioula-Margari 2016; Jiménez-Zamora et al., 2016; Zoric et al. 2017).

Coloured products especially for children are widely available on the shelves. The use of natural colorants in foods has increased as a result of the obligation within the scope of the revisions on the Turkish Food Labelling Regulation of a warning addition on the food label which is "may cause hyperactivity in children" when artificial colorants are used. The aim of the study was to examine the 2-week shelf life of cream cheeses produced with black carrot, blackberry and beetroot juice concentrates, which are rich in antioxidant and phenolic substances, in

a transparent food package. The shelf-life analysis focused on antioxidant capacity and total phenolic content of the cream cheeses.

MATERIALS and METHODS

Production of cream cheeses

The unsalted Turkish curd cheese (lor) and milk cream (45% milk fat) was supplied from dairy plant Murat Süt ve Süt Ürünleri (Sakarya). Lechitin and carrageenan were purchased from Smart Kimya (İzmir). Sugar and UHT milk were supplied from local markets in Sakarya. The black carrot and blackberry juice concentrates were supplied from the company Erkon Konsantre (Konya), and beetroot from Aroma (Bursa). The formulations of the cream cheeses are given in Table 1.

Table 1. The formulations in grams of cream cheese samples

	Control (C)	BC5	BC10	BB5	BB10	BR5	BR10
Lor	56.1	56.1	56.1	56.1	56.1	56.1	56.1
Cream	18.7	18.7	18.7	18.7	18.7	18.7	18.7
Milk	24.3	24.3	24.3	24.3	24.3	24.3	24.3
Lechitin	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Carrageenan	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Sugar	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Black carrot juice concentrate	-	5.0	10.0	-	-	-	-
Blackberry juice concentrate	-	-	-	5.0	10.0	-	-
Beetroot juice concentrate	-	-	-	-	-	5.0	10.0

The ingredients were mixed and pasteurized at 70°C for 4 minutes. Then stored at 4°C, 25°C and 25°C under fluorescent lamb until the analyses. The sensorial analysis, pH and colour values were measured on the first day of production, antioxidant activity and total phenolic contents were analysed once a week during two weeks of storage. Sensorial analysis was carried out by 8 panellists according to hedonic scale of 1-9 points. pH was measured by Bante 220 at 25°C and colour was analysed by a tintometer Lovibond RT300 in terms of *L*, *a*, *b* values.

Preparation of aqueous sample extracts for antioxidant activity and total phenolic content

Extracts were prepared according to the literature procedure, with a few modifications of Wojdyło et al. (2007). Samples (8 g) were weighed into a test tube. A total of 10 ml of 70% aqueous methanol was added, and homogenized with IKA T18 homogenizer. Tubes were sonicated twice for 15 min at room temperature (20°C) The extract was centrifuged for 10 min (8000 rpm 4°C), and supernatants were used for total phenolic content and antioxidant activity.

In preliminary tests, the phenolic and antioxidant contents exceeded the upper measurement limits. The extracts were diluted by volume of 1:10 in 70% methanol solution, and these diluted extracts were then used in the further measurements.

Determination of total phenolic content

Total phenolic content was measured using Folin–Ciocalteu colorimetric method described previously (Singleton and Rossi, 1965). Sample extracts prepared for total phenolic content (100 µl) were mixed with 0.2 ml of Folin–Ciocalteu reagent and 2 ml of H_2O, and incubated at room temperature for 3 min. Following the addition of 1 ml of 20% sodium carbonate to the mixture, total polyphenols were determined after 1 h of incubation at room

temperature. The absorbance of the resulting blue colour was measured at 765 nm with a spectrophotometer (Shimadzu UV–1240). Quantification was done with respect to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), mg per 100 g of dry weight (dw). All determinations were performed in triplicate.

Determination of DPPH free radical scavenging

The DPPH radical-scavenging activity was determined using the method described previously (Brand-Williams et al., 2005). DPPH (5 µg) was dissolved in 70% methanol (250 ml). The radical stock solution was prepared fresh daily. The DPPH solution (3 ml) was added to 200 µl of phenolic extracts. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 minutes and the resulting colour was measured spectrophotometrically at 517 nm against blanks. Methanol was used for blank measurement. The amount of DPPH discoloration was given as in Trolox equivalents (TEAC), in mg per 100 g of dry weight. All determinations were performed in triplicate.

Statistical analysis

The data of total phenolic content and antioxidant capacity were analysed statistically with SPSS 20.0 software using one-way ANOVA according to Tukey's test at 95% confidence level.

RESULTS and DISCUSSION

Sensorial results

The sensorial scores of cream cheese samples were given in Table 2. According to the results, blackberry gave an undesirable colour to cream cheese, when compared to control sample. Textures were generally improved by concentrate addition. Flavour of cream cheeses also developed by juice concentrate addition. Especially the blackberry added sample was very desirable, but also unfavourable foreign taste was noticed in that sample. Comparison of the general acceptability of the samples revealed that increasing amount of blackberry juice concentrate was undesired in cream cheese samples, however increasing amounts of beetroot juice concentrate was scored higher than control sample.

Table 2. Sensorial scores of cream cheese samples

	Colour	Texture	Flavour	Unfavourable flavour	General acceptability
C	6.6 ± 1.5	6.6 ± 0.9	4.6 ± 0.9	5.0 ± 0.9	5.3 ± 1.0
BC5	7.1 ± 0.8	7.4 ± 1.2	4.8 ± 1.9	5.9 ± 1.6	5.6 ± 1.4
BC10	7.6 ± 1.0	7.3 ± 1.0	4.6 ± 2.3	5.5 ± 1.9	5.6 ± 1.9
BB5	6.1 ± 1.4	7.0 ± 1.5	5.0 ± 1.5	5.4 ± 1.0	5.2 ± 1.6
BB10	5.4 ± 1.3	6.4 ± 1.1	4.1 ± 1.5	4.6 ± 1.7	4.3 ± 1.3
BR5	7.6 ± 1.2	7.3 ± 0.9	6.0 ± 2.1	6.2 ± 1.8	6.4 ± 1.7
BR10	7.7 ± 0.9	7.4 ± 1.1	6.4 ± 1.7	6.1 ± 2.2	6.7 ± 1.7

BC: Black carrot, BB: Blackberry, BR: Beetroot

pH and colour properties of the cream cheeses

The pH and the colour properties of samples are given in Table 3. pH decreased with juice concentrate addition. Beetroot and black carrot increased *a* value while decreasing *L* and *b* values. Blackberry colour values were more similar to the control sample.

Table 3. pH and colour properties of cream cheese samples

	pH	*L*	*a*	*b*
C	5.20 ± 0.02	77.7 ± 0.6	1.3 ± 0.0	9.3 ± 0.1
BC5	5.05 ± 0.01	48.0 ± 0.0	20.0 ± 0.0	-5.3 ± 0.0
BC10	4.88 ± 0.00	37.3 ± 0.3	24.2 ± 0.2	-4.8 ± 0.0
BB5	5.03 ± 0.01	73.6 ± 0.0	2.4 ± 0.0	6.70 ± 0.0
BB10	4.91 ± 0.01	70.7 ± 0.1	3.3 ± 0.0	5.5 ± 0.0
BR5	4.86 ± 0.01	46.5 ± 0.0	37.4 ± 0.1	1.6 ± 0.1
BR10	4.74 ± 0.00	38.7 ± 0.4	41.3 ± 0.8	6.4 ± 0.1

BC: Black carrot, BB: Blackberry, BR: Beetroot

Total phenolic content

The total phenolic content was expected to decrease with temperature increase and probably with light absorption. The natural colouring materials are known to be sensitive to UV-light. However, in this study, the phenolic and antioxidant contents were measured under market light (fluorescent lamb) to simulate real shelf conditions. The total phenolic content results are given in Table 4.

Table 4. The total phenolic contents (mg GAE / 100 g dw) of cream cheese samples during 14 days.

	Day 0	Day 7 at 4°C	Day 7 at 25°C	Day 7 at 25°C under fluorescent	Day 14 at 4°C	Day 14 at 25°C	Day 14 at 25°C under fluorescent
C	-1.05	2,34	2.49	-0.45	-0.45	-1.65	2.08
	± 1.69	± 2.69	± 1.33	± 0.63	± 2.33	± 1.63	± 2.13
	A d	A d	A d	A e	A c	A d	A d
BC5	87.64	92.35	72.95	66.14	97.64	120.79	83.86
	± 10.42	± 1.76	± 8.87	± 4.24	± 6.29	± 11.17	± 10.42
	AB b	AB b	B b	B b	AB b	A a	AB ab
BC10	159.92	167.37	150.98	142.05	165.66	140.37	92.30
	± 18.95	± 8.30	± 8.43	± 1.28	± 17.96	± 10.91	± 5.50
	A a	A a	AB a	AB a	A a	AB a	B a
BB5	6.91	20.27	15.92	11.13	18.76	11.82	10.23
	± 3.84	± 6.44	± 0.14	± 1.48	± 1.45	± 3.03	± 3.40
	A d	A cd	A cd	A de	A c	A cd	A d
BB10	15.77	30.36	14.57	15.42	24.13	12.71	16.52
	± 4.48	± 0.37	± 4.50	± 1.61	± 4.06	± 11.13	± 9.98
	A cd	A cd	A cd	A d	A c	A cd	A cd
BR5	30.27	47.90	45.17	41.77	86.99	73.65	50.89
	± 1.45	± 6.98	± 4.65	± 4.17	± 2.76	± 7.88	± 8.10
	C cd	BC c	BC bc	C c	A b	AB b	BC bc
BR10	63.74	85.73	68.35	67.25	100.52	47.24	67.70
	± 6.12	± 8.27	± 4.50	± 2.80	± 11.86	± 5.48	± 2.67
	AB bc	A b	AB b	AB b	A b	B bc	AB ab

BC: Black carrot, BB: Blackberry, BR: Beetroot

A, B and C capitalized referred the differences between storage conditions of the same sample at $P<0.05$ level.
a, b, c and d lowercase referred the differences between samples stored in the same conditions at $P<0.05$ level.

The control sample's phenolic content was below the detection limits. Juice concentrate added samples has significantly higher phenolic content compared to the control. The total phenolic content of black carrot juice added cream cheese was significantly higher than blackberry and beetroot juice added cream cheeses. On the first week, storage temperature did not affect the total phenolic content, however on the second week, storage temperature caused a general decrease in phenolic content of cream cheeses. Fluorescent use did not affect the total phenolic content during the two weeks of storage. A further study can be made for longer shelf life foods other than cream cheese. In Figure 1, the total phenolic contents are illustrated to make a better comparison.

Wootton-Beard and Ryan (2011) revealed that 100 ml of beetroot juice contain 97.7 mg GAE total phenolic compound and the value increased 5-fold following gastrointestinal system. Suzme et al. (2014) declared a 70% decrease in total phenolic compound and 67% decrease in antioxidant capacity of black carrot juice as a result of concentrate processing. Results of Wang and Xu (2007) showed that, the anthocyanin loses of blackberry juice stored at 5°C for 50 days was measured within 4-5 days at 25°C. However, our values were more stable. Cream cheese can be a protective carrier which needs further analyses.

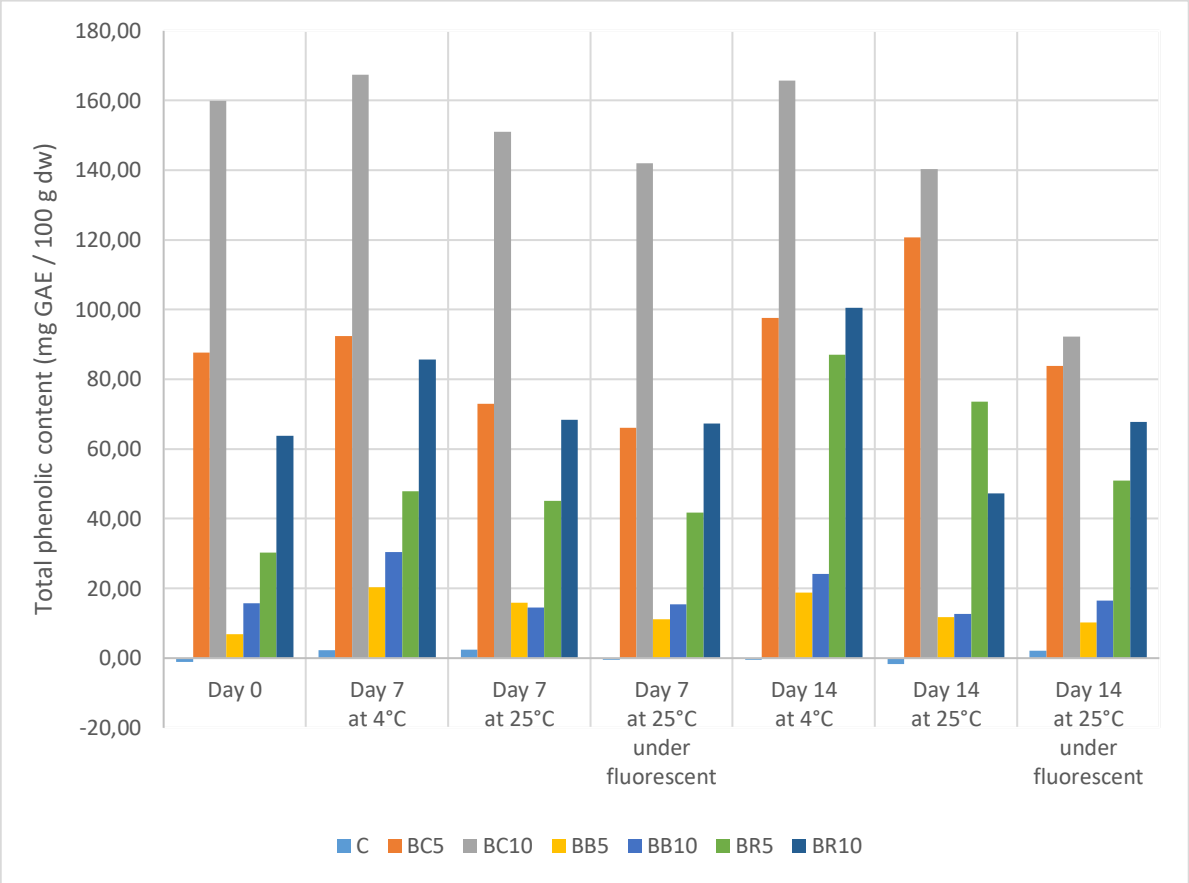

Figure 1. The total phenolic contents (mg GAE / 100 g dw) of cream cheese samples during 14 days.

Total antioxidant capacity

The total antioxidant capacity was measured according to the method of DPPH scavenging capacity and results evaluated in terms of TEAC are given in Table 5. Black carrot and beetroot juice concentrates added cream cheeses revealed significant changes during the storage period. However, in general of the samples, fluorescent lamb did not affect the antioxidant measurements, as in the total phenolic content results. Blackberry added cream cheese samples BB5 and BB10 had antioxidant capacities of 12 and 20 mg TEAC / 100 g dry matter, respectively. The control sample did not contain antioxidant. However, statistical analysis showed that blackberry juice concentrate added cream cheese samples had similar results with the control sample and had no antioxidant enough to declare.

CONCLUSION

This study is a research emphasizing the importance of using natural colorants in meeting today's health concept. Contrary to the discovered damages of artificial colorants, it has been revealed that the natural colorants we use within the scope of the study can make foods such as cream cheese, which is an important nutritional source for children, more enjoyable, and can increase the antioxidant and phenolic substance contents, as well. In addition, it has been shown that these effects can be protected for a long time without being affected by fluorescent market shelf light even in transparent packaging. According to the shelf stability test, all the three natural colouring sources can be used in cream cheese, however as a result of this study the use of black carrot concentrate rather than blackberry will be a good choice considering the health benefits.

Table 5. The total antioxidant capacity (mg TEAC / 100 g dw) of cream cheese samples during 14 days.

	Day 0	Day 7 at 4°C	Day 7 at 25°C	Day 7 at 25°C under fluorescent	Day 14 at 4°C	Day 14 at 25°C	Day 14 at 25°C under fluorescent
C	-2.68	-1.31	0.16	-1.40	-6.55	-3.23	0.30
	± 2.65	± 1.12	± 0.87	± 2.05	± 5.96	± 3.11	± 2.25
	A d	A g	A e	A e	A e	A e	A d
BC5	62.76	128.40	117.35	114.04	108.46	88.77	99.03
	± 5.35	± 1.50	± 3.00	± 2.52	± 3.36	± 2.84	± 2.84
	E b	A b	AB a	ABC a	BC b	D c	CD b
BC10	105.72	144.81	117.87	116.75	244.25	224.82	225.71
	± 3.33	± 1.20	± 3.78	± 4.74	± 5.40	± 2.30	± 3.70
	C a	B a	C a	C a	A a	A a	A a
BB5	12.12	10.76	6.66	10.22	13.61	10.01	5.99
	± 2.86	± 3.17	± 2.25	± 2.14	± 2.51	± 2.77	± 3.25
	A d	A f	A e	A de	A de	A e	A d
BB10	20.07	28.12	23.42	15.87	19.35	16.46	10.27
	± 2.34	± 0.13	± 0.23	± 3.41	± 6.63	± 6.78	± 0.82
	A cd	A e	A d	A d	A d	A e	A d
BR5	30.84	57.77	40.11	35.45	74.75	60.03	53.04
	± 2.63	± 1.46	± 4.45	± 1.27	± 3.37	± 2.22	± 1.26
	D c	B d	CD c	D c	A c	AB d	BC c
BR10	58.72	115.62	84.04	78.65	111.30	120.11	102.49
	± 1.46	± 2.15	± 1.24	± 3.67	± 2.45	± 2.65	± 4.33
	D b	AB c	C b	C b	AB b	A b	B b

The results of antioxidant capacity analyses of the samples given in Table 5 are illustrated in Figure 2 during the two weeks of storage.

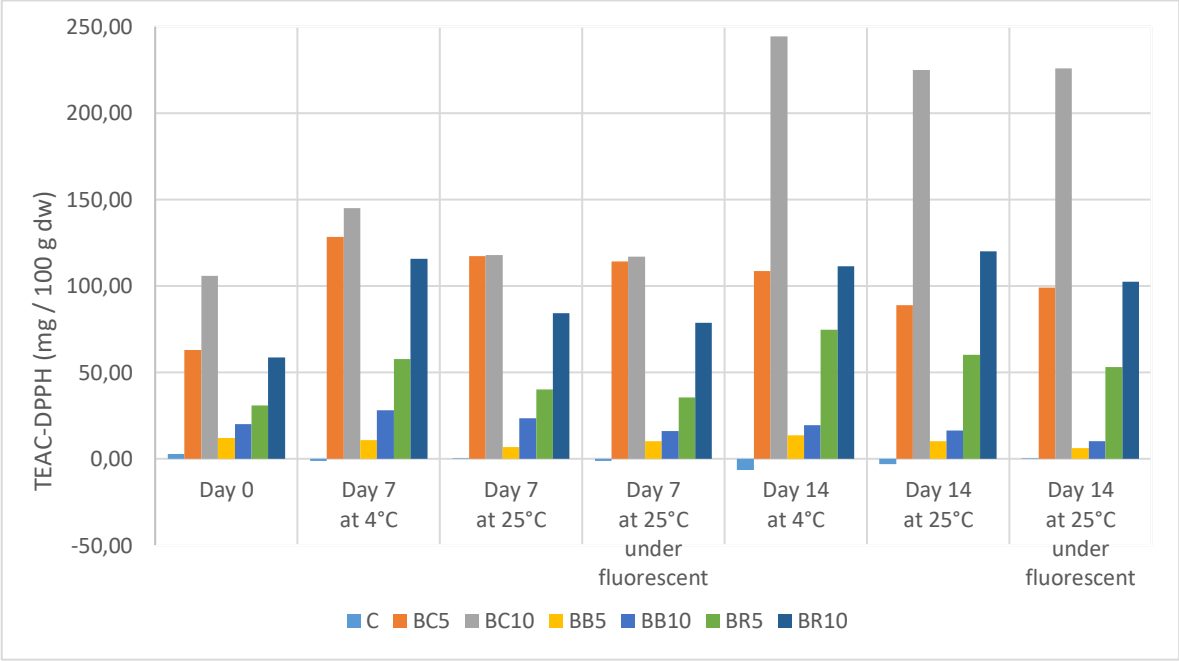

Figure 2. The total antioxidant capacity (mg TEAC / 100 g dw) of cream cheese samples during 14 days.

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➢ *ORAL PRESENTATION*

Investigation of chemical and microbiological quality of traditional tarhanas produced in Bilecik

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Abstract
In this study, some microbiological analysis (total aerobic mesophilic bacteria, mold and yeasts, lactic acid bacteria, *Enterobacteriaceae*, coliform bacteria, *Staphylococcus aureus* and *Salmonella-Shigella* and some chemical analysis pH, acidity (passed from 67 ml/100 ml ethyl alcohol, %), ash (%), dry matter (%), fat (%), salt (%) of a total 58 tarhana samples prepared traditionally at homes in Bilecik city were determined. TS 2282 Tarhana Standard is used as reference. Total aerobic mesophilic bacteria, mold and yeasts, coliform bacteria and lactic acid bacteria contents of tarhana; were 2.00-6.89 log cfu/g, <1.00-5.70 log cfu/g, <1.00-6.54 log cfu/g, 2.08-7.11 log cfu/g respectively and *Staphylococcus aureus* and *Salmonella-Shigella* was not detected in none of the samples. According to the findings of this study; 6 (10.34 %) samples were not appropriate in terms of coliform bacteria and 8 (13.79 %) samples in terms of mold and yeasts according to Tarhana Standard of Turkish Standards Institution. The ratios of pH, acidity (%), ash (%), dry matter (%), fat (%) and salt (%) of these tarhana samples were found in the range of 3.80–5.32, 7.25-17.50 %, 1.15-10.03 %, 82.86-97.67 %, 0.35-8.14 %, 2.55-9.01 % respectively. The result of the obtained findings showed that 14 of the samples (24.14 %) and 11 of the samples (18.97 %) were not in accordance with TS2282 Tarhana standard of Turkish Standards Institute in terms of acidity and dry matter, respectively.
Keywords: Chemical properties, Microbiological properties, Tarhana

1. INTRODUCTION

Fermentation is one of the oldest and the most economical method known in food production and preservation (Caplice and Fitzgerald, 1999; Ross et al., 2002). Since the fermented products produced since ancient times are consumed reliably, they have an important place in the nutrition of humans. Considering that metabolites that prevent and kill most of the pathogenic and food spoilage microorganisms are formed as a result of fermentation, these kinds of foods can be stated as safe foods. Fermented foods, leading to consumption one of the reasons is its reliability as well as the contribution of various nutritional qualities and flavors (Demir, 2018; Karahan et al., 2019). Tarhana, which was first produced by Turks living in Central Asia, is a Turkey specific cereal based fermented product (Siyamoglu, 1961). Tarhana, whose raw materials are flour and yoghurt, can be added regionally to different grains and their derivatives. As a result of the addition of these raw materials, it is a source of protein and vitamins, as well as balanced each other by mainly amino acids. Its factor is important in your diet because it contains various minerals. Tarhana, a food suitable for diversification, is consumed as chips in its dry form or as a soup after diluting, boiling the dry powder (Ertop and Atasoy, 2019). Tarhana is prepared by mixing wheat flour, yoghurt, cooked various vegetables (tomatoes, onions, peppers, etc.) and spicy mixtures followed by lactic acid bacteria and yeast fermentation for one to seven days. After fermentation, the mixture is dried in the sun and usually preserved after being ground to powder. Tarhana is prepared by this process during the end of summer and consumed as a sop in winter. It is high nutritional value food with protein, vitamins and minerals amounts it contains (Campbell-Platt,1994; Daglioglu, 2000; Ekinci,2005; Erbas et al., 2005; Ibanoglu et al., 1995; Pirkul,1988) According to Turkish standards Tarhana is defined as a food substance obtained by first mixing wheat flour, semolina or the mixture of these products, yogurt, pepper, salt, onions, tomatoes and flavoring, aromatizes and some harmless herbs and by kneading these products, then by fermentation, drying, grinding and sieving (Anonymous, 1981). Methods for preparation of tarhana and the compounds may vary from one place to another and some tarhana types with different properties are prepared (Flour, Göce, İrmik and mixed tarhana) (Anonymous, 1981; Ozbilgin, 1983; Siyamoglu, 1961; Temiz and Pirkul, 1990; Temiz and Pirkul, 1991; Yucecan et al., 1988).

Many researchers have been studied on tarhana (Bilgicli, 2009; Bilgicli and Türker, 2004; Bilgicli et al.,2005; Erbas et al., 2005; Funda, 2009; Gökmen, 2009; Ibanoglu et al., 1999; Siyamoglu, 1961; Tamer et al., 2007; Temiz and Pirkul, 1991; Yörükoğlu and Dayısoylu, 2016; Yucecan et al., 1988).The conducted studies showed that tarhana is not prepared in our country with the same quality and standard. However, production errors and preservation conditions can cause some problems in tarhana. Standard and quality production depends on the quality of the raw materials used in production, production technology; the hygienic conditions applied in production, proper packaging and storage conditions. This study has been conducted in order to determine the chemical and microbiological quality of tarhana samples prepared at home made available for consumption and put on the market in Bilecik city.

2. MATERIALS AND METHODS

Total 58 tarhana samples prepared traditionally at homes in Bilecik city were chosen by random sampling method without interfering with sales procedure and packaging material, brought to the laboratory under cold chain and kept under storage condition (4 ^{o}C) until the analyses were completed.

2. 1. MICROBIOLOGICAL ANALYSIS

Plate count agar (PCA, Merck) was used in total mesophilic aerobic count of tarhana samples and tarhana was incubated at 37±1 ^{o}C for 3 days. Man Ragosa Sharpe Agar (MRS, Merck) was used in lactic acid bacteria count and it was incubated at 35±1 ^{o}C under anaerobic conditions for three days .Violet Red Bile Agar (VRBA, Merck) was used in coliform group bacteria count and the colonies formed after 2-day incubation period at 35±1 ^{o}C were evaluated. Baird Parker Agar (BPA, Merck) was used in *Staphylococcus aureus* count and colonies formed after 2-day incubation period at 37±1 ^{o}C were evaluated. *Salmonella-Shigella* Agar (SSA, Merck) was used in *Salmonella-Shigella* count and colonies formed after 2-day incubation period at 37±1 ^{o}C were evaluated (Harrigan, 1998). Rose Bengal Chloramphenicol Agar (RBC, Merck) was used in yeast and mold count and colonies formed after 5-day incubation period at 22±1 ^{o}C were evaluated (Jarwis, 1998).

2. 2. CHEMICAL ANALYSIS

The total dry matter (%), fat (%), ash content (%), acidity (%), salt (%) and pH analyzes of tarhana samples were performed according to the method suggested in Tarhana standard (Anonymous, 1981).

3. RESULTS AND DISCUSSION

As a result of the study, the average values of the chemical properties of tarhana samples are given in Table 1 and the percentage distribution and frequencies are given in Figure 1. The average values of the microbiological properties are given in Table 2. In the study it was found that salt, acidity, total ash, dry matter, oil amounts and values of tarhana samples were 2.55-9.01 % on average of 5.06±1.75 %, 7.25-17.50 % on average of 11.67±2.63 %, 1.15-10.03 % on average of 3.92±2.05 %, 82.86-97.67 % on average of 91.54±2.34 %, 0.35-8.14 %, on average of 2.59±1.95 % and 3.80-5.32 on average of 4.40±0.46 respectively. According to Tarhana Standard maximum moisture level and salt amount in tarhana must be 10 % and the acidity level found by using 67 % must be between 10-35 %. 14 of the samples (24.14 %) and 11 of the samples (18.97 %) were not in accordance with Tarhana Standards in terms of acidity and dry matter, respectively (Anonymous, 1981). On the other hand, all the samples were found to be in accordance with the standards in terms of salt amount.

While acidity values of tarhana samples were similar (passed from %67 ethyl alcohol) with the findings obtained by Tamer et al. (2007) Şengün and Karapınar (2012) they were lower than the findings obtained by Çoşkun (2002), Erbaş et al. (2006). On the other hand, the findings were found to be higher than the ones obtained by Ekinci (2005), İbanoğlu et al. (1999), Yörükoğlu and Dayısoylu (2016), Değirmencioğlu et al. (2005), Çopur et al. (2001), Gül (2010), Demirci et al. (2016), Avcı et al. (2019). While the average pH value of the samples showed similarities with the findings obtained by Siyamoğlu (1961), İbanoğlu et al. (1995), Erbaş et al. (2005), Temiz ve Pirkul (1990), Temiz ve Pirkul (1991), İbanoğlu et al. (1999), Yörükoğlu and Dayısoylu (2016), Şengün and Karapınar (2012), Çoşkun (2002), Değirmencioğlu et al. (2005), Bilgiçli and İbanoğlu (2007), Bilgiçli et al. (2006), Sengün et al. (2009), Karagözlü et al. (2008), Çelik et al. (2005), Çelik et al. (2010), Magala et al. (2013), Demirci et al. (2016), they were found to be higher than the findings obtained by Çolak et al. (2012), Özdemir et al. (2012). The average dry matter value of the samples showed similarities with the findings obtained by (%), Yörükoğlu and Dayısoylu (2016), Gül (2010), Çolakoğlu (1977), but they were found to be lower than the ones

obtained by Coşkun (2002). While the total average ash values of the samples (%) showed similarities with the findings obtained by Yücecan et al. (1988), Yörükoğlu and Dayısoylu (2016), Gül (2010), they were higher than the ones obtained by Çolakoğlu (1977), Gürdaş (2002), and lower than the ones obtained by Siyamoğlu (1961), Bilgiçli and Türker (2004), Türker and Elgün (1995), Kıvanç and Funda (2017). The average total fat values (%) of the samples showed similarities with the findings obtained by Gül (2010), Tamer et al. (2007) and found to be lower than the findings obtained by Siyamoğlu (1961), Yücecan et al. (1988) Bilgiçli and Türker (2004), Yörükoğlu and Dayısoylu (2016), Çolakoğlu (1977), Kıvanç and Funda (2017) and lower than the findings obtained by Türker and Elgün (1995). The average salt values of the samples % were found to be lower than the values obtained by Erbaş et al. (2005) and salt values of tarhana samples were similar with the findings obtained by Kıvanç and Funda (2017). The reason for the similarities and differences of the findings of the study compared with other studies on tarhana may cause from the raw material compound of the analyzed tarhana samples or different production techniques.

Table 1: Chemical properties of tarhana samples.

Properties	N	Minimum	Maximum	Avarage
Salt (%)	58	2.55	9.01	5.06±1.75
Acidity (%)*		7.25	17.50	11.67±2.63
Ash (%)		1.15	10.03	3.92±2.05
Dry matter (%)		82.86	97.67	91.54±2.34
Fat (%)		0.35	8.14	2.59±1.95
pH		3.80	5.32	4.40±0.46

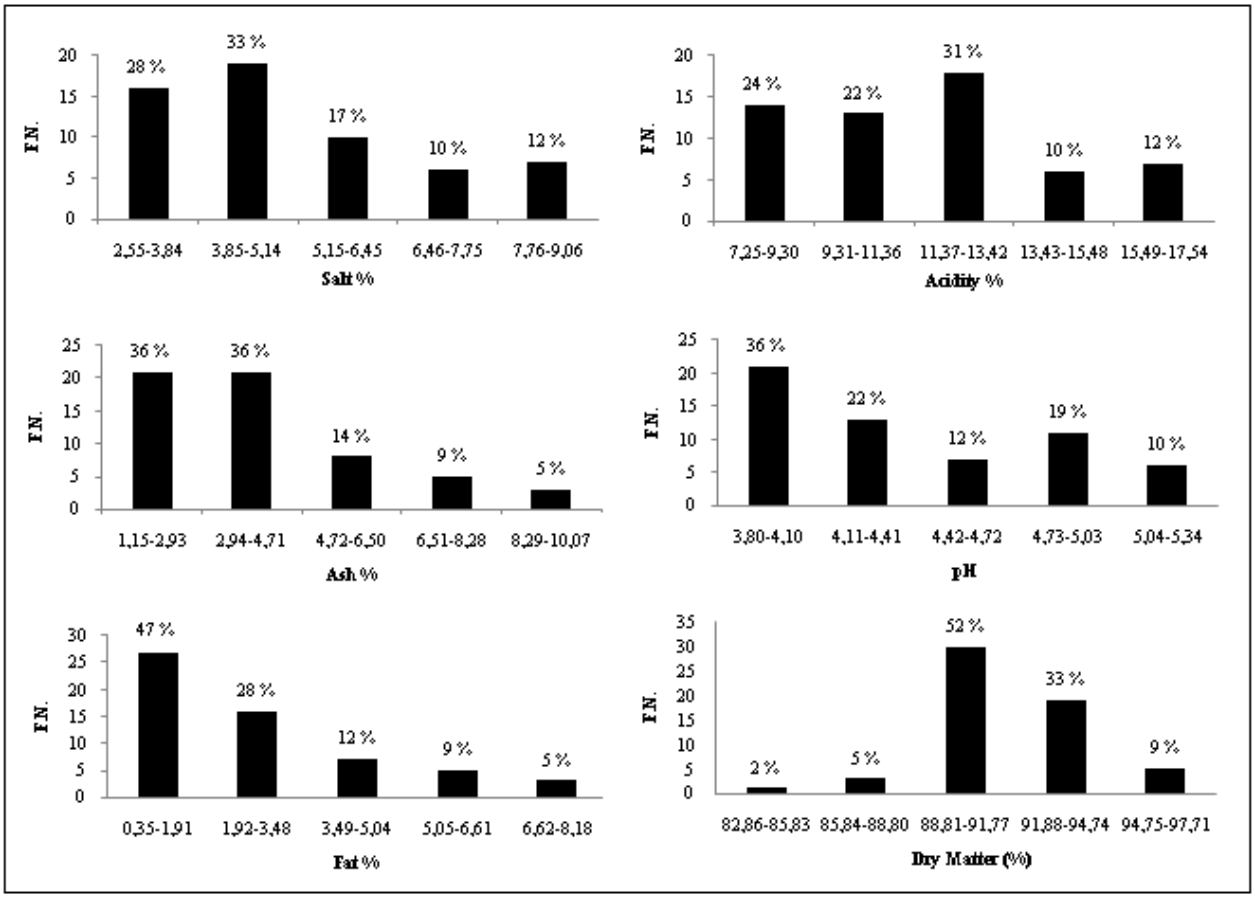
*Acidity (passed from 67 ml/100 ml ethyl alcohol)

Table 2: Microbical properties of tarhana samples. (log cfu/g)

Properties	N	Minimum	Maximum	Avarage
TMAB*	58	2.00	6.89	2.86±1.09
Coliform bacteria		<1.00	6.54	0.42±1.36
Mould and Yeast		<1.00	5.70	1.67±1.83
LAB**		2.08	7.11	3.28±1.41
Salmonella-Shigella			<1.00	
Staphylococcus aureus			<1.00	

*Total mesophylic aerobic bacteria
** Lactic acid bacteria

Total aerobic mesophilic bacteria number in tarhana samples was found to be between 2.00-6.86 log kob/g, on average 2.86±1.09 log kob/g. So, this number was lower than the findings obtained by İbanoğlu et al. (1999), Sengun and Karapınar (2012), Çoşkun (2002), Soyyiğit (2004), Taşoğulları (2017) and while coliform group bacteria was found to be lower than the detection limit in 52 (89.66 %) samples (<1.00 log kob/g), it was found to be 4.10 log kob/g on average in 6 (10.34 %) samples. Acid was not produced during fermentation due to lactic acid bacteria and yeast activation in yoghurt (Ibanoglu et al., 1999), therefore coliform group bacteria growth was prevented due to the acidic form of tarhana and drying process. These results showed similarities with the findings obtained by Çoşkun (2002), Soyyiğit (2004). Yeast-mold number, lactic-acid bacteria number were found to be between <1.00-5.70 log kob/g on average 1.67±1.83 log kob/g and 2.08-7.11 log kob/g on average 3.28±1.41 log kob/g, respectively. Yeast-mold number was determined as 3.00-4.00 log kob/g in Tarhana standard (Anonymous, 1981). Therefore, it was concluded that 50 of the tarhana samples (%86.21) were in accordance with Tarhana standard (Anonymous, 1981). Yeast-mold number of the analyzed samples were lower than the values obtained from many studies (Coskun, 2002; Dayioglu et al., 2002; Ibanoglu et al., 1999; Taşoğulları, 2017). *Staphylococcus aureus* and *Salmonella-Shigella* were found to be below the limit of detection (<1.00 log kob/g) in tarhana samples. Since pH value decreased to 3.8-4.2 levels by organic acid production and moisture content reached to 6-9 % ratio during the fermentation of tarhana, tarhana had bacteriostatic effect on many pathogenic and spoilage microorganisms and its shelf life was extended.



*F.N.: Frequency Number

Figure 1: Tarhana samples are percentage distribution and frequencies

Consequently, some differences were found in the chemical component since good-quality raw material was not used and the standard methods were not applied in the preparation of tarhana consumed in the region. Moreover, some tarhana samples examined in the study were beyond the legal standards. Microbial load was found to be higher than the determined limit due to the equipment used in the production, lack of hygiene of personnel and the environment, underestimation of the security, the use of unhygienic packaging materials and the unsuitable preservation conditions. Therefore, extension of the shelf life of the product and proving standardization and hygiene during the production are very important in terms of public health.

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➢ *ORAL PRESENTATION*

Zeytin (*Olea europaea*) meyvesi etilasetat ekstresinin *Bacillus subtilis* ve *Candida glabrata*'ya karşı farklı yöntemlerle antimikrobiyal aktivitesinin belirlenmesi

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Özet

Bu çalışmada, Ayvalık Yağlık çeşidi zeytin meyvesinden elde edilen etilasetat ekstresinin antimikrobiyal aktivitesi, gıda kaynaklı patojen *Bacillus subtilis* RSKK 244 ve klinik kökenli fungal patojen *Candida glabrata* RSKK 04019'a karşı araştırılmıştır. Antimikrobiyal aktivite, disk difüzyon, mikro-dilüsyon ve makro-dilüsyon yöntemleri kullanılarak test edilmiştir. Disk difüzyon yöntemi sonuçlarına göre, zeytin meyvesinin etilasetat ekstresinin inhibisyon zon çapları *B.subtilis* RSKK 244'e karşı 12.91 mm ve *C. glabrata* RSKK 04019'a karşı 9.65 mm olarak gözlenmiştir. Zeytin meyvesi etilasetat ekstresinin *B. subtilis* RSKK 244 ve *C. glabrata* RSKK 04019 üzerinde Minimum İnhibisyon Konsantrasyon (MİK) ve Minimal Bakterisidal/Fungusidal Konsantrasyonu (MBK/MFK) 20-40 mg/ml olarak belirlenmiştir. Makro-dilüsyon yönteminden elde edilen veriler, 20 mg/ml ve 50 mg/ml konsantrasyonundaki ekstrenin, patojenik test mikroorganizmalarının büyümesi üzerinde engelleyici bir etkiye sahip olduğunu göstermiştir. Sonuçlar, Ayvalık Yağlık çeşidi meyve etilasetat ekstresinin tıp ve ilaç endüstrilerinin yanı sıra gıda endüstrisinde de doğal bir antimikrobiyal katkı maddesi olarak kullanılabilme potansiyeli taşıdığını göstermiştir.

Anahtar kelimeler: Antibakteriyel, antifungal, zeytin meyvesi, etilasetat ekstresi, canlı hücre sayımı

Determination of the antimicrobial activity of olive (*Olea europaea*) fruit ethylacetate extract against *Bacillus subtilis* and *Candida glabrata* with different methods

Abstract

In this study, the antimicrobial activity of ethylacetate extract obtained from olive fruit of Ayvalık Yağlık variety grown in İzmir was investigated against the foodborne pathogen *Bacillus subtilis* RSKK 244 and clinical origin fungal pathogen *Candida glabrata* RSKK 04019. Antimicrobial activity was tested using disk diffusion, micro-dilution and macro-dilution methods. According to the disk diffusion method results, the inhibition zone diameters of the ethylacetate extract of olive fruit were 12.91 mm against *B.subtilis* RSKK 244 and 9.65 mm against *C. glabrata* RSKK 04019. The Minimum Inhibition Concentration (MIC) and Minimal Bactericidal/Fungucidal Concentration (MBC/MFC) of olive fruit ethylacetate extract on *B. subtilis* RSKK 244 and *C. glabrata* RSKK 04019 were determined as 20-40 mg/ml. The data obtained from the macro-dilution method showed that the extract at a concentration of 20 mg/ml and 50 mg/ml has an inhibitory effect on the growth of pathogenic test microorganisms. The results showed that Ayvalık Yağlık variety olive fruit ethylacetate extract collected from İzmir has the potential to be used as a natural antimicrobial additive in the food industry as well as in the medical and pharmaceutical industries.

Keywords: Antibacterial, antifungal, olive fruit, ethylacetate extract, viable cell count

GİRİŞ

Tıbbi bitkiler, biyolojik olarak aktif bileşenlerinden dolayı çeşitli hastalıkların tedavisinde yaygın olarak kullanılmaktadır. Zeytin (*Olea europaea*), *Oleaceae* familyası, Olea cinsinde yer alan, yaprağını dökmeyen herdem yeşil bir bitkidir (Al-Othman ve ark., 2012; Ibrar ve ark., 2017; Samejo ve ark., 2017). Çoğunlukla Akdeniz kıyılarında yetişmektedir. *O. europaea*, antik çağlardan beri iyi bilinmekte olup yüksek besin değerine sahip olması nedeniyle zamanla önemini arttırmıştır. Zeytin meyvesinin, yapraklarının ve yağının tıbbi özellikleri, ilaç ve beslenmenin gerekli bir parçası olarak kabul edilmektedir (Erbay ve ark., 2010; Galanakis 2011; Ghanbari ve ark., 2012; Hashmi ve ark., 2015). Zeytin meyvesi ve yaprak ekstreleri antibakteriyel, antifungal ve antioksidan özellikler göstermektedir (Ryan ve ark., 1998). Literatürde, zeytin meyvelerinde bulunan fenolik bileşiklerin antimikrobiyal aktivite ile bağlantılı olduğu belirtilmiştir (Bisignano ve ark., 1999; Soni ve ark 2006). Zeytin, antimikrobiyal aktiviteleri nedeniyle enfeksiyon hastalıklarının tedavisinde etkin bir şekilde kullanılmaktadır. *O. europaea*'nın yüksek kolesterol, kan şekeri ve ürik asit seviyelerini düşürdüğü bilinmektedir. Ayrıca şeker hastalığı, enflamasyon, ishal, hipertansiyon, idrar yolu ve solunum yolu enfeksiyonları, mide ve bağırsak hastalıkları, hemoroid ve romatizmayı tedavi etmek için kullanılmaktadır (Hashmi ve ark 2015). Günümüzde gıda ve klinik kökenli patojenlerin neden olduğu hastalıklar büyük sorun teşkil etmektedir. *Bacillus subtilis*, aerobik, Gram (+), endospor oluşturabilen toprak kökenli saprofit bir bakteridir. Vejetatif formu dayanıksız olup spor formu kaynama sıcaklıklarına birkaç saat süre ile dayanabilmektedir (Kaya, 2012). *B. subtilis* gıda işletmelerinde üretimin her aşamasında gıdaya kontamine olabilmektedir. *B. subtilis* bazı besin maddelerinde "subtilisin" adı verilen düşük toksisiteye sahip proteolitik enzim salgılamaktadır. Subtilisin toksisitesine maruz kalan kişilerde çeşitli alerjik reaksiyonlar ortaya çıkmaktadır (Kaya, 2012). Candida türleri ise insanda en yaygın mantar patojenleri olup dünya genelinde fırsatçı mantar enfeksiyonuna neden olmaktadır. Kandidiyaz da dahil olmak üzere mantar enfeksiyonları, özellikle immün yetmezliği olan hastalarda ciddi tehditler oluşturmaktadır (Yapar, 2014). *Candida albicans, C. glabrata, C. parapsilosis, C. tropicalis* ve *C. krusei* gibi Candida türleri, invaziv kandidiyazın %90'ından sorumludur (Yapar 2014; Sardi ve ark., 2013). *C. albicans*'ın dünya çapındaki klinik enfeksiyon sayısı son yıllarda önemli ölçüde artmakta ve geleneksel antifungal tedavilere dirençlilik gözlenmektedir (Lai ve ark., 2008). İlaçla ilişkili toksisite, önemli ilaç etkileşimleri ve geleneksel antifungallara karşı dirençlilik nedenleri ile doğal ürünlerden yeni alternatiflerin araştırılması gerekmektedir (Cavaleiro ve ark., 2006). Bu çalışmamızda, İzmir'den temin edilen Ayvalık Yağlık çeşidi zeytin meyvesinin etilasetat ekstresinin gıda kaynaklı bakteriyel *B. subtilis* RSKK 244 ve klinik kökenli fungal *C. glabrata* RSKK 04019 patojen mikroorganizmaları üzerine antimikrobiyal aktivitesi farklı yöntemler kullanılarak araştırılmış ve bu ekstrelerin gıda ve farmasötik alanda kullanım potansiyeli belirlenmiştir.

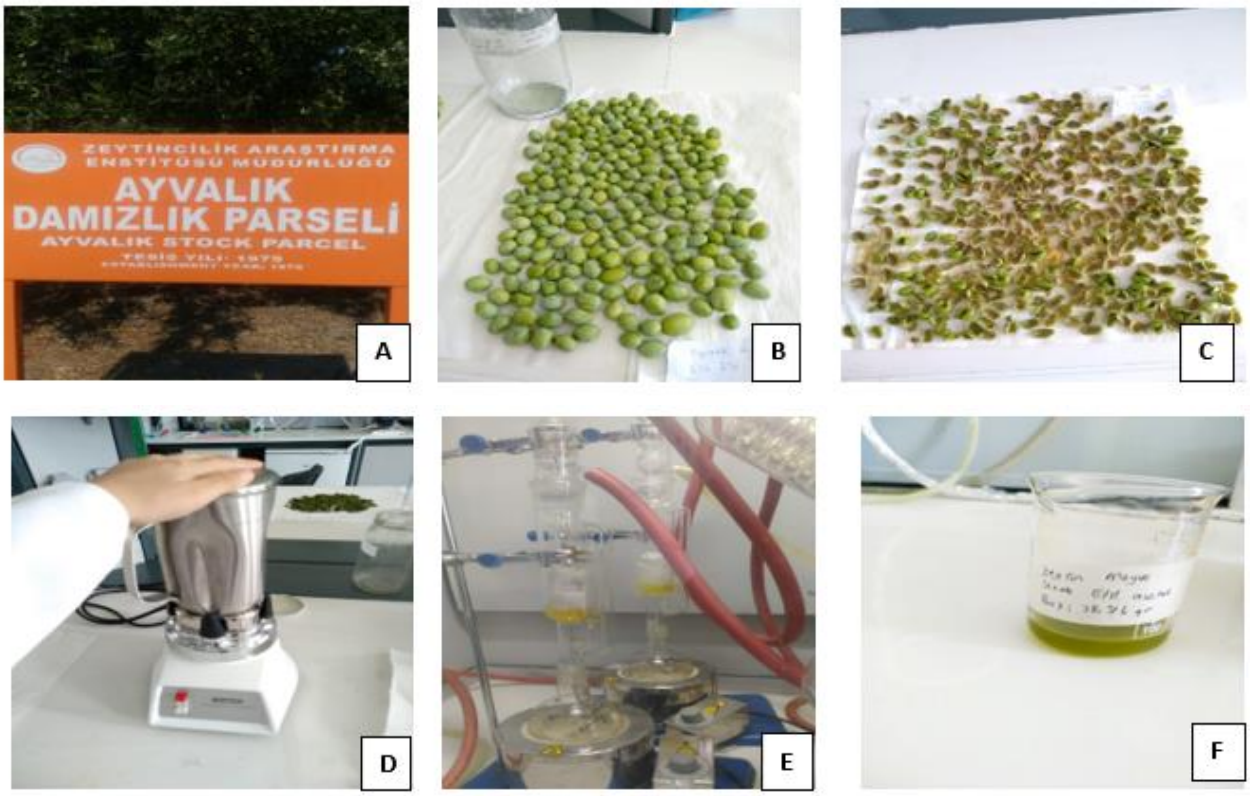
MATERYAL VE METOT

Zeytin meyvesimeyve örneklerinin temini

Ayvalık Yağlık çeşidine ait meyve örnekleri 29 Ağustos 2019 tarihinde İzmir Zeytincilik Araştırma Enstitüsü Müdürlüğü Bornova yerleşkesinden temin edilmiştir (Şekil 1-A).

Zeytin meyvesi ekstrelerinin hazırlanması

Zeytin meyveleri toplandıktan sonra yıkanıp kurutulmuş (Şekil 1-B ve C) ve Waring blendır ile öğütülmüştür. Öğütülen meyve örnekleri soksilet sistemi ile etilasetat ile ekstrakte edilmiştir (Şekil 1-D ve E). Çözücü rotary evaporatör kullanılarak uçurulmuş ve kuru ekstre elde edilmiştir (Şekil 1-F). Zeytin meyvesi etilasetat ekstresi Dimetil sülfoksit (DMSO) ile çözülmüş ve 0.45 µm'lik filtre ile steril edilmiştir. Elde edilen ekstre kullanılıncaya kadar kuru koşullar altında 4°C'de muhafaza edilmiştir.



Şekil 1. Zeytin meyvesi etilasetat ekstresinin hazırlanması
(A) Zeytin meyvelerinin toplanması
(B ve C) Zeytin meyvelerinin kurutulması
(D) Zeytin meyvelerinin Waring blendır ile öğütülmesi
(E) Soksilet sistemi ile zeytin meyvesi ekstresinin hazırlanması
(F) Zeytin meyvesi ekstresinin çözücüsünün uçurulması

Kullanılan test mikroorganizmaları
Çalışmada gıda ve klinik kökenli *Bacillus subtilis* RSKK 244 ve *Candida glabrata* RSKK 04019 test mikroorganizmaları kullanılmıştır. Test mikroorganizmalarının 24 saatlik aktif kültürleri ile tüm deneyler yapılmıştır.

Antimikrobiyal Aktivitenin Belirlenmesi
Disk difüzyon yöntemi ile antimikrobiyal aktivitenin belirlenmesi
Zeytin meyvesi etilasetat ekstrenin antimikrobiyal aktivitesinin belirlenmesi için disk difüzyon yöntemi kullanılmıştır. *B. subtilis* RSKK 244 Nutrient/agar ve *C.glabrata* RSKK 04019 YPD/agar besi yerinde sırasıyla 37°C ve 30°C'lerde kültürleri yapılmıştır. Test mikroorganizmalarının aktif kültürleri serum fizyolojik ile iki defa yıkandıktan sonra konsantrasyonları 0.5 McFarland'a ayarlanmış ve daha sonrasında uygun katı besi yerine ekimleri yapılmıştır. Ekimi yapılan petrilere steril diskler yerleştirildikten sonra disklere 20 µl (2000 µg/disk) zeytin meyvesi etilasetat ekstresi damlatılmıştır. Petriler 24 saatte uygun sıcaklıklarda inkübe edilmiştir. İnkübasyon süresi sonunda diskler etrafındaki zonlar kumpas ile ölçülerek kaydedilmiştir. Tüm deneyler üç tekrarlı olarak yapılmıştır.

Mikro-dilüsyon yöntemi ile minimal inhibisyon (MİK) ve bakterisidal/fungusidal (MBK/MFK) konsantrasyonlarının belirlenmesi
Ekstrelerin MİK, MBK ve MFK değerleri her iki test mikroorganizmasına karşı mikro-dilüsyon yöntemi ile belirlenmiştir. Ekstre ve besiyeri içeren her tüpe 0.5 McFarland konsantrasyonunda test mikroorganizmaları eklenmiş ve yavaşça karıştırılmıştır. Daha sonra karışımı içeren tüpler 24 saat uygun sıcaklıklarda inkübe edilmiştir. İnkübasyondan sonra, sıvı besi yerinde gelişmenin olmadığı konsantrasyon MİK değerleri olarak

kaydedilmiştir. Tüplerden örnekler alınarak spesifik agar besi yerine spot ekimleri yapılmış ve 24 saat uygun sıcaklıkta inkübe edilmiştir. İnkübasyon süresinin sonunda, katı besi ortamı üzerinde bakterilerin gelişimini önleyen ekstre konsantrasyonları MBK veya MFK değerleri olarak değerlendirilmiştir.

Makro-dilüsyon yöntemi ile antimikrobiyal aktivitenin belirlenmesi

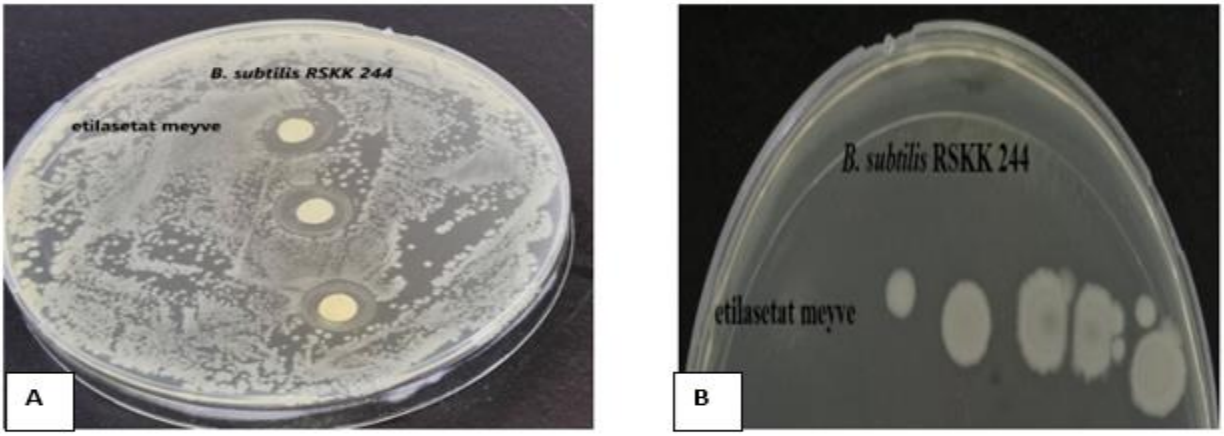
Zeytin meyvesi etilasetat ekstresinin makro-dilüsyon yöntemi ile antimikrobiyal aktivitesinin belirlenmesinde spektrofotometrik ölçüm ve canlı hücre sayımı yapılmıştır. *B. subtilis* RSKK 244 ve *C.glabrata* RSKK 04019 test mikroorganizmalarının 0.5 McFarland konsantrasyonunda hazırlanmış süspansiyonlarına değişik konsantrasyonlarda (10-20-50 mg/ml) zeytin meyvesi etilasetat ekstresi ilave edilmiştir. Kontrol grubu olarak ekstre ilave edilmemiş hücre süspansiyonu kullanılmıştır. *B. subtilis* RSKK 244, 37°C'de ve *C.glabrata* RSKK 04019, 30°C'de inkübe edilmiştir. Mikroorganizma gelişimi inkübasyonun 0, 4, 8, 24 ve 48. saatlerinde spektrofotometre (Beckman Coulter) ile OD 540 nm'de belirlenmiştir (Sousa ve ark., 2006). Ayrıca 48. saatte örnekler alınarak uygun dilüsyonları yapılarak katı besi yerine ekimleri yapılmıştır. Petriler uygun sıcaklıklarda (37°C ve 30°C) 24 saat inkübe edilmiştir. İnkübasyon sonrası canlı hücreler sayılmış ve $\log_{10}$ değeri olarak ml'deki koloni oluşturan birim (KOB/ml) olarak sunulmuştur. Tüm ölçümler üç tekerrürlü ve aseptik koşullarda yapılmıştır.

BULGULAR ve TARTIŞMA

Zeytin meyvesi etilasetat ekstresinin antimikrobiyal aktivitesi, disk difüzyon, mikro-dilüsyon ve makro-dilüsyon yöntemleri ile belirlenmiştir. Disk difüzyon sonuçlarına göre, zeytin meyvesi etilasetat ekstresinin *B. subtilis* RSKK 244 ve *C. glabrata* RSKK 04019 test mikroorganizmaları üzerinde oluşturduğu inhibisyon zon çapları 12.91 ve 9.65 mm olarak kaydedilmiştir (Tablo 1, Şekil 2-A ve 3-A). Etilasetat ekstresinin MİK ve MBK/MFK değerleri *B. subtilis* RSKK 244 ve *C. glabrata* RSKK 04019 için 20 ve 40 mg/ml olarak tespit edilmiştir (Tablo 1, Şekil 2-B ve 3-B). Disk difüzyon sonuçları değerlendirildiğinde, zeytin meyvesi etilasetat ekstresinin *B. subtilis* RSKK 244 üzerinde daha yüksek inhibisyon zon çapı oluşturduğundan *C. glabrata* RSKK 04019'a kıyasla daha fazla antimkrobiyal aktivite gösterdiği belirlenmiştir. Ancak etilasetat ekstresinin MİK ve MBK/MFK değerleri her iki test mikroorganizması için aynı çıkması mikro-dilüsyon yöntemiyle belirlenen antimikrobiyal aktivitenin benzer düzeyde olduğunu göstermiştir. Makro-dilüsyon yönteminde, kontrol grubuna nazaran ekstre ilave edilen test gruplarının zeytin meyvesi etilasetat ekstresinin renkli olmasından dolayı 0.saatte daha yüksek OD değeri ile başladığı tespit edilmiştir (Şekil 4). Spektrofotometrik ölçüm sonuçları, *B. subtilis* RSKK 244'de kontrol grubu 48. saate kadar OD değerinin arttığını göstermiştir. 10 mg/ml ekstre ilave edilen deney grubunda ise 48. saatin sonunda başlangıçtaki (0. saat) OD değerinin bir miktar üzerine çıktığı belirlenmiştir. 20 ve 50 mg/ml ekstre ilave edilen deney grubunda ise 48. saat sonunda başlangıçtaki OD değerinin altına düştüğü tespit edilmiştir. *C. glabrata* RSKK 04019 için spektrofotometrik ölçüm sonuçları kontrol grubunun *B. subtilis* RSKK 244'de olduğu gibi 48. saate kadar OD değerinin arttığı, 10 mg/ml ekstre uygulanan test grubunun ise bir miktar OD değerinde artış gösterdiği ancak kontrol grubuna kıyasla daha az arttığı belirlenmiştir. 20 mg/ml ekstre ilave edilen test grubunda ise 48. saatin sonuna kadar OD değerinin hemen hemen sabit kaldığı ancak 50 mg/ml ekstre ilave edilen test grubunda 48. saat sonunda başlangıç OD değerinin (0. saat) altına düştüğü tespit edilmiştir. Makro-dilüsyon yöntemiyle elde edilen spektrofotometrik veriler değerlendirildiğinde kontrol grubuna kıyasla özellikle 20 ve 50 mg/ml ekstre konsantrasyonun test mikroorganizmalarının gelişimi üzerinde engelleyici etkisi olduğu belirlenmiştir (Şekil 5 ve 6). Spektrofotometrik ölçüm sonuçlarında antimikrobiyal aktivitenin daha belirgin gözlendiği 20 ve 50 mg/ml ekstre ilave edilen deney grubunun 48. saat canlı hücre sayım deneyinde; *B. subtilis* RSKK 244'de 20 mg/ml ekstre ilave edilen deney grubunda 8.45 KOB/ml ve 50 mg/ml ekstre ilave edilen deney grubunda 8.16 KOB/ml olarak bulunmuştur. Ekstre ilave edilmeyen kontrol grubunda ise daha yüksek canlı sayım değerleri (9.37 KOB/ml) tespit edilmiştir. *C. glabrata* RSKK 04019'da 48. saat sonunda kontrol grubunda (9.82 KOB/ml) yine 20 mg/ml (8.57 KOB/ml) ve 50 mg/ml (8.53 KOB/ml) ekstre ilave edilen test gruplarına göre daha yüksek canlı hücre sayısı belirlenmiştir (Şekil 7 ve 8). Spektrofotometrik veriler ve canlı hücre sayım sonuçları değerlendirildiğinde kontrol grubuna kıyasla 20 ve 50 mg/ml ekstre ilavesinin gıda ve klinik kökenli patojen mikroorganizmalarının gelişimini sınırlandırdığı tespit edilmiştir.

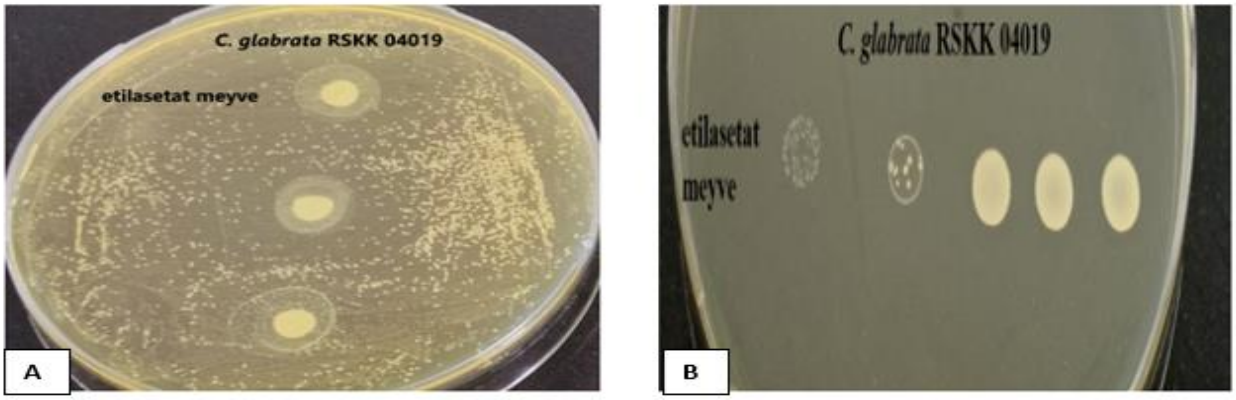
Tablo 1. Zeytin meyvesi etilasetat ekstresinin antimikrobiyal aktivitesi

Ekstre	İnhibisyon zon çapı (mm)		MİK (mg/ml)		MBK/MFK (mg/ml)	
	***B. subtilis* RSKK 244**	***C. glabrata* RSKK 04019**	***B. subtilis* RSKK 244**	***C. glabrata* RSKK 04019**	***B. subtilis* RSKK 244**	***C. glabrata* RSKK 04019**
Zeytin meyvesi etilasetat	12.91±0.93	9.65±0.61	20	20	40	40



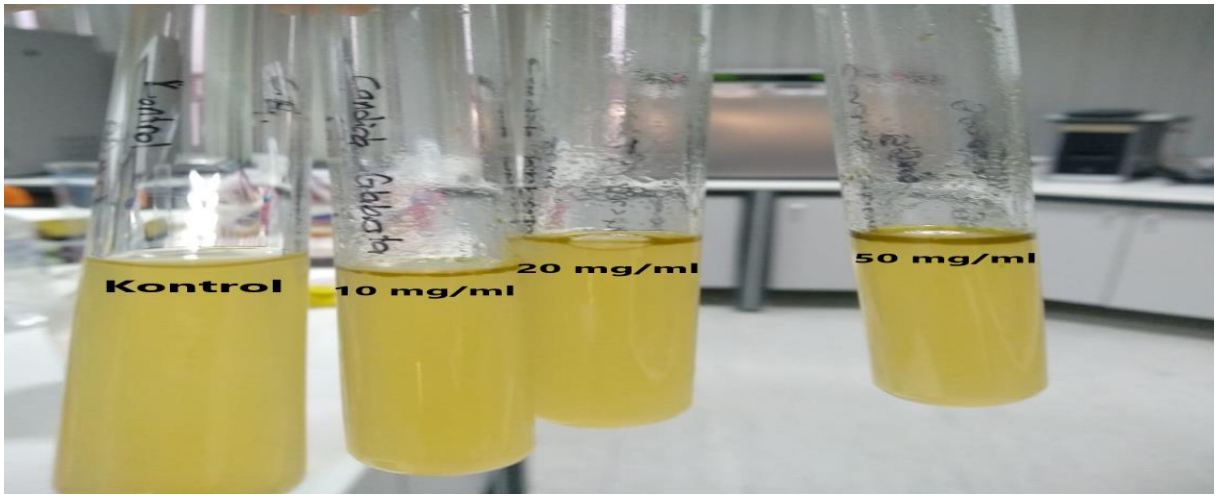
Şekil 2. Zeytin meyvesi etilasetat ekstresinin *B. subtilis* RSKK 244 üzerinde antimikrobiyal aktivitesi

(A) Etilasetat ile hazırlanan zeytin meyvesi ekstresinin disk difüzyon sonucu
(B) Etilasetat ile hazırlanan zeytin meyvesi ekstresinin MBK sonucu

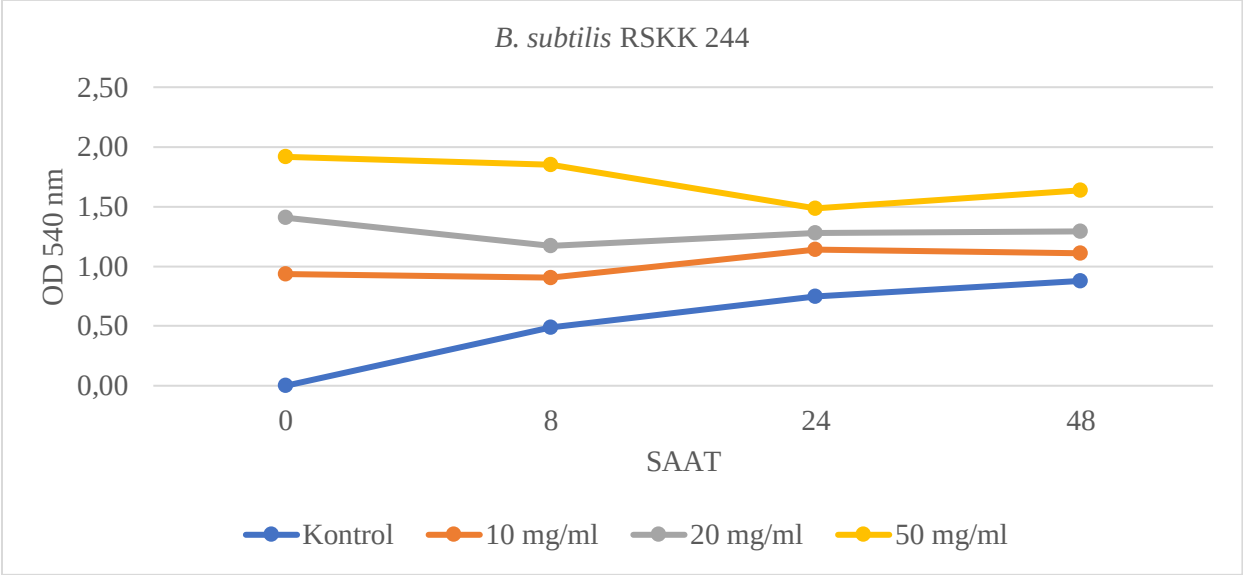


Şekil 3. Zeytin meyvesi etilasetat ekstresinin *C. glabrata* RSKK 04019 üzerinde antimikrobiyal aktivitesi

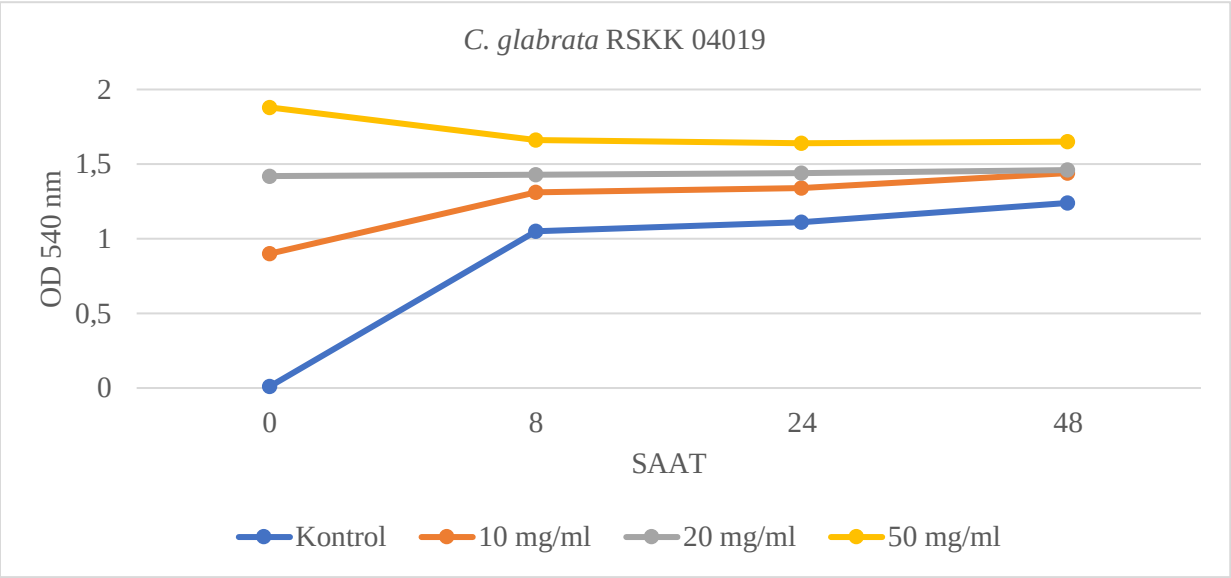
(A) Etilasetat ile hazırlanan zeytin meyvesi ekstresinin disk difüzyon sonucu
(B) Etilasetat ile hazırlanan zeytin meyvesi ekstresinin MBK sonucu



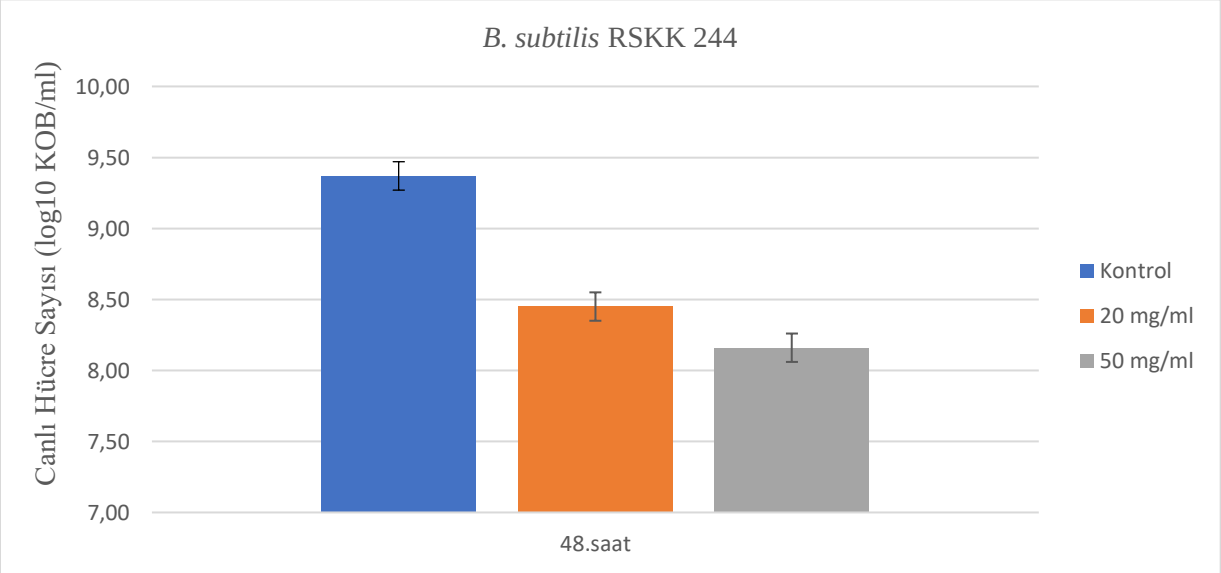
Şekil 4. Makro-dilüsyon yöntemi ile hazırlanan kontrol, 10 mg/ml, 20 mg/ml ve 50 mg/ml zeytin meyvesi etilasetat ekstresi içeren test grupları



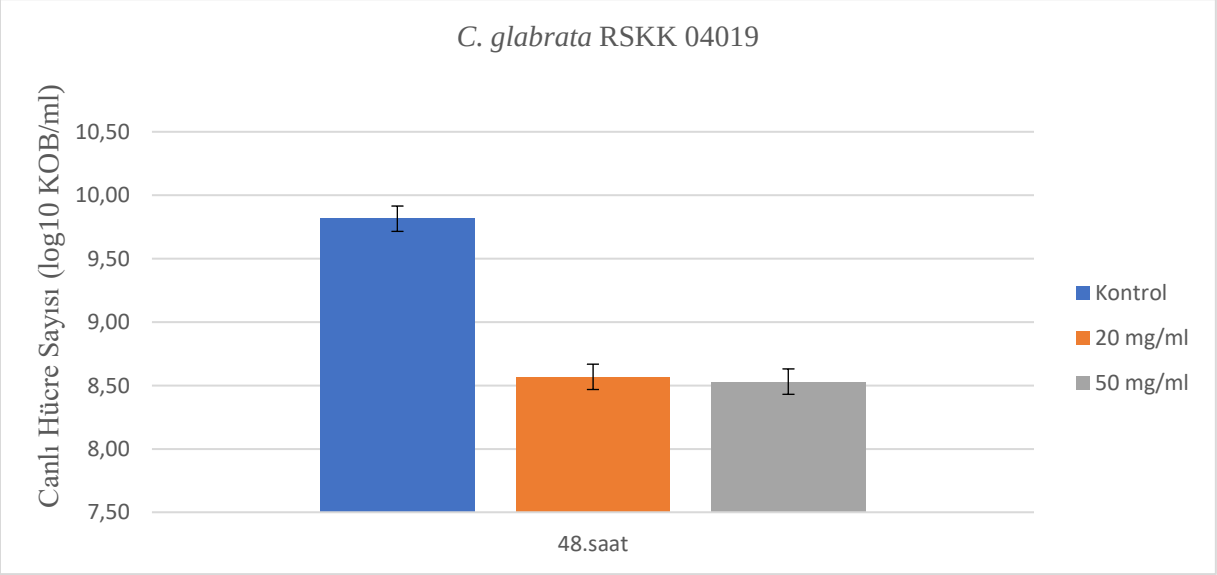
Şekil 5. Zeytin meyvesi etilasetat ekstresinin *B. subtilis* RSKK 244'nin gelişimi üzerindeki etkisinin spektrofotometrik veri grafiği



Şekil 6. Zeytin meyvesi etilasetat ekstresinin *C. glabrata* RSKK 04019 'nın gelişimi üzerindeki etkisinin spektrofotometrik veri grafiği



Şekil 7. Zeytin meyvesi etilasetat ekstresinin 48.saatte *B. subtilis* RSKK 244'nin gelişimi üzerindeki etkisini gösteren canlı hücre sayım grafiği



Şekil 8. Zeytin meyvesi etilasetat ekstresinin 48.saatte *C. glabrata* RSKK 04019'nın gelişimi üzerindeki etkisini gösteren canlı hücre sayım grafiği

Yapılan bir çalışmada, ticari olarak temin edilen dört farklı sofralık zeytinin meyve su ekstrelerinin farklı konsantrasyonlarının (10-100 mg/ml) antimikrobiyal aktivitesi araştırılmıştır. *B. subtilis* CECT 498 test mikroorganizması üzerinde iki çeşitte 100 mg/ml ekstre konsantrasyonunda antimikrobiyal aktivite gözlenmezken diğer iki çeşitte 50 mg/ml ekstre konsantrasyonunda antimikrobiyal aktivite tespit edilmiştir. *C. albicans* CECT 1394 üzerinde ise sadece bir sofralık zeytin meyvesi ekstresinde 100 mg/ml ekstre konsantrasyonunda antimikrobiyal aktivite tespit edilirken test edilen diğer ekstrelerin değişik konsantrasyonlarının antifungal etki göstermediği bildirilmiştir (Pereira ve ark., 2006). Çalışmamızda ise Ayvalık Yağlık çeşidi olgunlaşmamış zeytin meyvesi etilasetat ekstresinin *B. subtillus* RSKK 244 ve *C. glabrata* RSKK 04019 üzerinde oldukça iyi antimikrobiyal aktivite gösterdiği belirlenmiştir. Birçok doğal üründe olduğu gibi coğrafi konum, bitki beslenmesi ve çeşit özelliği gibi faktörler ekstrelerin kompozisyonunu değiştirebilmekte ve bu durumlara ilave olarak kullanılan test mikroorganizmalarının suş değişiklikleri ve kullanılan farklı test yöntemleri de antimikrobiyal aktivitede etkili olabilmektedir. Ayrıca bitkilerin toplanma alanlarının farklı toprak oluşumundan dolayı antimikrobiyal aktiviteyi etkilediği de öne sürülmektedir (Pereira ve ark., 2006; Sousa ve ark., 2006).

SONUÇ

Ayvalık Yağlık çeşidi zeytin meyvesinin etilasetat ekstresinin test mikroorganizmaları *B. subtilis* RSKK 244 ve *C. glabrata* RSKK 04019'a karşı oldukça iyi antimikrobiyal aktivite gösterdiği değişik yöntemlerle tespit edilmiştir. Toplumun bilinçlenmesiyle birlikte doğal ürünlerin kullanıma olan ilgi giderek artmaktadır. Gıda ve sağlık sektörlerinde kullanılan sentetik antimikrobiyallerin sağlık üzerine olumsuz yan etkileri ve zamanla bu antimikrobiyallere karşı gelişebilen direnç nedenleriyle doğal kökenli yeni antimikrobiyallere ihtiyaç duyulmaktadır. İzmir ilinden temin edilen Ayvalık Yağlık çeşidi meyve ekstresi yapılacak ileri çalışmalarla günümüzde var olan antimikrobiyallere alternatif olarak bitkisel içerikli doğal bir antimikrobiyal olarak, gıda ve farmasötik alanlarında daha etkili formülasyonların geliştirilmesine katkı sağlayabilecektir.

TEŞEKKÜR

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➢ *ORAL PRESENTATION*

Fermantasyonlarda FDM bazlı üç boyutlu küplerin kullanılabilirliği

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Özet

Prototip üretimi için en pratik yaklaşım olan 3D yazıcılar her geçen gün yeni alanlar için kullanılmaktadır. Fermantasyon işlemlerinde de destek veya immobilizasyon malzemesi olarak üç boyutlu yazdırılmış malzemeler kullanılabilmektedir. Bu yaklaşım sayesinde araştırmacılar benzersiz destek materyalleri tasarlayabilmektedirler. Araştırmanın amacı sıcak eriyik ekstrüzyon tekniği ile üretilen 3D küplerin fermantasyonlarda kullanılabilirliğini belirlemektir. Küplerin modellenmesinde 1,5 mm ve 2,5 mm kalınlık değerine sahip yüzey kafes tipi kullanılmıştır. 3D küplerin kullanıldığı etanol fermantasyonlarında maksimum tüketim ve üretim oranları istatistiksel olarak önemli düzeyde artmıştır. Ayrıca 48 saat süren fermantasyon sonucunda küplerin yüzeyinde maya hücrelerinin tutunması ise bu küplerin immobilizasyon amaçlı kullanılabileceğini göstermiştir.

Anahtar Kelimeler: 3D küpler, yüzey kafes tipi, fermantasyon, seçici lazer sinterleme, sıcak eriyik ekstrüzyonu

The usability of FDM-based 3D printed cubes for fermentation

Abstract

3D printers, which are the most practical way of prototype production, are used for new areas every day. 3D printed materials can be also used as the support or immobilization material for fermentation processes. The unique support materials can be designed by the researchers with these printers. The aim of the study was to determine the usability of 3D cubes produced by fusion deposition modeling for fermentation. Surface lattice type with 1.5 mm and 2.5 mm of thickness value was used in modeling the cubes. The results showed that using 3D cubes improve the maximum consumption and production rates of ethanol fermentations statistically. In addition, the attachment of yeast cells on the surface of the cubes after 48 hours of fermentation showed that these cubes could also be used for immobilization.

Keywords: 3D cubes, surface lattice type, fermentation, selective laser sintering, fusion deposition modeling

GİRİŞ

Petrol kaynaklarının azaldığı ve yenilenebilir enerji kaynaklarına yönelimin hızla devam ettiği günümüzde enerji ihtiyacının karşılanması ülkelerin en temel sorunları arasında yer almaktadır. Sanayi devriminden günümüze kadar gelinen süreçte sürekli artan enerji ihtiyacını karşılamada alışılagelmiş yaklaşım fosil yakıtların kullanımı iken çevre kirliliğindeki artış ülkeleri farklı kaynaklara yönelmeye zorlamıştır. Bu amaçla tercih edilen enerji kaynakları içerisinde rüzgâr türbinleri, güneş panelleri, verimi arttırılmış hidroelektrik santraller, jeotermal santraller, hidrojen enerjisi ve biyokütle enerjisi sayılabilir. Biyoteknolojik açıdan değerlendirildiğinde uygulanabilir en iyi yöntem mikrobiyal etanol üretimidir. Bu amaçla farklı mikroorganizmalar kullanılarak atıklardan veya ucuz şeker kaynaklarından etanol üretimi tüm dünyada yaygın olarak uygulanmaktadır. Etanol birden fazla kullanım alanına sahip değerli bir üründür. Enerji üretimi açısından yakıt olarak kullanılmakla birlikte gıda katkı maddesi, içecek üretimi, dezenfeksiyon ve bilimsel çalışmalarda da yaygın olarak kullanılmaktadır.

Etanol üretimi son yıllarda gittikçe artmakta olup bunun başlıca sebebi araçlarda yakıt olarak kullanılabilirliğinin ön plana çıkmış olmasıdır. Bu kullanımının yanı sıra dezenfektan üretiminde de ana girdilerden birisi olduğundan özellikle günümüzde görülmekte olan Covid-19 salgını sürecinde de ihtiyaç miktarı artmıştır. Yakın gelecekte fosil kaynaklı yakıtların bitebilecek olması ve çevre kirliliğinin azaltılması adına tüm gelişmiş ve gelişmekte olan

ülkeler yeni enerji kaynakları arayışına girmişlerdir. Bu doğrultuda ihtiyaç miktarı her geçen gün artan etanolün üretim miktarları da yıldan yıla artmıştır (Tablo 1).

Tablo 1. Yıllara göre etanol üretim oranları (RFA, 2020)

Ülkeler	Üretim miktarı (milyon litre)				
	2015	**2016**	**2017**	**2018**	**2019**
Amerika Bir. Dev.	56051	58345	60324	60911	59726 (%54,41)
Brezilya	27255	25552	25173	30245	32517 (%29,62)
Avrupa Birliği	5148	5148	5375	5489	5186 (%4,72)
Çin	2915	2536	3028	2915	3785 (%3,45)
Kanada	1703	1741	1741	1741	1968 (%1,79)
Diğer ülkeler	4213	5099	5050	6204	6594 (%6,01)
Dünya Toplamı	**97285**	**98421**	**100691**	**107505**	**109776**

Mikrobiyal üretimlerde kullanılan her bir mikroorganizmanın besin talepleri ve gelişim sürecindeki morfolojik özellikleri birbirinden farklıdır. Özellikle yüksek konsantrasyonlarda hücre yoğunluğunun gerektiği üretimlerde farklı yaklaşım ve stratejilerin oluşturulması hedeflenen ürünün üretilebilmesi açısından önemlidir. Biyoteknolojik üretimlerin temel amacı daha fazla ürünü daha ekonomik bir yöntem ve kısa sürede üretmektir. Bu amaçla kesikli, yarı-kesikli, sürekli fermantasyon tekniklerinin yanı sıra immobilizasyon ve biyofilm gibi yüksek hücre yoğunluğunda çalışmayı sağlayan teknikler kullanılmaktadır. Mikrobiyal gelişimi teşvik etmek veya kontrol etmek adına denenen yeni tekniklerden birisi de farklı kafes tiplerine veya üç boyutlu tasarıma sahip 3D yazıcıda üretilmiş materyallerin fermantasyonlarda kullanılmasıdır. Bu tekniğin temelinde iki amaç yatmaktadır. Bunlar; immobilizasyon destek materyali olarak görev yapması ve yüksek yüzey alanı sağlamasıdır. Üç boyutlu materyallerin fermantasyon ortamına ilave edilmesi üzerine gerçekleştirilen iki çalışmada da SLS (Selective Laser Sintering; Seçici Lazer Sinterleme) teknolojisi ile üretilen materyaller kullanılmıştır. SLS teknolojisi, katmanlar halinde toz tabakaların özel lazer teknolojisi ile işlenmesi sonucu yüksek karmaşıklığa ve çok küçük detaylara sahip 3D materyallerin yazdırılmasını sağlayan ileri seviye bir teknolojidir. Fermantasyonlarda bu teknolojinin tercih edilmesinin bir diğer sebebi ise üretimlerde kullanılan hammaddenin gıda ile temasa uygun ve sterilizasyon sıcaklığına dayanıklı olmasıdır.

Bu çalışmalardan ilki atık suların arıtımı için fulleren tipte küreler tasarlanması ile ilgilidir. Çalışma kapsamında üç farklı küre tasarlanmış ve SLS teknolojisi ile üretilmiştir. K3 isimli biyotaşıyıcı ile karşılaştırılan 3D kürelerin daha pürüzlü bir yapıya sahip olduğu taramalı elektron mikroskobu ile belirlenmiştir. Denemeler sonucunda ise 3D taşıyıcıların biyofilm oluşumunda daha iyi sonuçlar verdiği (yüksek mikrobiyal aktivite ve daha güçlü yapışma kabiliyeti) ve atık suların arıtımında başarıyla kullanılabileceği bildirilmiştir (Dong ve ark., 2015). Diğer bir çalışmada ise *Propionibacterium acidipropionici* hücrelerinin 15 mm çapa sahip kürelerle birlikte fermantasyona bırakıldığında fermantasyon süresinin serbest hücrelerle yapılan fermantasyona göre azaldığı, üretim (25,8 g/L) ve üretkenlik (0,46 g/L/sa) değerlerinin arttığı belirlenmiştir (Belgrano ve ark., 2018).

Bu çalışma kapsamında üretim maliyeti kısmen ucuz olarak değerlendirilebilmesine karşın ilk yatırım maliyeti oldukça yüksek olan SLS teknolojisi yerine ilk yatırım maliyeti oldukça düşük olan FDM (Fusion Deposition Modeling; Sıcak eriyik ekstrüzyonu) teknolojisi ile üretilen küplerin fermantasyonlarda kullanılabilirliğinin belirlenmesi amaçlanmıştır. Geleneksel olarak kullanılan ABS ve PLA gibi materyaller gıda ile temasa uygun olmamalarının yanı sıra sterilizasyon sıcaklığına da dayanıklı değillerdir. Bu nedenle alışılagelmiş filamentler ile FDM cihazında üretilecek materyallerin fermantasyonda kullanımı mümkün değildir. STH filamentler ise, bitkisel bazlı üretilen yeni bir filament olmasının yanı sıra hem gıda ile temasa hem de sterilizasyon sıcaklığına dayanıklı olduğundan FDM bazlı 3D materyallerin fermantasyonlarda kullanılabilirliğinin önünü açmıştır. Gerçekleştirilen bu çalışmada FDM teknolojisi ile üretilen küplerin fermantasyonlarda kullanılabilirliğinin belirlenmesi amaçlanmıştır. Çalışmada kapsamında yüzey kafes tipi uygulanmış küpler ile gerçekleştirilen etanol fermantasyonları ile üç boyutlu küplerin verim, maksimum tüketim ve maksimum üretim oranına etkisinin ve ilerleyen çalışmalarda FDM bazlı küplerin immobilizasyon destek materyali olarak kullanılabilirliğinin belirlenmesi amaçlanmıştır.

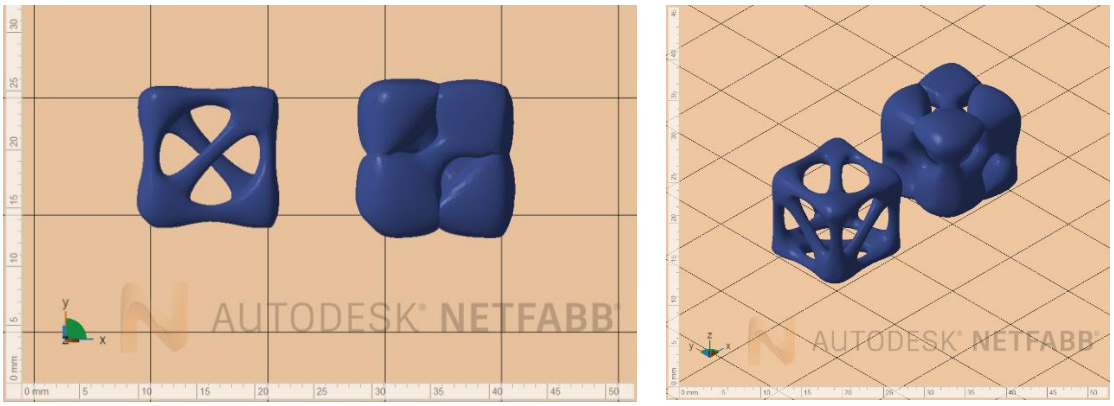
MATERYAL VE METOD

Kullanılan mikroorganizma

Bu çalışmada gerçekleştirilen etanol denemelerinde *Saccharomyces cerevisiae* (ATCC 36858) kullanılmıştır. Stok ve ön kültürler için 50 g/L glukoz, 6 g/L maya ekstraktı, 0,3 g/L $CaCl_2.2H_2O$, 4 g/L $(NH_4)_2SO_4$, 1g/L $MgSO_4.7H_2O$ ve 1,5 g/L KH_2PO_4 içeren besiyeri kullanılmış ve 30°C'de 18-24 saat inkübe edilerek stok ve ön kültürler oluşturulmuştur. Stok kültürler 15 günde bir yenilenerek kullanım anına kadar +4°C'de muhafaza edilmiştir.

Üç boyutlu küplerin tasarımı ve üretimi

Çalışma kapsamında FDM teknolojisi ile üretilecek olan küplere ait özellikler daha önce yapılmış çalışmalar (Belgrano vd., 2018; Dong vd., 2015) referans alınarak tasarlanmıştır. Çalışma flask denemeleri şeklinde yapılacağı için çap değeri 10 mm olarak alınmıştır. Tüm çizimler Autodesk Fusion 360 2019 (Student Edition) ve Autodesk Netfabb 2019 (Student edition) programı kullanılarak çizilmiş, alan ve hacim değerleri hesaplanmıştır. 10 mm'lik küplere farklı kafes tipleri uygulanmış ancak üretimde kullanılacak olan teknoloji yeterli gelmediğinden yüzey kafes tipinde çizimler gerçekleştirilmiştir. Yüzey kafes tipi çizimlerinde kalınlık değerleri 1-3 mm aralığında alınmış ve fermantasyonda kullanılacak olan küpler 1,5 mm ve 2,5 mm kalınlığa sahip küpler olarak belirlenmiştir (Şekil 1).



Şekil 1. Fermantasyon denemelerinde kullanılacak olan küplerin yandan görünümü (Soldan sağa sırayla: 1,5 ve 2,5 mm kalınlığa sahip küpler)

Tasarlanan küplerin hacim ve alan değerleri incelendiğinde kalınlık değeri ile doğru orantılı bir artış olduğu görülmüştür. 3D küplerin kullanımındaki asıl amaç mikroorganizmaların tutunabileceği yüzey alanını arttırarak mikrobiyal faaliyeti hızlandırmaktır. Durum böyle olunca da daha az malzeme ile daha yüksek yüzey alanı sağlayabilmek adına 3D materyallerden faydalanmak akıllıca bir yaklaşım olmaktadır. Bu amaçla tasarlanan küpler TEVO Tarantula 3D Yazıcı ile üretilmiştir. 3D yazıcıda kullanılan yazdırma parametreleri; 0,1 mm xyz eksen hassasiyeti, 0,1 mm katman kalınlığı, 50 mm/sn yazma hızı, 60ºC tabla sıcaklığı, 220ºC basım sıcaklığı şeklindedir. Üretimlerde ABS ve PLA filamentler yerine bitkisel bazlı ve sterilizasyon sıcaklığına dayanıklı STH filament (ABG filaments, Türkiye) kullanılmıştır. Üretilen küpler referans küp ile karşılaştırıldığında daha yüksek hacim ve alan değerlerinin daha düşük ağırlık değerlerinde elde edilebildiği görülmüştür (Tablo 2). Tasarlanan küpler referans küpe kıyasla daha az hacim kaplamalarına karşın referans küpe göre daha fazla alan sağlamaktadırlar.

Tablo 2. Üretilen 3D küplere ait hacim, alan ve ağırlık değerleri

	Hacim (mm^3)	**Alan (mm^2)**	**Ağırlık (mg)***
Referans küp	1000	600,00	751,83±3,70
1,5 mm kalınlıkta üretilen küp	491	846,10	505,39±0,76
2,5 mm kalınlıkta üretilen küp	1444	1250,60	1293,43±1,38
*Ağırlık verileri "değer±stdhata" türünden verilmiştir.			

Tablodan da görüleceği üzere referans küpten iki adet fermantasyon ortamına eklendiğinde 1200 mm^2'lik bir alan sağlanırken 2,5 mm'lik küp bu alandan daha fazlasını tek başına sağlamaktadır. Hem de 210,23 mg daha az filament harcanarak filament sarfiyatının da azalması sağlanmıştır.

Fermantasyon

Üç boyutlu küplerin etanol fermantasyonuna etkisini belirlemek amacıyla farklı sayılarda küp içeren besiyerlerinde fermantasyonlar gerçekleştirilmiştir (Tablo 3). Bu amaçla kontrol fermantasyonunun yanı sıra 1,5 mm ve 2,5 mm kalınlığa sahip küplerden 4 ve 12 adet içeren flasklarda fermantasyonlar gerçekleştirilmiştir.

Tablo 3. Fermantasyon denemelerine ait deneme deseni

Deneme	Kalınlık (mm)	Küp sayısı (ad)	Şeker kons. (g/L)	Kullanılan küplerin sağladığı yüzey alanı (mm^2)
Kontrol	-	-	100	-
1	1,5	4	100	3384,4
2	2,5	4	100	5002,4
3	1,5	12	100	10153,2
4	2,5	12	100	15007,2

Fermantasyonlarda kullanılan besiyeri içeriği; 100 g/L glukoz, 6 g/L maya ekstraktı, 0,3 g/L $CaCl_2.2H_2O$, 4 g/L $(NH_4)_2SO_4$, 1g/L $MgSO_4.7H_2O$ ve 1,5 g/L KH_2PO_4 olup hazırlanan besiyeri flasklara konduktan sonra 121ºC'de 15 dakika sterilize edilmiştir. Aynı zamanda temizlenen küpler de saf su içeren kaplarda steril edilerek inokülasyondan hemen önce fermantasyon ortamına belirlenen tür ve sayıda eklenmiştir. Ardından inokülasyon yapılarak 30°C'de 48 sa sürecek fermantasyon başlatılmıştır. Fermantasyon başladıktan sonra başlangıç ve sıfır zamanı örneklerine ek olarak 3, 6, 9, 12, 15, 24, 30, 36 ve 48. saatlerde de örnekler alınarak biyokütle, etanol ve indirgen şeker analizleri yapılmıştır. Tüm örnekler analiz anına kadar +4ºC'de muhafaza edilmiştir.

Toplam hücre kütle (biyokütle) miktarı

Fermantasyon ortamından alınan örneklerde toplam biyokütle miktarını belirlemek için 1 ml örnek üzerine 9 ml saf su eklenerek inoküle edilmemiş besiyeri örneğine karşı spektrofotometrede (ThermoScientific Evolution, Shanghai, China) 620 nm'de okutulmuştur. Absorbans değerleri denklemde yerine koyularak biyokütle değerleri hesaplanmıştır (Denklem 1) (Turhan ve ark., 2010).

- $Biyokütle\ (g\ kuru\ ağ./L) = (0{,}666019 \times Abs_{620\,nm}) + 0{,}10635$ (1)

Etanol ve kalıntı şeker analizi

Etanol ve şeker analizleri Thermo Scientific Ultimate 3000 HPLC (Drejeich, Almanya) cihazı ile gerçekleştirilmiştir. Analizlerin gerçekleştirilmesinde Transgenomic ICSep ORH-801 HPLC kolonu (6,5x300 mm, Apple Valley, ABD) kullanılmış ve analizler 0,01 N H_2SO_4 mobil faz, 0,5 ml/dk akış hızı, 20 µL enjeksiyon hacmi ve 70°C kolon sıcaklığında gerçekleştirilmiştir.

Kinetik parametrelerin belirlenmesi

Gerçekleştirilen fermantasyonlar süresince alınan örnekler ve gerçekleştirilen analizler sonucunda elde edilen veriler doğrultusunda aşağıda verilen denklemler ile kinetik parametreler hesaplanmıştır (Shuler ve Kargi, 2008).

- $Şeker\ tüketimi\ \left(\frac{g}{L}\right); S(Substrat) = S_1 - S_0$ (2)
- $Etanol\ üretimi\ \left(\frac{g}{L}\right); P(Ürün) = P_1 - P_0$ (3)
- $Ürün\ verimi\ \left(Y_{\frac{P}{S}}\right)(\%) = \left[Etanol\ üret.\left(\frac{g}{L}\right) \Big/ Şeker\ tük.\left(\frac{g}{L}\right)\right] \times 100$ (4)
- $Üretim\ oranı\ \left(\frac{g}{L\ sa}\right) = Etanol\ kurvesinin\ en\ dik\ kısmının\ eğimi$ (5)
- $Tüketim\ oranı\ \left(\frac{g}{L\ sa}\right) = Şeker\ kurvesinin\ en\ dik\ kısmının\ eğimi$ (6)

İstatistiksel değerlendirmeler

Etanol üretiminde gerçekleştirilen denemelere ait sonuçlar SAS programı (University Edition, Online Version) kullanılarak gerçekleştirilmiştir. Farklı çıkan uygulamalar çoklu karşılaştırma testlerine tabi tutulup sonuçlar %95 güven aralığında istatistiksel olarak değerlendirilmiştir. Tüm denemeler ve analizler en az iki tekerrürlü olarak gerçekleştirilmiştir ve sonuçlar "değer±stdhata" şeklinde verilmiştir (Düzgüneş ve ark., 1987).

BULGULAR

Üç boyutlu yazıcıda üretilen küplerin şeker tüketimi, etanol üretimi ve verim değerlerine etkisi

Çalışma kapsamında ilk olarak küplerin eklenmediği kontrol fermantasyonu gerçekleştirilmiştir. Kontrol fermantasyonunun haricinde ise 1,5 mm ve 2,5 mm kalınlığa sahip küplerle dört fermantasyon gerçekleştirilerek elde edilen sonuçlar Tablo 5'te verilmiştir.

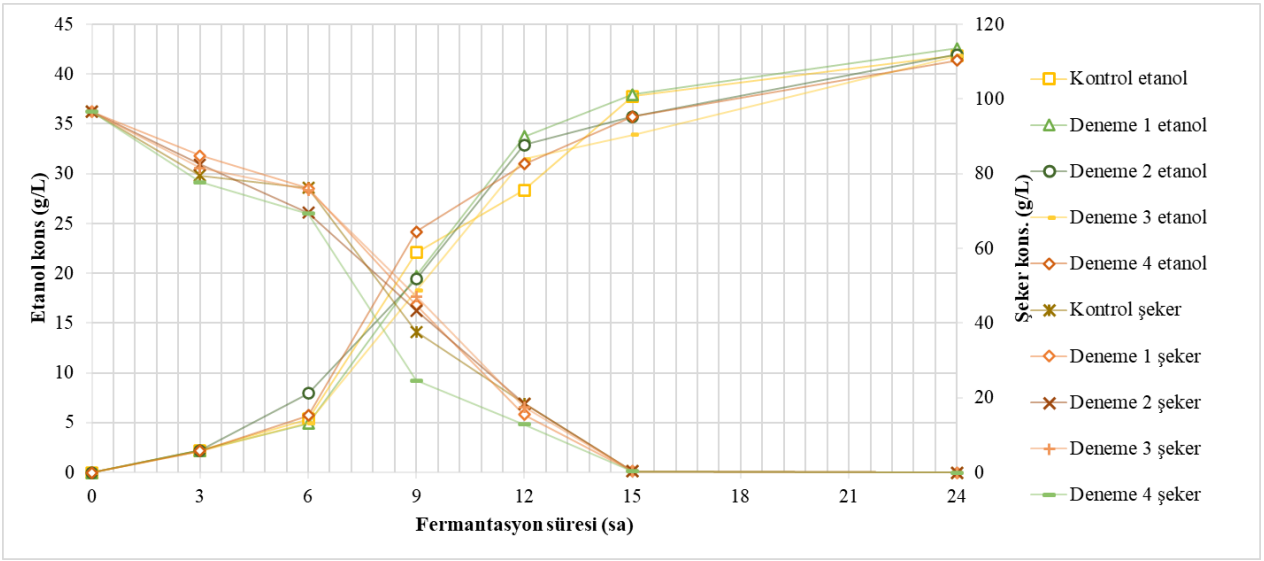
Tablo 5. Fermantasyonlara ait kinetik sonuçlar

	Şeker tüketimi (g/L)	Etanol üretimi (g/L)	Verim (%)	Biyokütle (g/L)
Kontrol	96,57^{a}±0,98	41,86^{b}±0,17	43,35^{a}±0,62	17,68^{a}±0,04
1	96,57^{a}±0,98	42,57^{a}±0,18	44,08^{a}±0,63	17,39^{c}±0,05
2	96,57^{a}±0,98	41,94^{ab}±0,11	43,43^{a}±0,55	17,58^{ab}±0,00
3	96,57^{a}±0,98	41,79^{b}±0,32	43,28^{a}±0,77	17,46^{bc}±0,01
4	96,57^{a}±0,98	41,38^{b}±0,02	42,85^{a}±0,45	16,83^{d}±0,05

*Veriler "değer±stdhata" olarak verilmiştir.

**Aynı sütunda farklı harfleri taşıyan değerler arasındaki fark önemlidir ($p<0,05$)

Veriler incelendiğinde şeker tüketimi ve verim değerleri arasındaki farklılığın istatistiksel olarak önemli olmadığı belirlenmiştir ($p<0,05$). Tüm fermantasyonlarda ortama ilave edilen şekerin tamamı tüketilmiş ve etanol üretimleri başarıyla gerçekleştirilmiştir. Biyokütle ve etanol üretim değerlerine bakıldığında ise elde edilen veriler arasındaki farkın istatistiksel olarak önemli olduğu ($p<0,05$) ve en yüksek etanol üretim değeri 42,57 g/L olarak 1,5 mm kalınlığa sahip küplerin kullanıldığı 1 numaralı denemede elde edildiği belirlenmiştir. Fermantasyonlar 48 saat sürdürülmesine karşın şeker kullanımı ve etanol üretimleri 24. saatte son bulmuştur (Şekil 3).



Şekil 3. Fermantasyonlara ait zamana bağlı şeker tüketim ve etanol üretim grafikleri

Fermantasyonlara ait grafikler incelendiğinde etanol üretim değerlerinin benzer olduğu görülmekle birlikte üretim hızlarının farklı olduğu da eğrilerden açıkça görülmektedir. Besiyerine 1,5 mm veya 2,5 mm kalınlığa sahip üç boyutlu küplerin ilave edilmesinin maksimum üretim ve tüketim değerlerini istatistiksel olarak önemli düzeyde arttırdığı belirlenmiştir (Tablo 6). En yüksek tüketim ve üretim oranları sırasıyla 9,68 g/L/sa olarak 3 numaralı denemede ve 4,80 g/L/sa olarak 1 numaralı denemede hesaplanmıştır. Bu değerler kontrol denemesinde elde edilen 8,23 g/L/sa ve 3,84 g/L/sa değerlerinden sırasıyla %17,62 ve %25 daha yüksektir. Elde edilen sonuçlar fermantasyon ortamına ilave edilen küplerin fermantasyon performansını substrat kullanım ve ürün üretim yönünden istatistiksel olarak önemli düzeyde arttırdığını göstermiştir ($p<0,05$).

Tablo 6. Fermantasyonlara ait maksimum tüketim ve üretim oranları

	Maksimum tüketim oranı (g/L/sa)	Maksimum üretim oranı (g/L/sa)
Kontrol	$8,23^{d}\pm0,05$	$3,84^{c}\pm0,41$
1	$8,54^{c}\pm0,06$	$4,80^{a}\pm0,03$
2	$8,50^{cd}\pm0,15$	$4,16^{ab}\pm0,08$
3	$9,68^{a}\pm0,01$	$4,41^{ab}\pm0,01$
4	$9,39^{b}\pm0,03$	$4,21^{ab}\pm0,10$

*Veriler "değer±stdhata" olarak verilmiştir.

**Aynı sütunda farklı harfleri taşıyan değerler arasındaki fark önemlidir ($p<0,05$)

Fermantasyon sonrası alınan küplerin stereomikroskopta yapılan incelemelerinde de kısmen immobilize olduğu görülmüştür (Görüntüler verilmemiştir). Mikroskobik incelemeler sonucunda SLS küreler gibi FDM küplerin de immobilizasyon destek materyali olarak kullanılabileceği düşünülmektedir.

TARTIŞMA

Literatürde yer alan iki çalışmada SLS tekniği ile üretilen farklı 3D materyallerin fermantasyon performansını arttırdığı bildirilmiştir (Dong ve ark., 2015; Belgrano ve ark., 2018). Bu çalışmalarda kullanılan teknolojinin ilk yatırım maliyeti yüksek olmakla birlikte sterilizasyona dayanıklı ve gıda ile temasa uygun hammadde kullanımına olanak sağladığından fermantasyon uygulamaları için SLS teknolojisi ön plana çıkmaktadır. Gerçekleştirilen çalışmada en ucuz 3D teknolojisi olan FDM teknolojisi ile gıda ile temasa uygun ve sterilizasyon sıcaklığına dayanıklı olduğu üretici firma tarafından bildirilen bitkisel bazlı STH filament ile oluşturulan küplerin fermantasyonlarda kullanılabilirliği araştırılmıştır. Elde edilen sonuçlar FDM teknolojisi ile üretilen küplerin de biyoteknolojik proseslerde rahatlıkla kullanılabileceğini göstermiştir.

SONUÇ

Etanol fermantasyonunda FDM teknolojisi ile üretilen küplerin etanol fermantasyonunda kullanımının maksimum tüketim ve üretim oranlarını arttırdığı görülmüştür. Ayrıca hücrelerin kısmen küpler üzerinde immobilize olması da SLS bazlı üç boyutlu materyaller gibi FDM bazlı üç boyutlu materyallerin de immobilize destek materyali olarak kullanılabileceğini göstermektedir. Bu çalışma en ucuz üç boyutlu yazıcı teknolojisi olan FDM teknolojisinin fermantasyonlarda kullanılacak materyallerin üretiminde STH filament ile rahatlıkla kullanılabileceğini göstermiştir. İlerleyen çalışmalarda FDM bazlı üç boyutlu materyallerin immobilizasyon destek malzemesi olarak kullanımı üzerine denemelerin yapılması bu tip materyallerin uygunluğunun belirlenmesi adına önemlidir.

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➢ ***ORAL PRESENTATION***

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Özet
Obezite her geçen gün artış gösteren küresel bir sorun olmaya başlamıştır. Ayrıca obezite görülen bireylerin yaş ortalaması da hızla küçülmektedir. Beslenme bozukluklarının getirdiği hastalıkların da görülme oranı artmıştır. Bu durum obezite, yeme bozuklukları, aşırı kilo, iştah gibi konulara dikkat çekmiş ve yapılan araştırmalar yemek yeme isteğini artıran ya da azaltan peptid hormonların literatüre kazandırılmasını sağlamıştır. Keşfedilen bu peptidler iştah açıp yemek yeme isteğini arttırıyorsa oreksijenik, yemek yeme isteğini azaltıyorsa anoreksijenik olarak isimlendirilirler. Bu çalışmada; söz konusu peptidler ve bunların çalışma mekanizmaları hakkındaki bilgiler derlenerek sunulacaktır.
Anahtar Kelimeler: Obezite, Peptid, İştah

Orexigenic and Anorexigenic Peptides

Abstract
Obesity has become a global problem that is increasing day by day. Besides, the average age of obese patients is falling rapidly. The incidence of diseases caused by eating disorders has also increased. This has drawn attention to issues such as obesity, eating disorders, overweight, appetite, and studies that have provided peptide hormones that increase or decrease the desire to eat. These discovered peptides are called orexigenic if they increase appetite and desire to eat, and anorexigenic if they reduce the desire to eat. In this study Information about the mentioned peptides and their working mechanisms will be compiled and presented.
Keywords: Obesity, Peptide, Appetite

GİRİŞ

Günümüzde enerji yoğun gıda tüketimi ve fiziksel aktivitedeki azalma nedeniyle aşırı kiloluluk ve obezite prevelansı artmıştır. Dünya sağlık örgütü raporlarında obezitenin 1980 yılından sonra iki kat arttığını bildirmiştir, 2008 yılındaki raporunda ise 1,8 milyar insanın aşırı kilolu, 500 milyon insanın ise obez olduğunu bildirmiştir (WHO 1998). 2012 yılında ise 5 yaş altındaki 40 milyon çocuğun aşırı kilolu veya obez olduğunu bildirmiştir.
Değişen beslenme alışkanlıklarıyla yüksek kalorili mısır şurubu ve margarinler gibi gıdalar bolca tüketilmeye başlanmıştır. Dünya Sağlık Örgütü 2016 verilerine göre, tüm dünyada erişkinlerin %13'ünün obez, %39'unun ise aşırı kilolu oldukları hesaplanmıştır ve bu oranlar bir pandemiye işaret etmektedir (WHO 2018). Dünyada görülme sıklığı giderek artan obezite, yaşam süresini kısaltan ve önemli tedavi maliyetlerine neden olan tip 2 diyabet, koroner arter hastalığı, hipertansiyon, kronik karaciğer hastalığı, meme kanseri, kolon kanseri, depresyon, infertilite, uyku apnesi, osteoartrit ve inme gibi birçok hastalık ile ilişkilidir (Şahin ve Yalnız 2018)

BULGULAR ve TARTIŞMA

Asprosin

Romere ve arkadaşları tarafından 2016 yılında tanımlanan peptid yapılı bir hormondur (Romere ve ark.2017). Asprosinin salınımının açlık durumunda arttığı ve karaciğerden kana G protein –cAMP –PKA yolu aracılığı ile glikoz geçişini sağladığı bildirilmiştir. Plazma asprosinin, plazma glikoz ve lipid metabolizmasıyla ilişkili olduğunu ayrıca insülin direnci ve pankreatik β-Hücrelerinin fonksiyonuyla ilişkili olduğunu bildirmiştir. Dolaşımdaki asprosinin kan-beyin bariyerini geçtiği ve oreksijenik AgRP + nöronlarını cAMP'ye bağlı bir yolla doğrudan aktive ettiği gösterilmiştir. Bu sinyalleşme, anoreksijenik proopiomelanokortin pozitif nöronların GABA'ya bağlı bir şekilde inhibisyonuna neden olur ve bu da daha sonra iştah uyarılmasına ve adipozite ve vücut

ağırlığını biriktirme dürtüsüne yol açar. İnsanlarda, asprosinde genetik bir eksiklik, iştahsızlık ve aşırı zayıflıkla karakterize bir sendroma neden olur (Romere ve ark.2017; Que ve ark 2017; Zhang ve ark,2017; Duerrcchmid ve ark, 2017).

Glukojenik fonksiyon gerçekleştirmenin yanı sıra, asprosin, hem obezite hem de diyabet tedavisinde potansiyel bir terapötik hedef olan, merkezi olarak etkili oreksijenik bir hormondur (Duerrcchmid ve ark, 2017).

Apelin

Apelin 1993 yılında önce reseptörü ardından 1998 yılında endojen ligandının keşfiyle Totemato ve arkadaşları tarafından tanımlanmıştır. Apelinin kalp, akciğer ve böbreklerde daha fazla işlev gördüğü tespit edilmiştir. Obez bireylerde apelinin HDL, LDL, Trigliserit ve kolesterol başta olmak üzere bazı biyokimyasal parametreleri olumsuz yönde etkilediği aterosklerotik plak oluşumunu ve kalp damar hastalıklarını ortaya çıkardığı ileri sürülmektedir (Sandal ve Tekin, 2013).

Apelin merkezi sinir sisteminde, özellikle hipotalamusta ve birçok periferik dokuda ifade edilir. Apelin'in, kardiyovasküler ve sıvı homeostazının, gıda alımının, hücre proliferasyonunun ve anjiyogenezin düzenlenmesinde rol oynadığı gösterilmiştir. Her yerde bulunan bir peptit olmasının yanı sıra, apelin ayrıca adipositler tarafından üretilir ve salgılanır ve bu nedenle bir adipokin olarak kabul edilir. Apelin, anti-obezite ve anti-diyabetik özelliklere sahip faydalı bir adipokin olarak ve dolayısıyla metabolik bozukluklarda umut verici bir terapötik hedef olarak kabul görmektedir (Marta ve ark, 2018).

Ghrelin

Oreksijenik bir hormon olarak bilinen ghrelin; insan ve hayvanlarda büyümeyi situmile eder ve büyüme hormonu salgılatır. Enerji dengesinin düzenlenmesi ve glikoz metabolizmasında etkilidir. 44 amino asitten oluşur ve hipotalamik bir peptitdir (Dabak ve ark. 2008).

Hipotalamus, hipofiz, tükrük bezi tiroit bezi, ince bağırsak, böbrekler, kalp pankreasın α hücreleri, gonadlarda sentezlenen bu peptidin esas sentezlendiği yer midedir. Ghrelin hayvanlarda beslenme davranışında, insanlarda ise iştahın düzenlenmesinde görev alır (Aydın 2006).

Leptin

Ob genden kopyalanan 167 aminoasitli bir protein olan leptin, obeziteye eğilimli bir suş olan ob / ob mouse'daki moleküler kusuru tanımlamaya yönelik araştırma sırasında fareden klonlanmıştır. Leptin esas olarak beyaz yağ dokusunda üretilir; kahverengi yağ dokusunda çok küçük miktarlarda bulunur. İlk başta leptin, gıda alımını sınırlayan ve enerji tüketimini artıran, adiposit türevi sinyal veren bir molekül olarak görülmüştür. Leptinin bir adiposit olduğu iddiasını destekleyen kanıtlar, leptin ile enjekte edilen genetik veya diyete bağlı obez olan kemirgenlerde vücut ağırlığındaki azalma ve metabolik kontrolün iyileştirilmesiyle sağlanmıştır. Leptinin dikkate değer bir etkisi, laboratuar hayvanlarına enjekte edildiğinde glikoz homoeostazını geliştirme kapasitesidir (O'Donella ve ark. 2006).

Nesfatin-1

Nesfatin-1, 2006 yılında homeostatik beslenmenin düzenlenmesinde rol oynayan güçlü bir anoreksijenik peptit olarak tanımlanmıştır. Hipotalamusta melanokortin yoluyla yemek alımını baskıladığı düşünülmektedir (Algül ve Özçelik, 2012). Beyinde ve periferal dokularda nesfatin-1'in lokalizasyonunun belirlenmesinde ilerlemeler olmuştur. Hipotalamus ve beyin sapında nesfatin-1, anoreksijenik özelliklerini iletmek için oksitosin, melankortin ve diğer sistemleri görevlendirir. Başlangıçta keşfedilen anoreksijenik özelliklerinin yanı sıra, son yıllarda nesfatin-1'in diğer önemli işlevleri keşfedilmiştir, bunların çoğu enerji homeostazı ile ilgilidir. Nesfatin-1 sadece bu fizyolojik süreçleri etkilemekle kalmaz, aynı zamanda metabolik durumdaki değişikliklerinde, (örneğin yağ kütlesi, glisemik durum) nesfatin-1 sentezi ve salınımı üzerinde etkisi vardır. Dahası, nesfatin-1, kardiyovasküler ve sindirim sistemleri düzeyinde pleiotropik işlevler uygulamakla birlikte stres yanıtı, davranış, uyku ve üremede de rol oynar (Algül ve Özçelik;2012, Dore ve ark. 2017).

Adropin

Adropin 2008 yılında kumar ve arkadaşları tarafından keşfedilmiş76 aminoasitten oluşmuş enerji homeostasisinde ve diyabette etkili polipeptid yapılı bir hormondur. Beyin, karaciğer ve plazma enerji hemoastasisinde görev yapar (Aydın 2014).

Karaciğer ve beyinde ifade edilen Enerji Homeostaziyle İlişkili Gen tarafından kodlanır, ancak varlığı kas, kalp, pankreas ve böbreklerde de kanıtlanmıştır. Yapılan aştırmalarda, adropinin, glikoz metabolizmasının ve yağ asitlerinin oksidasyonunun fizyolojik bir düzenleyicisi olama rolüne işaret edilmiştir. Diyetle uyarılmış obeziteye sahip farelerin, adropin ile tedavi edildiğinde, artmış glukoz toleransı, azalmış insülin direnci ve oksidatif reaksiyonlarda karbonhidrat artışı gösterdiği bulunmuştur. Obezite ve insülin direnci olan kişilerde adropin konsantrasyonunun azaldığı ve vücut ağırlığı kaybının adropin düzeylerinde artışa neden olduğu bulunmuştur (Akçılar; 20015).

Resistin

Resistin, ilk olarak 2001 yılında farelerde keşfedilmiştir ve insülin etkisine direnme kabiliyeti ile adlandırılmıştır. Başlangıçta adiposite özgü bir hormon olarak tanımlanan resistinin, obezite, insülin direnci ve diyabet arasında önemli bir bağlantı olduğu öne sürülmüştür (Aydın 2014). Ekspresyonu başlangıçta adipositlerde tanımlanmış olmasına rağmen, insanlarda esas olarak mononükleer lökositler, makrofajlar, dalak ve kemik iliği hücrelerinde önemli seviyelerde resistin ekspresyonu bulunur. Artan kanıtlar, resistinin çeşitli biyolojik süreçlerde insülin direnci ve diyabetteki rolü dışında önemli düzenleyici roller oynadığını göstermektedir: ateroskleroz ve kardiyovasküler hastalık, alkolsüz yağlı karaciğer hastalığı, otoimmün hastalık, malignite, astım, inflamatuar bağırsak hastalığı ve kronik böbrek hastalığı. Resistin'in hedef hücreleriyle etkileşime girdiği etki ve sinyal yolları anlaşılmaya başlandı resistin araştırması alanında klinik çeviriler, terapötik düşünceler ve gelecekteki yönlendirmeler tartışılmaktadır (Ergün 2003, Jamaluddin ve ark; 2012).

SONUÇ

Obezite araştırmalarının yoğunlaşmasıyla birlikte peptid yapılı hormonların araştırılmasıda artmıştır. Yeni üyelerin litaratüre kazandırılması dikkatleri; çalışma mekanizmalarının aydınlatılması ve birbirleriyle ve diğer enerji metabolizması unsurlarıyla ilişkisi üzerine çekmiştir. Büyük çoğunluğu yakın zamanda keşfedilmiş olan bu peptidlerin daha iyi anlaşılabilmesi için haklarında daha çok araştırma yapılması gerekmektedir.

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➢ *ORAL PRESENTATION*

Primula Vulgaris Ekstraktlarının Serviks Kanseri Hücrelerinde Hücre Proliferasyonu ile BCL2, MDR1 ve p53 Gen Ekspresyonları Üzerine Etkileri

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Özet

Serviks kanseri dünya çapında kadınlar arasında meme kanserinden sonra en yaygın ikinci kanser türüdür. HeLa hücreleri bir insan serviks kanseri hücre hattıdır. HeLa; kanserli hücrelerin moleküler biyolojik özelliklerini incelemek amacıyla en yaygın kullanılan model hücre hattıdır. Bu çalışmada, *Primula vulgaris* bitkisinin çiçek ve yaprak ekstraktları yüklü gümüş nanopartiküllerinin(AgNP) HeLa hücre hattında hücre proliferasyonu ile BCL2, MDR1 ve p53 gen ekspresyon seviyeleri üzerine etkilerinin araştırılması amaçlandı. *Primula vulgaris* bitkisinin çiçek ve yaprak ekstraktları yüklü AgNP IC_{50} doz değerleri iCELLigence gerçek zamanlı hücre analiz sistemiyle (RTCA) belirlendi. IC_{50} doz belirlenmesinin ardından, IC_{50} doz grubu (1. Grup), LD_{50} doz grubu (2. Grup), Sisplatin grubu (3. Grup), bitki ekstraktı yüklenmemiş AgNP doz grubu (4. Grup) ve kontrol grubu (5. Grup) olmak üzere 5 farklı grupta BCL2, MDR1 ve p53 gen ekspresyon seviyeleri qRT-PCR yöntemiyle analiz edildi. HeLa hücre hatlarına *Primula vulgaris* çiçek ekstraktı yüklü AgNP ilavesi ardından 12. saat ve 24. saatte belirlenen IC_{50} değerleri sırasıyla 58,39 ug/mL ve 57,65 ug/mL'dir (r^2= 1). *Primula vulgaris* yaprak ekstraktı yüklü AgNP ilavesi ardından 12. saat ve 24. saatte belirlenen IC_{50} değeri sırasıyla 42,14 ug/mL ve 48,50 ug/mL'dir (r^2= 1). *Primula vulgaris* çiçek ve yaprak ekstraktı yüklü AgNP uygulanan HeLa hücrelerinin ölüm oranlarının doza bağlı olarak artış gösterdiği belirlenmiştir($p<0.05$). *Primula vulgaris* çiçek ekstraktı yüklü AgNP IC_{50} doz grubunda kontrol grubuna göre; BCL2 gen ekspresyon miktarında 0,7 kat artış, MDR1 gen ekspresyon miktarında 1,2 kat artış ve p53 gen ekspresyon miktarında 2,6 kat azalma gözlenmiştir. Sonuç olarak, *Primula vulgaris* çiçek ve yaprak ekstraktları yüklü AgNP'lerin HeLa hücre hattı üzerinde dozla orantılı olarak hücre ölümüne neden olduğu belirlenmiştir. *Primula vulgaris* çiçek ekstraktı yüklü AgNP dozundaki artışla orantılı olarak BCL2 ve MDR1 gen ekspresyon seviyelerinde ortaya çıkan artışlar ilaç direnci gelişimi açısından değerlendirilmelidir. *Primula vulgaris* ekstraktlarının serviks kanserinin tedavisinde kullanımı konusunda daha detaylı ve in vivo araştırmaların yapılmasına ihtiyaç bulunmaktadır.

Anahtar Kelimeler: *Primula vulgaris*, HeLa, p53, BCL2, MDR1, Gen, Ekspresyon

Effects of Primula Vulgaris Extracts on Cell Proliferation and BCL2, MDR1 and p53 Gene Expressions in Cervical Cancer Cells

Abstract

Cervical cancer is the second most common type of cancer among women worldwide after breast cancer. HeLa cells are a human cervical cancer cell line. HeLa cell lines are the most widely used model cell line to study the molecular biological properties of cancerous cells. In this study, it was aimed to investigate the effects of silver nanoparticles (AgNP) loaded with flower and leaf extracts of *Primula vulgaris* plant on cell proliferation and BCL2, MDR1 and p53 gene expression levels in HeLa cell line. IC_{50} dose values of AgNP loaded with flower and leaf extracts of *Primula vulgaris* plant with were determined by iCELLigence real time cell analysis system (RTCA). After IC_{50} dose determination, IC_{50} dose group (1st Group), LD_{50} dose group (2nd Group), Cisplatin added group (3rd Group), AgNP dose group without plant extract loaded (4th Group) and control group (5. Group), BCL2, MDR1 and p53 gene expression levels in 5 different groups were analyzed by qRT-PCR method. After the addition of AgNP loaded with *Primula vulgaris* flower extract to HeLa cell lines, the IC_{50} values determined at the 12th hour and 24th hour are 58,39 ug/mL ve 57,65 ug/mL, respectively (r^2= 1). After the addition of AgNP loaded with *Primula vulgaris* leaf extract, the IC_{50} value determined at the 12th and 24th hour is 42,14 ug/mL ve 48,50

ug/mL'dir, respectively (r^2= 1). It was determined that the death rate of HeLa cells treated with AgNP loaded with flower and leaf extract of *Primula vulgaris* increased depending on the dose ($p < 0.05$). According to the control group in IC_{50} dose group of AgNP loaded with *Primula vulgaris* flower extract; A 0.7-fold increase in the amount of BCL2 gene expression, a 1.2-fold increase in the MDR1 gene expression amount and a 2.6-fold decrease in the amount of p53 gene expression were observed. As a result, it was determined that AgNPs loaded with flower and leaf extracts of *Primula vulgaris* caused cell death depending on the dose in HeLa cell line. Increases in BCL2 and MDR1 gene expression levels in proportion to the increase in the dose of AgNP loaded with *Primula vulgaris* flower extract should be evaluated in terms of the development of drug resistance. More detailed and in vivo studies are needed on the use of *Primula vulgaris* extracts in the treatment of cervical cancer.

Keywords:*Primula vulgaris,* HeLa, p53, BCL2, MDR1, Gene, Expression

1. GİRİŞ

Kadın üreme sistemi organlarından servikste meydana gelen kanserler serviks kanseri olarak adlandırılır. Serviks kanseri; kadınlar arasında meme kanserinden sonra en yaygın görülen ve ölüme neden olan kanser tipidir [1]. Dünya genelinde 2018 yılı istatistiklerine göre serviks kanseri kadınlarda kansere bağlı ölümlerinin %7,5 ini oluşturur ve her yıl tahmini yeni vaka sayısı 570.000 ile kadınlar arasında meme kanserinden sonra ikinci en sık görülen kanser türüdür [2] ve bunların %85'inden fazlası az gelişmiş bölgelerde meydana gelmektedir. Serviks kanseri risk faktörleri dikkat alındığında önlenebilir bir hastalıktır[3]. Serviks kanserinde hücre proliferasyonundan sorumlu sinyal yollarındaki kritik proteinlerin regülasyonu bozulmaktadır. Örneğin, p53 ve pRb gibi iki önemli supresor proteini inhibe olmaktadır [4]. Günümüzde serviks kanserinin tedavisinde cerrahi müdahale, radyoterapi ve sisplatin ve tedavi kombinasyonları gibi platin bazlı kemoterapi kullanılmaktadır [5].

BCL2 bir proto-onkogendir ve apoptozu engelleyici protein kodlar. Bu gen ilk defa B hücreli Lenfoma'da 14. ve 18. kromozomlar arasındaki translokasyon kırılma noktalarında keşfedilmiştir [6]. BCL2 ailesi BAX, BAK, BAD, BCL-Xs, BİD, BİK, BİM ve HRK gibi pro-apoptotik; BCL2, BCL-XL, BCL-W, BFL-1 ve MCL-1 gibi anti-apoptotik proteinlerden oluşmaktadır [7]. BCL2 ekspresyonunda azalma antikanser ilaçlara karşı apoptotik tepkiyi yükseltirken ekspresyonunun artması radyasyon terapisine ve kemoterapötik ilaçlara karşı dirence neden olur. Pro-apoptotik BCL2 üyeleri apoptoz destekleyici olarak çalışırlarken anti-apoptotik üyeler sitokrom C'nin salınımını engeller ve apoptozu önleyici olarak çalışırlar. BCL2'nin intrinsik yolakta over-ekspresyonu, ekstrinsik kontrollü apoptozu baskılar [8]. Serviks kanserinin gelişiminde HPV enfeksiyonu önemli rol oynar[9]. HPV E6 ile kompleks oluşturarak p53'ü inaktif eder ve apoptozu baskılar [10]. Çoklu ilaç direnci (MDR1) geni; ABCB1 olarak da adlandırılır. Transmembran taşıyıcı bir gen olan P-glikoproteini (P-gp) kodlar [11]. P-glikoprotein hem birçok ilacın hücre dışına taşınmasında hem de metabolizma atıklarının hücre dışına taşınmasında önemli rol oynar [12,13]. P-gp; kanser hücrelerinde gen ifadesinin yükselmesi ve kemoterapi ilaçlarına karşı gelişen kemoterapötik direnç fenotipi sonucu keşfedilmiştir. P-gp, sadece kanser hücrelerine çoklu ilaç direnci sağlamanın yanı sıra normal dokulardaki birçok ilacın farmakokinetiğinide etkilemektedir [12]. Serviks kanserli MDR1 gen ekspresyonu azalmış olan bireylerin yaşam ömürleri, MDR1 gen ekspresyonu artmış olan bireylere göre daha uzundur [14]. p53 geni; 17. kromozomun p kolunda yerleşen (17p13.1) bir tümör supresor bir gendir. DNA sentez ve tamiri, transkripsiyon ve apoptoz gibi önemli biyolojik aktivitelerde görev alır. Birçok kanserin gelişimde p53 geninde ortaya çıkan mutasyonlar yatmaktadır [15]. Normal koşullarda inaktif halde bulunan p53 geni DNA hasarı ile aktifleşir. p53 geni DNA transkripsiyonu üzerinde etkisi bulunan birçok genin ekspresyonunu düzenler [16]. DNA hasarı ile aktifleşen p53 geni hasara; hücre döngüsünün durdurulması, DNA hasar tamiri ve programlı hücre ölümü (apoptozis) ile karşılık verir [17]. Serviks kanserinin gelişiminde rol oynayan HPV [9], E6 ile kompleks oluşturarak p53'ü inaktif eder ve apoptozu baskılar [10]. Serviks kanserinin tedavisinde kullanılabilecek kemoterapötik etkili yeni tedavi seçeneklerinin geliştirilmesi son derece önemlidir. Bu çalışmada, *Primula vulgaris* bitkisinin çiçek ve yaprak ekstraktları yüklü gümüş nanopartiküllerinin (AgNP) HeLa hücre hattında hücre proliferasyonu ile BCL2, MDR1 ve p53 gen ekspresyon seviyeleri üzerine etkilerinin belirlenmesi amaçlanmıştır.

2. GEREÇ VE YÖNTEM

2.1. HeLa Hücre Hattı Kültürü: HeLa (ATCC® CCL-2™) epitel hücrelerden ve adenokarsinomalardan türevlenen insan servikal kanser hücre hattıdır [30].HeLa hücre hattı; %10 FBS (FetalBovineSerum),%1 Pen-Strep (Penicilin-Streptomycin) ve %1 L-Glutamin içeren RPMI-1640 Mediumile 37^{o}C'de %5'lik CO_2'li ortamda kültüre edildi.

2.2. RTCA Sisteminde HeLa Hücrelerine *Primula Vulgaris* Ekstraktı Yüklü AgNP'lerin Uygulanması: Tüm uygulamalar %5'lik CO_2 içeren ortamda 37^{o}C'lik inkübatörde yapıldı. RTCA sisteminde hücre analizleri E-plateler kullanıldı. E-platelerin her bir kuyucuğuna 250 ul kültür mediumu ilave edildikten sonra 37^{o}C'de hücre indeksi değeri sıfırlandı. RTCA sistemi için en uygun HeLa hücre sayısını belirlemek amacıyla thoma lamında hücre sayım işlemi yapıldı. Farklı kuyulara $1x10^4$, $2x10^4$ ve $3x10^4$ hücre/ml konarak hücre sayısı optimizasyonu yapıldı. RTCA sistemi için en uygun hücre sayısının $2x10^4$ olduğu belirlenmiştir. Daha sonra her kuyucukta $2x10^4$ hücre/mL ve totalde her kuyucukta 600 ul kültür mediumu olacak şekilde hücre ekim işlemi gerçekleştirildi. Hücrelerin zemine çökmesini sağlamak için toplamda 30 dk oda sıcaklığında plateler bekletildi ve 96 saat boyunca her 15 dk'da bir ölçüm yapacak şekilde sistem başlatıldı. 20. saatte E-plateler sistemden çıkarılarak; 25 ug/mL, 50 ug/mL, 75 ug/mL, 100 ug/mL ve 125 ug/mL *Primula vulgaris* bitkisinin çiçek ve yaprak ekstraktı yüklü AgNP'ler ilave edildi. 96 saat boyunca yapılan hücre indeksi ölçümleriyle IC_{50} değerleri belirlendi.

2.3. RNA İzolasyonu ve cDNA Sentezi: Total RNA HeLa hücrelerinden Wizprep^{TM}Total RNA Mini Kit (Tissue) protokolüne uygun olarak izole edildi.İzole edilen total RNA'lar --86^{0}C'de saklandı.RNA'nın OD ölçümü için JenwayGenova cihazı kullanıldı. Primer olarakrandomhekzamerler kullanılarak WizScriptcDNASynthesisKit (HigCapacity) ile birinci, ikinci ve üçüncü örnekler için 6 mL, dördüncü ve beşinci örnekler için 5 mL total RNA'dan üretici firmanın protokolü takip edilerek cDNA sentezi yapıldı. cDNA'lar -20^{o}C'de saklandı.

2.4. Gerçek Zamanlı Polimeraz Zincir Reaksiyonu(qRT-PCR) Analizi: RocheLightCycler 480 cihazında kantitatif olarak gerçek zamanlı polimeraz zincir reaksiyonuna (qRT-PCR) alındı. Her grup için PCR cihazında 3 tekerrür çalışıldı. Çalışmada RocheLightCycler 480 II qPCR cihazına uygun SensiFAST^{TM}SYBR^{R}No-ROX kiti kullanıldı. Araştırılan genlerin cDNA'larına özgül olan primer dizileri Tablo 1'de verilmiştir.Reaksiyonun ardından spesifik gen ürünlerinin varlığı basicrelativequantification analizi ile doğrulandı ve elde edilen BCL2, MDR1 ile p53 genlerine ait Ct değerleri GAPDH housekeeping gen ekspresyon düzeylerine göre normalize edildi. Sistem üzerinden elde edilen C_t değerleri kullanılarak $2^{-\Delta\Delta Ct}$ metodu ile değişim farklılıkları analiz edildi.

2.5. İstatistiksel Analizler: 12 ve 24 saat boyunca elde edilen hücre indeks değerlerinin istatistiksel olarak karşılaştırılmasında parametrik olmayan bir test olan "Mann Whitney U" ve "SPSS 21.0 Paket Programı" kullanılmıştır. $p<0,05$ değerindeki sonuçlar istatistiksel olarak anlamlı kabul edilmiştir. Gen ekspresyon sonuçlarının değerlendirilmesinde $2^{-\Delta\Delta Ct}$ metodu kullanılmıştır.

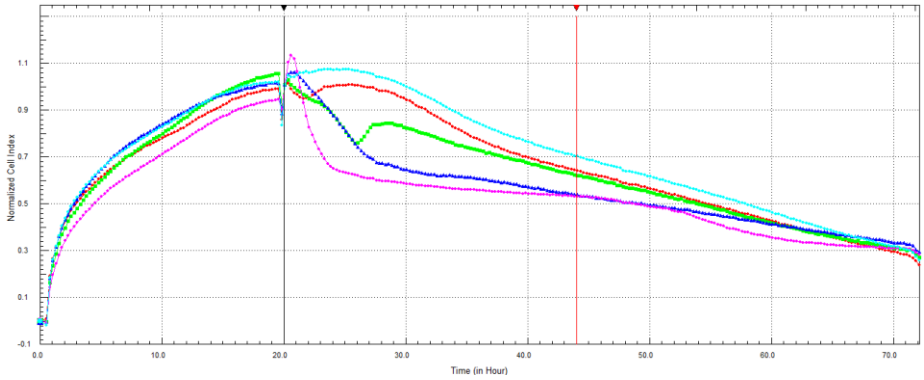
Tablo1: Bcl-2, MDR1 ve p53 gen ekspresyon analizinde kullanılan primerlerin baz dizilimleri.

BCL2- F	**5'-TGT TTT GAG AGC GTC AAC CG-3'**
BCL2- R	5'- TCA GGT AGA GGC CGC ATG CTG-3'
GAPDH- F	5'- GTC GTA TTG GGC GCC TGG TCA-3'
GAPDH- R	5'- GCC AGC ATC GCC CCA CTT GAT- 3'
MDR-1- F	5'- AAC TTC TAT CCC ACC CGA CGG- 3'
MDR-1- R	5'- GTA CTG CAG TCA AAC AGA TGG TT-3'
p53- F	5'- CAC GAG CGC TGC TCA GAT AGC-3'
p53- R	**5'- ACA GGC ACA AAC ACG CAAAA-3'**

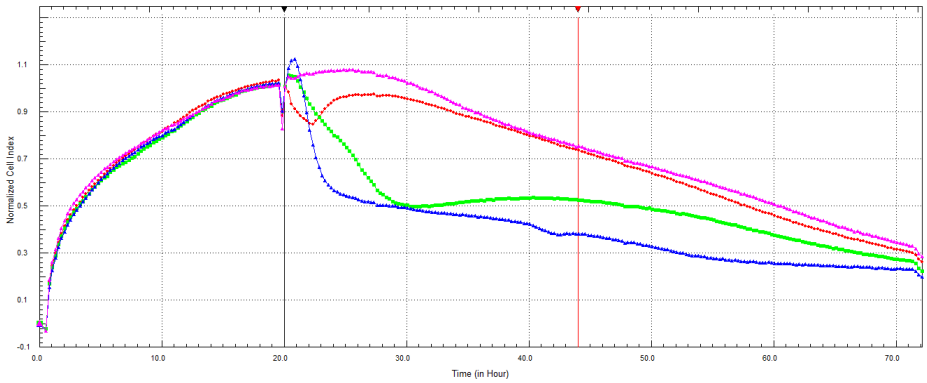
3 BULGULAR

RTCA sisteminde ekimi yapılan HeLa hücrelerine 20. saatte madde ilaveleri yapıldı. Madde ilavesinin yapılmasından 12 ve 24 saat (kültürün 32. ve 44. saatleri) sonra Sigmoidal dose response (variableslope) yöntemiyle IC_{50} değeri hesaplanmıştır. Çiçek ekstraktı yüklü AgNP için hesaplanan IC_{50} değeri sırasıyla 58,39 ug/mL ve 57,65 ug/mL olarak belirlenmiştir (r^2=1)(Şekil 1).Yaprak ekstraktı yüklü AgNP için hesaplanan IC_{50} değeri ise; sırasıyla 42,14 ug/mL ve 48,50 ug/mL belirlenmiştir (r^2=1)(Şekil 2). Kontrol hücre grubu ile 25, 50,

75 ve 100 ug/mL çiçek ekstraktı ve 25, 50 ve 75 ug/mL yaprak ekstraktı yüklü AgNP ile yapılan IC_{50} çalışmasında 12 ve 24 saat maruz bırakılan hücre indeks değerlerinin istatistiksel karşılaştırılmasında; kontrol grubuna göre çiçek ekstraktı yüklü AgNP sırasıyla 25, 50, 75 ve 100 ug/mL dozları ve yaprak ekstraktı yüklü AgNP 25, 50 ve 75 ug/mL dozları 12. ve 24. saatlerde istatistiksel olarak anlamlı seviyede HeLa hücrelerinin çoğalmasını durdurarak hücre ölümüne neden olmuştur ($p<0.05$).

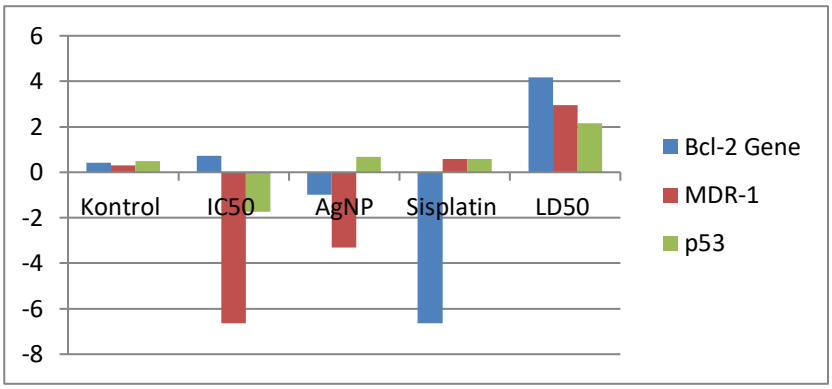


Şekil 1: iCELLigence sisteminde çiçek ekstraktı yüklü AgNP HeLa hücrelerinin hücre canlılığı optimizasyonu. Turkuaz: Normal hücre, Kırmızı: 25 ug/mL, Yeşil: 50 ug/mL, Mavi: 75 ug/mL, Pembe: 100 ug/mL



Şekil 2:iCELLigence sisteminde yaprak ekstraktı yüklü AgNP HeLa hücrelerinin hücre canlılığı optimizasyonu. Pembe: Normal hücre. Kırmızı: 25 ug/mL,Yeşil: 50 ug/mL ve Mavi: 75 ug/mL..

IC_{50} doz grubunda BCL2 gen ekspresyon miktarında kontrol grubuna göre 0,7 kat artış meydana gelmiştir. AgNP doz grubunda BCL2 gen ekspresyon miktarında kontrol grubuna göre 0,8 kat azalma görülmüştür. Sisplatin doz grubunda BCL2 gen ekspresyon miktarında kontrol grubuna göre 1,3 kat azalma görülmüştür. LD_{50} doz grubunda BCL2 gen ekspresyon miktarında kontrol grubuna göre 16,9 kat artış gözlenmiştir (Şekil 3). IC_{50} doz grubunda MDR1 gen ekspresyon miktarında kontrol grubuna göre 1,2 kat azalma meydana gelmiştir. LD_{50} doz grubunda MDR1 gen ekspresyon miktarında kontrol grubuna göre 6,5 kat artış gözlenmiştir. Sisplatin doz grubunda MDR1 gen ekspresyon miktarı kontrol grubuna göre 0,3 kat artış gözlenmiştir. AgNP doz grubunda MDR1 gen ekspresyon miktarında kontrol grubuna göre 1,1 kat azalma görülmüştür (Şekil 3).IC_{50} doz grubunda p53 gen ekspresyon miktarında kontrol grubuna göre 2,6 kat azalma meydana gelmiştir. Sisplatin doz grubunda p53 gen ekspresyon miktarında kontrol grubuna göre 1,4 kat azalma görülmüştür. AgNP doz grubunda p53 gen ekspresyon miktarında kontrol grubuna göre 1,3 kat azalma meydana gelmiştir. LD_{50} doz grubunda p53 gen ekspresyon miktarında kontrol grubuna göre 1,6 kat artış gözlenmiştir (Şekil 3).



Şekil 3: Göreceli gen ekspresyon sonuçlarının log2 tabanında gösterimi.

3. TARTIŞMA

Bu çalışmada, HeLa hücre hatlarında *Primula vulgaris* ekstraktı yüklü AgNP'lerin hücre çoğalması ile BCL2, MDR1 ve p53 gen ekspresyon seviyeleri üzerine etkisi incelenmiştir. Tümörler değişik fenotiplerde ve değişik metastatik özelliklerde olabilmektedir[18]. Serviks kanserinin tedavisinde etkili tedavi stratejilerinin geliştirmesi hastalığa bağlı ölümleri azaltmak açısından önemlidir[19].İnsanlığın varoluşundan itibaren bitkiler çeşitli hastalıkların tedavisinde kullanılmaya başlanmıştır. Son yıllarda ise bitkilerden elde edilen etken maddelerin özellikle insan kanserlerinin tedavisinde kullanımları ve hücreler üzerinde etkilerinin araştırılmaları yaygınlaşmaktadır[20, 21, 22]. Günümüzde, bitkisel kaynaklı etken maddelerin kanser hücre hatlarında antiproliferatif etkileri olduğu yönünde ciddi bulgular ortaya çıkmaktadır. Çeşitli çalışmalarda, farklı *Primula* türlerinin kanserli hücre hatları üzerinde sitotoksik etkileri araştırılmıştır[23, 24, 25, 26]. Literatürde; HeLa hücre hatlarında *Primula vulgaris* ekstraktının gen ekspresyon seviyeleri üzerine etkisini araştıran başka bir çalışma bulunmamaktadır. Bu çalışma; HeLa hücre hatlarında *Primula vulgaris* ekstraktı yüklü AgNP'lerin BCL2, MDR1 ve p53 gen ekspresyon seviyeleri üzerine etkisini araştırmayı amaçlayan ilk çalışma olma özelliğindedir.

Programlı hücre ölümü anlamına gelen apopitoz [27], hücrelerde DNA hasarı başta olmak üzere pek çok biyokimyasal veya fizyolojik olaya yanıt olarak ortaya çıkabilmektedir[28]. Parkinson, tip1 diyabet, hepatit C ve MI gibi hastalıklarda apoptoz hızlanırken kanserlerde ve otoimmün hastalıklarda yavaşladığı tespit edilmiştir[29]. Demir S. ve ark. (2018), *Primula vulgaris* çiçek ekstraktının normal fibroblast hücrelerine kıyasla HeLa hücrelerinde konsantrasyona bağlı olarak mitokondriyal membran potansiyelini azalttığı, hücrelerin siklusun S fazında durmasını sağlayarak seçici bir sitotoksik etki ortaya çıkardığını ve apoptotik hücrelerin sayısının arttığını belirlemişlerdir [23]. *Primula vulgaris* bitkisi çiçeklerinden dimetilsülfoksit kullanılarak elde edilen ekstraktın insan kolon (WiDr), akciğer (A549), karaciğer (HepG2), meme (MCF7), ve prostat (PC-3) kanser hücre hatları üzerine etkilerinin araştırıldığı bir çalışmada; tüm kanserli hücre hatları üzerinde seçici bir şekilde sitotoksik etkiye sahip olduğunu ortaya koymuşlar ve IC_{50} değerini 191,8-375,3 ug/mL olarak belirlemişlerdir [30]. Turan İ. ve arkadaşları (2017) *Primula vulgaris* bitkisinin yaprak ekstraktlarının insan akciğer (A549), karaciğer (HepG2), meme (MCF-7), prostat (PC-3) ve kolon (WiDr) kanser hücre hatları üzerinde normal fibroblast hücrelerine kıyasla sitotoksik etkileri olduğunu ortaya koymuş ve beş kanser hücre hattında IC_{50} değerinin 133,3-253,8 ug/mL arasında değiştiğini saptamışlardır [31]. Özkan ve ark. (2018), hidrofobik karakterli, sitoplazma içerisine kolayca difüze olarak hidroksil radikallerinin transformasyonuna neden olan H2O2'nin fibroblast hücrelerinde DNA kırıklarına neden olduğunu belirlemişlerdir. DNA kırığı oluşturulan fibroblast hücre kültürlerine *Primula vulgaris* ekstraktı ilavesiyle doza bağlı olarak %16-32 oranında DNA kırıklarında azalmaların olduğu belirlenmiştir[32]. Bu çalışmada; *Primula vulgaris* çiçek ve yaprak ekstraktı yüklü AgNP'lerin HeLa hücre hattı üzerine doza bağlı sitotoksik etki gösterdiği gözlenmiştir ($p<0.05$). Literatür bilgileri ışığında, *Primula vulgaris* çiçek ve yaprak ekstraktı yüklü AgNP'lerin HeLa hücre hatları üzerine proliferasyon profilleri ile ilgili bulgularımız daha önce gerçekleştirilen çalışmalarla uyumludur. Bu açıdan bakıldığında, *Primula vulgaris* çiçek ve yaprak ekstraktlarının kanserli hücreler üzerinde antiproliferatif etkileri olduğu görülmektedir.

Apopitoz, ölüm liganları ile tetiklenen dış (ekstrinsik) ölüm yolağı ve mitokondri yolu ile gerçekleşen iç (intrinsik) ölüm yolakları olmak üzere iki yolla gerçekleşmektedir. İntrinsik yolakta apoptozun başlatılması için kaspazkaskadın aktif hale gelmesi için sitokrom c'nin sitozole salınması gerekir. Apoptozda ilk evre olan sitokrom c'nin salınması, BCL2 aile üyeleri tarafından pozitif ve negatif olarak ayarlanmaktadır. Yine, mitokondriyal

geçirgenliğin düzenlenmesi, hücrede kalsiyum (Ca+2) iyonunun miktarındaki artış, DNA hasarı gibi hücre için stres oluşturan uyarılara cevap olarak oluşan intrinsik mitokondriyal yolağın düzenlenmesi Bcl-2 gen ailesi tarafından gerçekleşmektedir [33, 34]. Bcl-2 aile grubundan Bcl-2, bcl-xL, bcl-w ve mcl-1 anti-apopitotik iken, bax, bak, bid, bik, bim ve bad gibi genler pro-apopitotik özelliktedir [35,36]. Bcl-2 ve bcl-xL; kaspaz-3 ve kaspaz-7 üzerinde negatif düzenleyici etkileri ile apopitozu engellemektedir[37]. Bcl-2 proteinlerindeki mutasyonlar, tümör hücrelerinin kemoterapi veya radyoterapiye olan hassasiyetlerini etkiler. BCL2 gen ekspresyonunun artışı sonucunda apoptotik fonksiyonlar durmaktadır[38]. Ayrıca, farklı bitki ekstraktlarının HeLa hücre hatları üzerindeki moleküler seviyedeki etkileri incelenmiş ve *Colchicumumbrosum S.* bitki ekstraktının Bcl-2 gen ekspresyonlarını etkilemediği belirlenmiştir [39]. Literatür verileri ışığında, apopitoz miktarı ile Bcl-2 gen ekspresyonunun ters orantılı olduğunu ifade edebiliriz. Çalışmamızda, BCL2 gen ekspresyonu *Primula vulgaris* çiçek ekstraktı yüklü IC_{50} ve LD_{50} doz gruplarında sırasıyla 0,7 ve 16,9 kat artarken, sadece AgNP ve sisplatin uygulanan HeLa hücrelerinde BCL2 gen ekspresyonu sırasıyla 0,8 ve 1,3 kat azalmıştır. Buradan hareketle, çiçek ekstraktı yüklü AgNP IC_{50} ve LD_{50} doz grupları ile ekstrakt yüklü olmayan sadece AgNP uygulanan gruplar arasında BCL2 gen ekspresyonu seviyelerinde fark olması bitki ekstraktının etkilerini göstermektedir. Anti-apopitotik BCL2 gen ekspresyonunun *Primula vulgaris* çiçek ekstraktı yüklü AgNP dozunun artışına bağlı olarak artış gösterdiği belirlenmiştir. Bu durum, gerçek zamanlı hücre analiz sistemi ile gerçekleştirdiğimiz ve çiçek ekstraktı yüklü AgNP dozunun artışına bağlı olarak hücre ölümünün artış gösterdiği sonuçlarımızla uyum göstermemektedir. *Primula vulgaris* çiçek ekstraktı yüklü AgNP dozuna bağlı HeLa hücrelerinin ölüm oranı artmakta ve beklenilenin aksine BCL2 gen ekspresyonu seviyesinde de artış görülmektedir. BCL2 gen ekspresyonundaki artışla ilgili sonuçlarımızı; Apopitozu indükleyen kemoterapötik etkinin inhibisyonuna neden olan BCL2 ve BCL-XL gen ekspresyon miktarlarının artışlarla ilgili bilgilerle [40] birlikte değerlendirmek yerinde olabilecektir.

MDR1 (P-gp veya ABCB1); ABC proteininin en iyi taşıyıcısı olarak bilinir. ABC ailesinin bilinen en karakteristik özellikleri ATP bağlamalarıdır[41]. MDR1'in geniş substrat özgüllüğü ve taşıyıcı fonksiyonu kanser kemoterapisinde bazen problemlere neden olur[42,43]. Uzun süre kemoterapi ilaçlarına maruz kalma MDR1'in transkripsiyonel aktivasyonunu baskılar. Kanser hücreleri üzerinde artan MDR1 ekspresyonu önemli miktarda antikanser ilaçların hücre dışına pompalanmasına neden olur [44]. Birçok antikanser ilaç MDR1 için substrat özelliği taşır. Bu nedenle çok fazla sayıda antikanser ilaç uygulaması malign hücrelerde MDR1 aktivitesini kolayca baskılayabilir[45]. MDR1 ekspresyonundaki artış serviks kanserinde görülmektedir. Ayrıca serviks kanserli MDR1 gen ekspresyonu negatif olan bireylerin yaşam ömrünün pozitif olanlara göre daha uzun olduğu belirlenmiştir[14]. Yine, başka bir çalışmada invaziv karakterli serviks kanserli hastalarda MDR1 ekspresyon seviyesinin arttığı belirtilmektedir[46]. Kalsiyum bağlayıcı bir protein olan Sorcin indüklemesinin ortadan kaldırılması HeLa hücrelerinde MDR1 ekspresyonunda azalışa neden olur. Sorcin, ilaç dirençli tümör hücrelerinde veya MDR1 eksprese eden çeşitli lenfositlerde artan ekspresyon göstermektedir [47]. MDR1 gen ekspresyonunun artması kanserleşmede olumsuz bir prognostik faktör olarak karşımıza çıkmaktadır[48]. İnsan kanserlerinde MDR1 gen ekspresyonundaki artış kemoterapötik ilaçlara karşı gelişen ilaç direnci ile de sıklıkla ilişkilendirilmektedir[49]. Çalışmamızda, IC_{50} doz grubu ve sadece AgNP uygulanan grupta MDR1 ekspresyon seviyelerinin kontrol grubuna göre sırasıyla 1,2 ve 1,1 kat azaldığı belirlenmiştir. Sisplatin uygulanan grupta MDR1 ekspresyon seviyesinde bir değişiklik olmazken, LD_{50} doz grubunda MDR1 ekspresyon seviyesi 6,5 kat artmıştır. Sonuçlarımız literatür verileri ışığında değerlendirildiğinde; *Primula vulgaris* çiçek ekstraktı yüklü AgNP'lerin dozunun artmasına bağlı olarak HeLa hücre hatlarında ilaç direnci geliştiği görülmektedir. Kontrol grubuna kıyasla IC_{50} doz grubunda MDR1 ekspresyon seviyesi 1,2 kat azalmışken, LD_{50} doz grubunda MDR1 ekspresyon seviyesinin 6,5 kat artması bu fikrimize önemli bir dayanak oluşturmaktadır.

p53; kanserde apoptoz yolağında artış gösteren tümör baskılayıcı bir proteindir. BCL2 ve p53 apoptozun kontrolünü sağlayan önemli iki gendir. p53, DNA hasarlarına tepki olarak hücre ölümünü başlatır. DNA hasarlarında normal hücrelerde p53 düzeyinde artış meydana gelerek hücre döngüsü G1 fazında bloke edilir. Hücre döngüsünün durdurulması ile DNA hasarı giderilir ve S fazına geçmeden sorun ortadan kaldırılmaya çalışılır. Eğer hücrede meydana gelen hasar büyük boyutlarda ise hücre apoptoza girer[50]. Serviks kanserinde sisplatin etkinliğini inceleyen bir çalışmada; sisplatinin p53 ekspresyon seviyesinde artış sağladığı tespit edilmiştir[51]. Serviks kanserinde HPV enfeksiyonu önemli rol oynar[9]. HPV E6 ile kompleks oluşturarak p53'ü inaktif eder ve apoptozu baskılar [10]. Serviks kanserli 78 hasta üzerinde yapılan bir çalışmada; 48 hastada p53 ekspresyonu artarken, 30 hastada p53 ekspresyonu azalmıştır[18]. Ayrıca, radyoterapinin hastalarda p53 ekspresyon seviyesinde artış sağladığı ifade edilmektedir [52]. Serviks kanseri hastalarında %6,6 seviyesinde seyreden p53

ekspresyon seviyesi radyoterapi sonrası %13.9 oranına yükselerek kanser hücrelerinin apoptoza eğilimlerini artırdığı belirtilmektedir [52,53]. Çalışmamızda IC_{50}, AgNP ve Sisplatin doz gruplarında p53 gen ekspresyon seviyelerinde kontrol grubuna kıyasla sırasıyla 2.6, 1.3 ve 1.4 kat azalma olduğu belirlenmiştir. LD_{50} doz grubunda ise; kontrol grubuna kıyasla p53 gen ekspresyon seviyesinde 1,6 kat artış olduğu saptanmıştır. Bu bilgiler ışığında bir tümör süpressör gen olan p53'ün ekspresyonunda anlamlı bir artışa neden olmayan *Primula vulgaris* çiçek ekstraktı yüklü AgNP IC_{50} dozunda HeLa hücrelerinde apopitoza neden olmazken, LD_{50} dozundaki p53 gen ekspresyon artışı kanser hücrelerinde apopitoza neden olmaktadır.

Sonuç olarak, *Primula vulgaris* çiçek ve yaprak ekstraktları yüklü AgNP'lerin HeLa hücre hattı üzerinde dozla orantılı olarak hücre ölümüne neden olduğu belirlenmiştir. *Primula vulgaris* çiçek ekstraktı yüklü AgNP dozundaki artışla orantılı olarak BCL2 ve MDR1 gen ekspresyon seviyelerindeki artışlar ilaç direnci gelişimi açısından değerlendirilmelidir. Söz konusu ve muhtemel etkiden sorumlu hücresel mekanizmaların aydınlatılması serviks kanserinin tedavisi amacıyla kullanılabilecek yeni bitkisel kökenli ilaçların geliştirilmesine katkı sağlayabilecektir.

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➢ *ORAL PRESENTATION*

Change in anthocyanin profile of grape juice concentrate during storage

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Abstract

Anthocyanins, which give the distinctive fascinating colour to red grapes, are also responsible of the taste of grape products. However, they are easily decomposed due to several factors such as temperature, pH degree, concentration, light, oxygen, enzymes, SO_2, hydrogen peroxide and 5-hydroxymethylfurfural, ascorbic acid, flavonoid, protein and metal ion presence and degradation occurs during storage, which corresponds to loss in nutrition value and quality of products. Changes in anthocyanin profile of grape juice concentrates produced from Öküzgözü, Köhnü and Papazkarası varieties during a 6 months storage period have been studied by this research. Decreases in anthocyanin fractions particularly in the 1st, 2nd and 5th months of storage period are remarkable according to research findings. During the storage of grape juice concentrate, time-dependent changes in Mv-3-glu amount for Papazkarası variety, Mv-3-glu and Pn-3-glu amounts for Öküzgözü variety and Cy-3-glu, Pn-3-glu and Mv-3-glu amounts for Köhnü variety are found statistically important ($p<0.01$).

Keywords: anthocyanin profile, grape juice concentrate, storage, malvidin-3-glucoside, cyanidin-3-glucoside

Üzüm suyunun depolanması sırasında antosiyanin profilindeki değişim

Özet

Antosiyaninler siyah üzümlere ilgi çekici rengini vermelerinin yanı sıra üzüm ürünlerinin tadından da sorumludur. Ancak, antosiyaninler depolama sırasında sıcaklık, pH derecesi, konsantrasyon, ışık, oksijen, enzimler, SO_2, hidrojen peroksit, 5-hidroksimetilfurfural, askorbik asit, flavonoid, protein, metil iyon varlığı ve degredasyon oluşumu gibi çeşitli etkenlerle kolayca parçalandığı için ürünlerin besin değeri ve kalitesinde kayıplar oluşmaktadır. Bu araştırmada Öküzgözü, Köhnü ve Papazkarası çeşidi üzümlerden elde edilen siyah üzüm suyu konsantrelerin 6 ay depolama sırasındaki antosiyanin profili değişimi araştırılmıştır. Araştırma bulgularına göre özellikle 1., 2. ve 5. ay depolama sürelerinde antosiyanin fraksiyonlarındaki düşüşler dikkat çekicidir. Üzüm suyu konsantresinin depolanması sırasında Papazkarası çeşidi için Mv-3-glu miktarında, Öküzgözü çeşidi için Mv-3-glu ve Pn-3-glu miktarlarında ve Köhnü çeşidi için Cy-3-glu, Pn-3-glu ve Mv-3-glu miktarlarında zamana bağlı değişimler istatistiksel olarak önemli bulunmuştur.

Anahtar kelimeler: antosiyanin profili, üzüm suyu konsantresi, depolama, malvidin-3-glikozit, siyanidin-3-glikozit

INTRODUCTION

Anthocyanins occur in grapes by the colour change called as veraison. Anthocyanins, which are simple monomer and free form at this stage, start to accumulate and polymerised in the course of ripening process and reach its maximum level after ripening. 10-15% of anthocyanins are in polymer form. Factors such as light, temperature, etc. increase not only sugar amount but also anthocyanin amount (Toprak, 2011).

Number of anthocyanin components detected in grape is approximately 20 and major anthocyanidins participating in anthocyanin formation are delfinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn) and malvidin (Mv) (Fong et.al. 1971, Wulf and Nagel, 1978; Pomar et.al., 2005).
Anthocyanins, according to many research studies, are easily decomposed due to several factors such as temperature (Cemeroğlu et.al., 1994; Kırca and Cemeroğlu, 2003; Kırca et.al., 2003), pH degree (Mazza and Brouillard, 1987), SO_2 (Starr and Francis, 1968), hydrogen peroxide (Sondheimer and Kertesz, 1952, 1953; Özkan et.al., 2000, 2002), sugar decomposition compounds like furfural and 5-hydroxymethyfurfural (Daravingaz and Cain, 1968; Debicki-Pospısıl et.al., 1983) and ascorbic acid (Poei-Langstan and Wrolstad, 1981).

In this research study, change of anthocyanin distribution in grape juice concentrate production process has been examined and the most appropriate storage period for quality protection has been tried to be determined.

MATERIALS AND METHODS

Materials
Grape juice concentrates produced from Papazkarası, Öküzgözü and Köhnü varieties harvested during September and October months in 2012 and 2013 have been used, in order to determine change of anthocyanin profile during storage of grape juice concentrate.

Methods
The method identified by Durst and Wrolstad (2001) was modified and applied for the best differentiation of anthocyanin standard peaks.

Chemicals
Asetonitrile HPLC gradient (Sigma-Aldrich), o-phosphoric acid (Sigma Aldrich), methanol HPLC gradient (Sigma Aldrich), cyanidin-3-glucoside, peonidin-3-glucoside, delfinidin-3-glucoside, malvidin-3-glucoside, petunidin-3-glucoside (Sigma-Aldrich), 37% HCl (Sigma-Aldrich).

Preparation of anthocyanin standard substances
First, 1000 ppm stock solution for each anthocyanin standard substance was prepared with ultra pure water including 0.1% HCl. Solutions at different concentrations were prepared from each stock solution by using 4% phosphoric acid and injected into HPLC device then, for plotting anthocyanin standard substance curves.

Chromatography conditions
Solvent A : 4% Phosphoric Acid
Solvent B : %100 Asetonitrile
Flow rate : 1.0 mL/min
Wavelength : 520 nm
Linear gradient flow :

Time (min)	Solvent A (%)	Solvent B (%)
0	94	6
55	80	20
57	30	70
60	5	95
60.1	94	6
70	94	6

Column : Reversed phase C_{18} column (250 x 4.6 mm, 5µm)
Temperature : 30 ^oC
Analysis duration: 72 min

Identification and calculation

Acquired chromatograms have been evaluated by means of Agilent Chemstation software.
Primary peaks detected in chromatograms are identified by comparing them with the incidence time of standard substance of each anthocyanin (Table 1). Anthocyanin amounts are calculated quantitatively by using equations derived from standard substance curves.

Table 1. Incidence Time of Grape Juice Anthocyanins in HPLC Chromatogram

Anthocyanin standard	Incidence time (min)
Cyanidin-3-glucoside	29.82
Delfinidin-3-glucoside	37.18
Peonidin-3-glucoside	37.77
Malvidin-3-glucoside	40.68
Petunidin-3-glucoside	48.35

Statistical Evaluation

Composition variation of concentrates has been evaluated by means of SPSS 20 packaged software using repeated measurement variance analysis in factorial system. DUNCAN multiple comparison method was used for identifying different groups. Mstat-c packaged software was used for DUNCAN test (Kesici and Kocabaş 2007). Results of DUNCAN test are shown with letters next to average values.

RESULTS AND DISCUSSIONS

Changes in major anthocyanins in grape juice concentrates produced from Papazkarası, Öküzgözü and Köhnü varieties during 6 months storage period are graphically shown in Figure 1, Figure 2 and Figure 3.

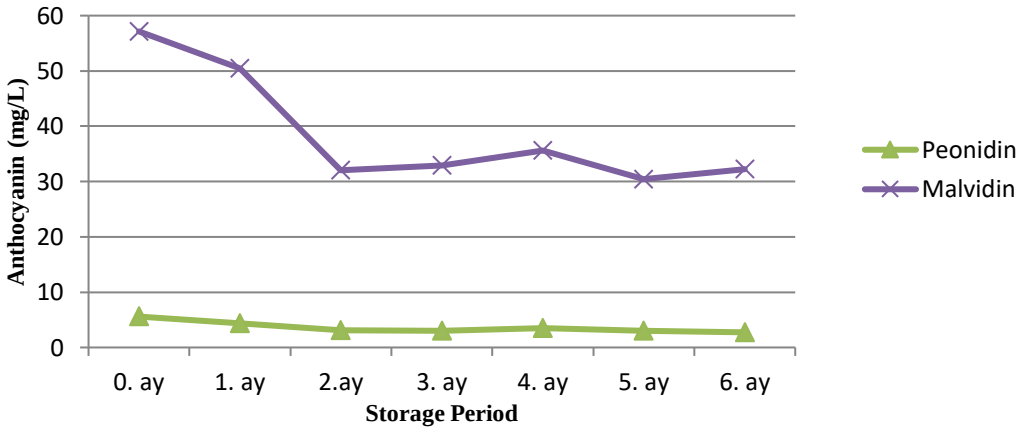

Figure 1. Change in Anthocyanin Profile During Papazkarası Grape Juice Concentrate

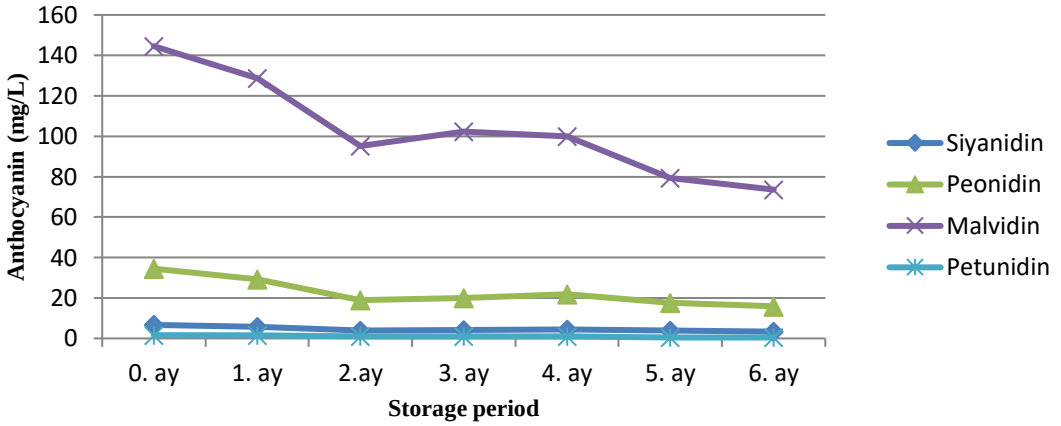

Figure 2. Change in Anthocyanin Profile During Öküzgözü Grape Juice Concentrate

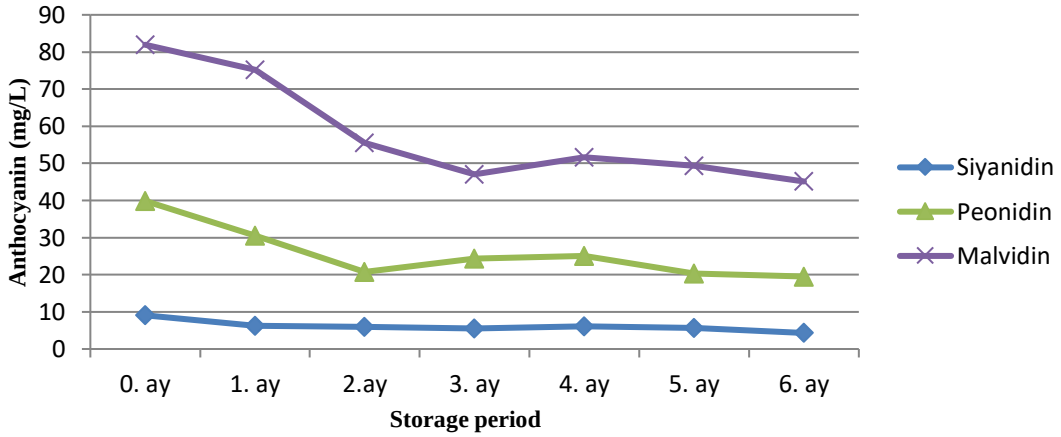

Figure 3. Change in Anthocyanin Profile During Köhnü Grape Juice Concentrate

Change in Pn-3-glu amount depending on storage period of Papazkarası grape juice concentrate is not statistically significant ($p>0.01$), whereas change in Mv-3-glu amount is statistically significant ($p<0.01$). Especially, decreases in 1st, 2nd and 5th months are remarkable.

Changes in Pt-3-glu and Cy-3-glu amounts depending on storage period of Öküzgözü grape juice concentrate are not statistically significant ($p>0.01$), whereas changes in Mv-3-glu and Pn-3-glu amounts are statistically significant ($p<0.01$). 1st and 2nd months for Pn-3-glu decrease and 1st, 2nd and 5th months for Mv-3-glu decrease are important.

Changes in Cy-3-glu, Pn-3-glu and Mv-3-glu amounts depending on storage period of Köhnü grape juice concentrate are statistically significant ($p<0.01$). 1st, 2nd and 5th months for Pn-3-glu decrease and 1st, 2nd and 3rd months for Mv-3-glu decrease are noticeable. Cy-3-glu decrease between 0 and 6th months is found important in general.

The difference between Papazkarası and other two varieties in terms of Pn-3-glu amount and the difference between three varieties in terms of Mv-3-glu amount are statistically significant ($p<0.01$), when the difference between same anthocyanin fraction in grape varieties during the same storage process is examined. The difference between Öküzgözü and Köhnü in terms of Cyn-3-glu amount is significant ($p<0.01$), whereas Cyn-3-glu is not detected in Papazkarası variety.

The difference between Mv-3-glu and Pn-3-glu amounts in Papazkarası variety during entire storage period is statistically significant ($p<0.01$), when the difference between anthocyanin fractions in the same grape varieties during the same storage process is examined. The difference between Mv-3-glu, Pn-3-glu and Cyn-3-glu anthocyanins and Mv-3-glu, Pn-3-glu and Pt-3-glu anthocyanins in Öküzgözü variety is statistically significant ($p<0.01$), whereas the difference between Cyn-3-glu and Pt-3-glu is not statistically significant ($p>0.01$). The difference between all anthocyanin fractions (Mv-3-glu, Pn-3-glu and Cyn-3-glu) in Köhnü variety is statistically significant ($p<0.01$).

Hellström et. al. (2013) carried out a research study on the importance of storage temperature for stability of anthocyanins which is one of the main quality factors in fruit juices. According to that study, it is reported that temperature has an important impact on degradation rate of anthocyanins and the degradation always occurs at its maximum rate particularly at room temperature (21 ˚C), which is followed by fruit juices stored at 9 ˚C and 4 ˚C respectively. In addition, it is detected that different anthocyanins in fruit juices have different degradation kinetics, Analysis of glucosides and rutinosides of cyanidin and delphinidin show that degradation rate of delphinins is higher than that of cyanins.

Fleschhut et.al. (2006) reported that increase in hydroxyl groups at B ring of anthocyanin core leads to decrease in stability. Moreover, any difference between stabilities of delphinins and cyanins in watermelon juice is not observed, while both of them are reported as more stable compared to petunins, peonins and malvines. This is interpreted as an indicator showing that methylation of hydroxyl groups at B ring increase the stability of anthocyanins.

CONCLUSION

It is known that anthocyanins are quite sensitive against several factors such as pH, concentration, storage temperature, light, oxygen and enzymes, flavonoid, protein and metal ion presence. Anthocyanins degrade during storage period. However, selection of storage conditions has an important impact on the stability of anthocyanins (Gimenez et al., 2001; Morais et al., 2002; Rubinskiene et al., 2005). Commercial fruit juices are generally produced by means of thermal processes, which have significant influence on the stability of anthocyanins (Hollands et al., 2008). Although many research studies on the stability of anthocyanins have been carried out up to date, still more information about the effects of storage periods, temperatures, matrices and structural characteristics of products produced from different fruits on anthocyanins is required. This research study contributes to determination of degradation for anthocyanin fractions during storage of grape juice concentrate and also optimum storage period regarding quality protection.

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➢ *ORAL PRESENTATION*

$Ca(OH)_2$ ile ön işlemin üzüm posası atıklarının biyogaz üretimine ve kompozisyonuna etkileri

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Özet
Biyogaz üretim verimini iyileştirmek için öğütülmüş üzüm posası atıklarının $Ca(OH)_2$ ile kimyasal ön işleme tabi tutulması olası bir metot olarak karşımıza çıkmaktadır, ancak günümüze kadar biyogaz üretimi ve kalitesi üzerindeki etkilerinin yeteri kadar araştırılmadığı görülmektedir. Bu çalışmanın amacı, $Ca(OH)_2$ ile kimyasal ön işlemin üzüm posasının biyogaz üretimi ve kalitesi üzerindeki etkilerini araştırmaktır. Çalışmada ön işlem uygulanmış ve uygulanmamış üzüm posalarının biyogaz üretimi ve kalitesi gözlemlenmiştir. Deneysel çalışmalar mezofilik şartlarda (37±0.5°C) 6.15 L aktif hacme sahip anaerobik reaktörlerde 40 gün süre ile batch olarak yürütülmüştür. Çalışmada kimyasal ön işlem uygulanan substratın spesifik biyogaz üretiminin ve metan içeriğinin önemli ölçüde daha yüksek olduğu gözlemlenmiştir. Üzüm posasının kimyasal ön işlemden geçirilmesi kümülatif biyogaz üretimini yaklaşık olarak 2 katına çıkarmıştır. 30.75 gr öğütülmüş kuru üzüm posası 0.615 L hacme kadar musluk suyu ile tamamlandıktan sonra elde edilen substrata 4 günlük sürede toplamda 3 gr $Ca(OH)_2$ ilavesi (%10) ile kimyasal ön işlem uygulanması durumunda kümülatif olarak 18751 mL biyogaz üretildiği tespit edilmiştir. Kimyasal ön işlem uygulanmaması durumunda kümülatif biyogaz üretimi miktarının 9669 mL olduğu görülmüştür. Kimyasal ön işlemin uygulandığı ve uygulanmadığı reaktörlerindeki biyogazın CH_4 - CO_2 oranlarının sırasıyla %69.5 - %16.5 ve %53.8 - %12.2 olduğu görülmüştür. pH kontrolüne de yardımcı olan $Ca(OH)_2$ ile üzüm posasına kimyasal ön işlem uygulamasının anaerobik süreç verimliliğini önemli ölçüde arttırarak biyogaz üretimi ve kalitesini artırdığı görülmüştür.
Anahtar Kelimeler: Biyogaz Üretimi, Üzüm Posası, Ön İşlem, Anaerobik Arıtma

Effects of $Ca(OH)_2$ pretreatment on biogas production and composition from grape pomace wastes

Abstract
Chemical pretreatment of ground grape pomace waste with $Ca(OH)_2$ appears to be a possible method to improve biogas production efficiency. However, it is seen that its effects on biogas production and quality have not been studied sufficiently until today. The aim of this study is to investigate the effects of chemical pretreatment with $Ca(OH)_2$ on biogas production and quality of grape pomace. Biogas production and quality of pretreated and unpretreated grape pomace were observed in the study. Experimental studies were conducted under mesophilic conditions (37 ± 0.5°C) in anaerobic reactors with an active volume of 6.15 L for 40 days as batch. In the study, it was observed that the specific biogas production and methane content of the chemically pretreated substrate were significantly higher. Chemical pretreatment of grape pomace approximately doubled its cumulative biogas production. 30.75 g of ground dried grape pomace has been completed with tap water to a volume of 0.615 L. Then, chemical pre-treatment has been applied to the substrate obtained by adding 3 g of Ca $(OH)_2$ (10%) in total within 4 days. As a result, it has been determined that 18751 mL of biogas was produced cumulatively. It was observed that the cumulative biogas production amount of the chemically unpretreated substrate was 9669 mL. It was obtained that the CH_4 - CO_2 ratios of the biogas in the reactors where chemical pretreatment was applied and not applied were 69.5% - 16.5% and 53.8% - 12.2%, respectively. It has been observed that chemical pretreatment of grape pomace with $Ca(OH)_2$, which also helps pH control, significantly increases the efficiency of the anaerobic process and increases biogas production and quality.
Keywords: Biogas Production, Grape Pomace, Pretreatment, Anaerobic Treatment

GİRİŞ

Üzüm, dünya çapında yaygın olarak yetiştirilen meyvelerden biridir ve hasat edilen ürünlerin yaklaşık %80'i şarapçılık sektöründe kullanılmaktadır (Drosou ve ark., 2015). Şarap endüstrisinde presleme sonrasında işlenen üzümlerin yaklaşık %13'ü yan ürün olarak ortaya çıkmaktadır (Clemente ve ark., 2014). Bitkisel üretim alanında bağcılık sektörü olarak bilinen üzüm üretimi Türkiye'de önemli bir yere sahiptir. Türkiye'de yetiştirilen üzümlerin çoğu kuru ve sofralık (yaş) olarak tüketilmektedir (Bashimov 2017). TÜİK verilerine göre Türkiye' 2019 yılı üzüm üretimi toplamda 4.1 milyon ton olup bu üretimin 451 bin tonu şaraplık-şıralık (%11), 1.599 milyon tonu kurutmalık (%39) ve 2.05 milyon tonu sofralık (%50) olarak kullanılmaktadır (TÜİK 2019). Ülkemizde yaş üzümler pekmez, pestil, sucuk, şıra ve şarap yapımı maksadıyla işlenmektedir. İşlenen bu üzümler %15-26 oranında kuru madde içeriğine sahiptir (Singh ve ark., 2012; Tufaner 2018). Kabuklar, tohumlar ve saplardan oluşan yan ürün atıklar üzüm posası olarak adlandırılmaktadır. Üzüm posası ya etanol elde etmek için damıtma tesislerine gönderilmekte ya da doğal atık olarak atılmaktadır. Bu atıklar kompost olarak veya hayvan yemi olarak kullanılabilmektedir (Drosou ve ark., 2015). Araziye atma biyolojik bozulmaya karşı direnci arttırmasının yanında posanın pH'ını düşüren fenolik bileşiklerin çevreye zararlı etkileri olabilir. Yüzey ve yer altı suyu kirliliği, kötü koku, hastalıkları yayabilen sinek ve zararlıların üremesi, tanenler ve diğer bileşikler tarafından toprak ve yer altı sularında oksijenin tükenmesi diğer çevre sorunları arasında sayılabilir (Beres ve ark., 2017).

Anaerobik prosesten önce, enzim erişilebilirliğini arttırıp sistem stabilitesini sağlayarak hidroliz ve fermatasyon safhalarının verimliliğini artırmak için mekanik ve kimyasal ön işlem yöntemleri genellikle kullanılmaktadır. Mekanik parçalama lignoselülozik materyalin anaerobik sindiriminde temel bir ön işlem olduğunu belirtmek gerekir. Mekanik ön işlem, substratın partikül boyutunu verimli bir şekilde azaltarak partiküllerin erişilebilir yüzey alanını artırmaktadır (Kratky ve Jirout 2011). Fiziksel olarak substratın öğütülmesi anaerobik sindirim verimliliğini etkili bir şekilde artırabilse de, etki hala sınırlıdır çünkü öğütme substratın yalnızca yapısal karakterizasyonunu değiştirmektedir. Substratın lignin içeriği gibi kimyasal özellikleri de biyokütlenin biyobuzunurluğunu etkileyen önemli bir faktördür. Alkali ön işlem, özellikle lignin giderimi yoluyla lignoselülozik maddelerin kimyasal karakterizasyonunu değiştirmek için etkili bir ön işlemdir (Krishania ve ark., 2013). Alkali ön işlem sırasında çözünme ve sabunlaşma reaksiyonları hızla gerçekleşir (Park ve Kim 2012). Bu durum, biyokütlenin şişmesine sebep olarak polisakkaritleri ayrıştırmaktadır. Böylece alkali ön işlem enzim erişilebilirliğini artırarak biyokütlenin biyobozunurluğunu arttırır (Hendriks ve Zeeman 2009). Alkali ön işlem sadece lignoselülozik biyokütlenin sindirilebilirliğini artırmakla kalmaz, aynı zamanda ekstra alkalinite sağlayarak asit üretimini nötrleştirir ve anaerobik sindirim işlemi sırasında pH düşüşünü engeller (Chen ve ark., 2014; Gu ve ark., 2015). NaOH ve $Ca(OH)_2$ kimyasal ön işlem uygulamalarında yaygın olarak kullanılan iki alkalidir. $Ca(OH)_2$, NaOH ile karşılaştırıldığında çok daha ucuz bir reaktiftir. Ayrıca, Ca^{2+}'nin anaerobik proses üzerinde inhibe edici etkisinin olmadığı da bildirilmiştir (Chen ve ark., 2008).

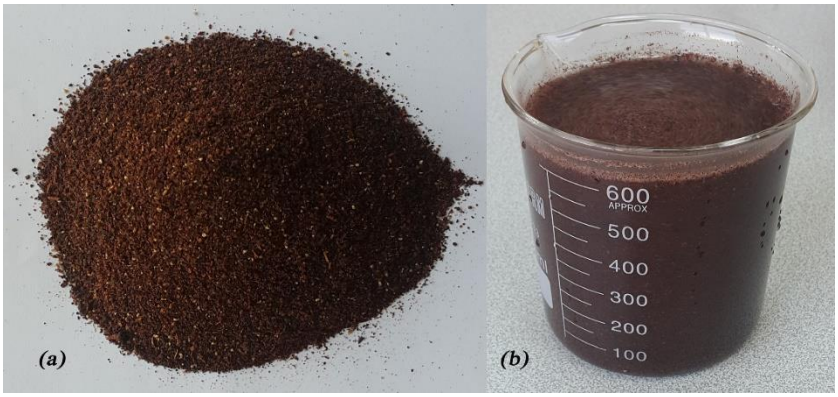
Biyogaz üretimi maksadıyla üzüm posası gibi lignoselülozik atıkların kullanımındaki genel zorluk, bu atıkların yapısı ve bileşimleri ile ilgilidir (Monlau ve ark., 2012). Üzüm posasının katı maddesi esas olarak selüloz, hemiselülozlar ve ligninden oluşmaktadır. Üzüm posasının oldukça düşük anaerobik biyobozunurluğu, muhtemelen kuru maddesinde yüksek miktarda lignin ve selüloz bulunmasından kaynaklanmaktadır. Bunlar içerisinde de lignin mikrobiyal bozunmaya ve oksidasyona en dirençli olan organiktir (Xu ve ark., 2014). Selüloz ise genellikle saf haliyle anaerobik koşullar altında parçalanabilir (Carlsson ve ark., 2012). Başka bir çalışmada, hemiselülozdaki karbonun kolayca kullanılabildiği ve bu nedenle mikroorganizmalar tarafından kolayca parçalanabildiği ifade edilmektedir (Mottet ve ark., 2010). Bu nedenle, lignin ve selülozun yapısal özelliklerini değiştirmek ve lignoselülozik fraksiyonların karmaşıklığını azaltmak için anaerobik prosesten önce üzüm posasına ön işlem uygulanması gerekli görülmektedir. Buna göre, substratın mikrobiyal ayrışmasını kolaylaştırmak, üzüm posasının biyobozunmasını ve dolayısıyla metan verimini arttırmak için yenilikçi ve düşük maliyetli ön işlem yöntemlerinin geliştirilmesi gerekmektedir (El Achkar ve ark., 2018). Dinuccio ve ark., (2010) üzüm posasının biyogaz potansiyelini inceledikleri çalışmalarında 0.116 m^3 CH_4/kg UKM üretiminin gerçekleştiğini belirtmişlerdir. Geršl ve ark., (2015) kırmızı ve beyaz şarap yapımında kullanılan üzüm posasının kuru bazda kilogramından sırasıyla 0.238 m^3 ve 0.246 m^3 biyogaz üretildiğini ve biyogazın CH_4 içeriğinin sırasıyla %59 ve %62 olduğunu ifade etmişlerdir.

Enzimatik hidrolizi ve organik atıklardan metan üretimini kolaylaştırmak için Hendriks ve Zeeman (2009), Carlsson ve ark., (2012) ve Carrere ve ark., (2016) tarafından farklı ön işlem yöntemlerinin geniş çapta incelendiği

görülmektedir. Bununla birlikte, yapılan literatür araştırmasına göre $Ca(OH)_2$ ön işlem uygulamasının üzüm posası atıklarından biyogaz üretimi ve metan verimi üzerindeki etkilerini değerlendiren herhangi bir çalışma bulunmamaktadır. Bu nedenle, bu çalışmada üzüm posası atıklarına $Ca(OH)_2$ ile kimyasal ön işlem uygulanmasının anaerobik proseste biyogaz üretimi ve metan verimi üzerindeki etkileri incelenmiştir.

MATERYAL VE METOD

Çalışmada pekmez yapımı sonrasında kalan atık üzüm posaları kullanılmıştır. Salkım halindeki üzümler ezilip sıkıldıktan ve suyu alındıktan sonra geriye üzüm salkımı sapı, üzümün çekirdekleri ve kabuk kısmı kalmaktadır. Üzüm posası ilk olarak bir hafta süreyle güneşte kurutulmuştur. Kurutulan üzüm posası kıyma makinesinde (EMES marka DA.12 model) öğütüldükten sonra 1 mm gözenek aralığına sahip bir elekten (LOYKA marka AEK20T model) geçirilmiştir. Şekil 1 (a)'da öğütülmüş ve elekten geçirilmiş üzüm posası gösterilmiştir. Şekil 1 (b)'de ise hazırlanan substrat gösterilmektedir.



Şekil 1. Çalışmada kullanılan öğütülmüş üzüm posası (a) ve üzüm posası ile hazırlanan susbstrat (b)

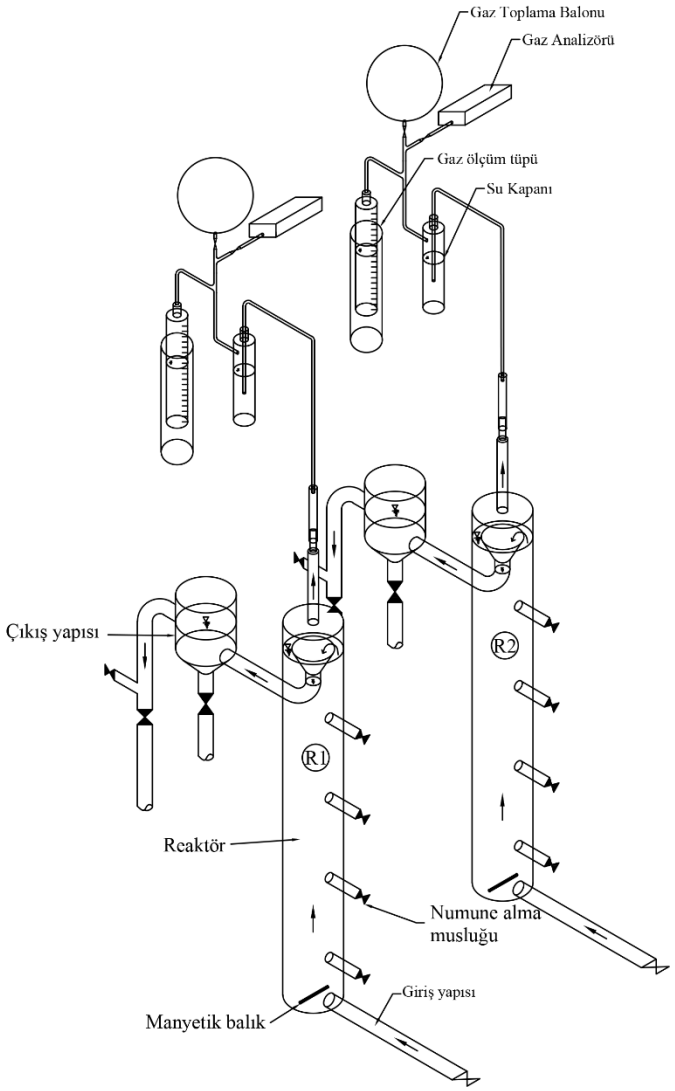
Çalışmada aşı çamuru olarak anaerobik reaktörlerde daha önceki çalışmalardan kalan ve reaktör içerisinde oda sıcaklığı şartlarında yaklaşık 4 ay süreyle bekleyen anaerobik çamur kullanılmıştır. İlk olarak reaktörlerdeki bütün çamur dışarı çıkartılmıştır. Daha sonra musluk suyu yardımı ile reaktörlerin içi temizlenmiştir. Bu temizleme suyu ile anaerobik çamur 11 litreye tamamlandıktan sonra karıştırılarak homojenize edilmiştir. Daha sonra Şekil 2'de şematik görünümü verilen ve 6.15 L aktif hacme sahip olan reaktörlere 5.5 L aşı çamuru doldurulmuştur. Reaktörler su ceketi içerisinde mezofilik şartlarda (37±0.5°C) işletilmiştir. Reaktörlere kesikli (batch) besleme yapılmış ve süreç 40 gün boyunca takip edilmiştir. Reaktör 1'e (R1) sönmüş kireç ($Ca(OH)_2$) ile kimyasal ön işlem görmüş susbstrat, reaktör 2'ye (R2) ise hiçbir ön işlem görmemiş olan susbstrat hazırlandıktan hemen sonra direkt olarak reaktöre beslenmiştir. Etüvde kurutulup desikatörde soğutulan 30.75 gram üzüm posası üzerine yaklaşık olarak toplamda 0.615 L olacak şekilde musluk suyu ilave edilerek besleme sıvıları (substrat) hazırlanmıştır. R1 için hazırlanan susbstrat kademeli olarak $Ca(OH)_2$ ilave edilerek 4 gün süre ile kimyasal ön işleme tabi tutulmuştur. R2 için hazırlanan susbstrat ise 4. günde hiçbir ön işlem uygulanmadan R1'e beslenen susbstrat ile aynı zamanda R2 reaktörüne beslenmiştir. Besleme sıvıları reaktörlere Şekil 2'de gösterilen numune alma musluğundan verilmiştir. Reaktörlere besleme yapıldıktan sonra reaktör tabanına bırakılan manyetik balık ile reaktörler her gün yaklaşık olarak yarım saat süreyle karıştırılmıştır. Tablo 1'de çalışmada kullanılan aşı çamuru ve susbstratın fizikokimyasal özellikleri verilmiştir.

Tablo1. Deneysel çalışmada kullanılan aşı çamuru ve susbstratın fizikokimyasal özellikleri

Parametreler	Aşı Çamuru	Susbstrat
pH	6.92	3.68
TKM (g/L)	26.05±0.21	50
UKM (g/L)	13.94±0.18	40.32
Kül (g/L)	12.11±0.03	9.68
KOİ (g/L)	22.85±0.56	57.62±2.41

Toplam Katı Madde (TKM); Uçucu Katı Madde (UKM); Kimyasal Oksijen İhtiyacı (KOİ)

Reaktörlerde üretilen biyogaz deplasman yöntemine göre çalışan gaz ölçüm tüpleri ile ölçülmüştür. Biyogazın gaz kompozisyonu ise taşınabilir biyogaz dedektörü (ETG-MCA 100 P, ETG Risorse e Tecnologia) ile belirlenmiştir. Çalışmada yapılan deneysel analizler Standart Metotlarda (SM) belirtilen yöntemlere göre yürütülmüştür (APHA 2012). pH tayini (Orion Star marka A211 model) pH metre ile yapılmıştır. TKM, SM'de (APHA SM (2012) 2540-B) belirtildiği gibi etüvde (Nüve marka FN 500 model) gerekli kurutma işlemi yapılıp numune sabit tartıma getirildikten sonra hassas terazi (Axis marka AGN 220 C model) kullanılarak gerekli tartım yapıldıktan sonra tayin edilmiştir. Aynı numunenin UKM değeri APHA SM (2012) 2540-E yönteminde tarif edildiği şekilde belirlenmiştir. KOİ analizleri gerekli seyreltme işlemleri yapıldıktan sonra SM Kapalı Reflux Kolorimetrik metoduna (APHA SM (2012) 5220-D) göre spektrofotometrede (Hach Lange DR 6000) 600 nm dalga boyunda gerçekleştirilmiştir.

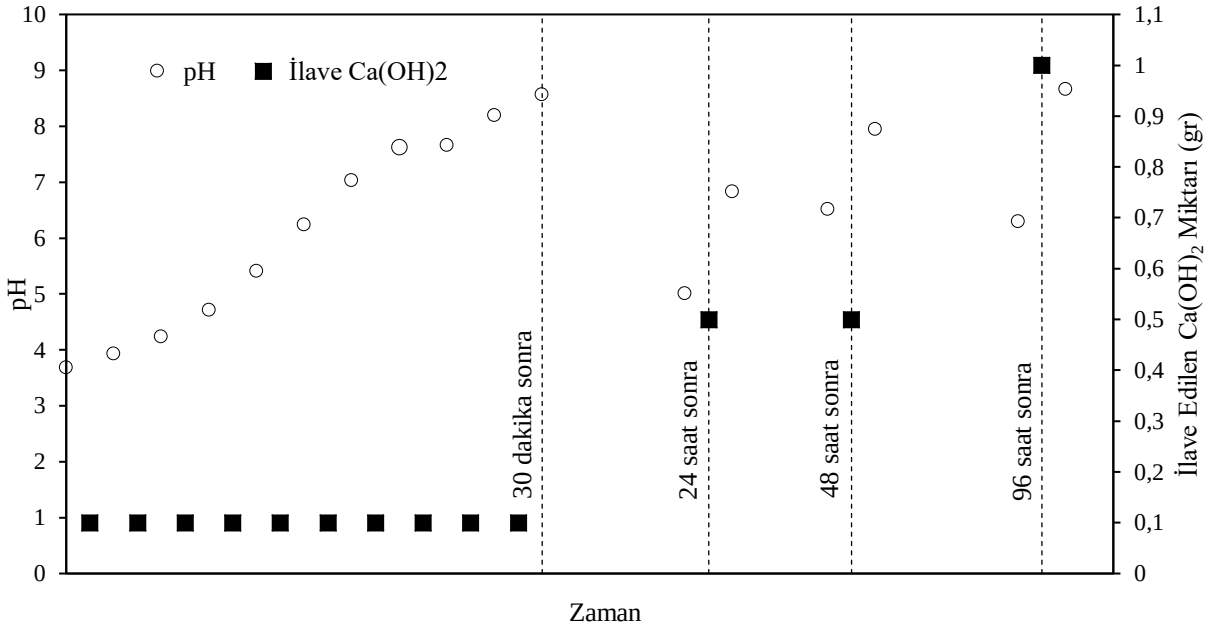


Şekil 2. Çalışmada kullanılan reaktörlerin şematik görünümü

BULGULAR VE TARTIŞMA

Çalışmada R1 reaktöründe gerekli asit tamponunu sağlamak için susbstratın pH değeri takip edilerek $Ca(OH)_2$ ilavesi yapılmıştır. Üzüm posası ve musluk suyu ile hazırlanan substratın ilk pH'ı 3.68 olarak belirlenmiştir. Daha sonra manyetik balık ile karıştırılan susbstrata ilk etapta toplamda 1 gr $Ca(OH)_2$ 0.1 gr'lık parçalar halinde 30 dakika içerisinde eklenmiştir. Şekil 3'te bu uygulama ile substrattaki pH değişimi gösterilmiştir. Kiremit kırmızısına yakın bir renkte olan susbstrata $Ca(OH)_2$ ilave edildikçe renk koyu kahve-siyaha yakın bir renge dönmüştür. 30 dakikanın sonunda pH değerinin 8.57 değerine ulaştığı görülmüştür. 24 saat sonra pH değerinin

5.01'e düştüğü görülmüştür. Bunu üzerine 0.5 gr $Ca(OH)_2$ ilavesi yapılarak pH'ın 6. 83 değerine çıktığı gözlemlenmiştir. 2. günün sonunda 6.52 seviyesine gerileyen pH yine 0.5 gr $Ca(OH)_2$ ilavesi ile 7.95 seviyesine yükseltilmiştir. Oda şartlarında beklemeye bırakılan susbstratın pH değerinin 4. günün sonunda 6.3 değerine düşmesi ile 1 gr daha $Ca(OH)_2$ ilave edilerek pH değeri 8.66'ya yükseltilmiştir. Bekleme esnasında üzüm posasının $Ca(OH)_2$ etkisi ile şişerek daha da parçalandığı görülmüştür. $Ca(OH)_2$ dozlamasının tamamının ilk gün yapılması durumunda substratın daha fazla parçalanacağı düşünülmektedir. Bu nedenle farklı $Ca(OH)_2$ dozları ile ve farklı sürelerde ön işlem uygulamasının sistem verimliliğine etkisinin incelenmesi daha sonra yapılacak çalışmalar için önerilmektedir. 4 gün süreyle ilave edilen $Ca(OH)_2$ miktarına göre susbstratın pH değerindeki değişim Şekil 3'te gösterilmiştir. Kimyasal ön işlem uygulanan susbstrat, R1 reaktörünün yaklaşık orta noktasında bulunan 2. numune alma musluğundan reaktöre beslenmiştir. R1 reaktörüne beslemenin yapıldığı gün R2 reaktörüne de hiçbir işlem uygulanmayan susbstrat yine 2. numune alma musluğundan beslenmiştir.

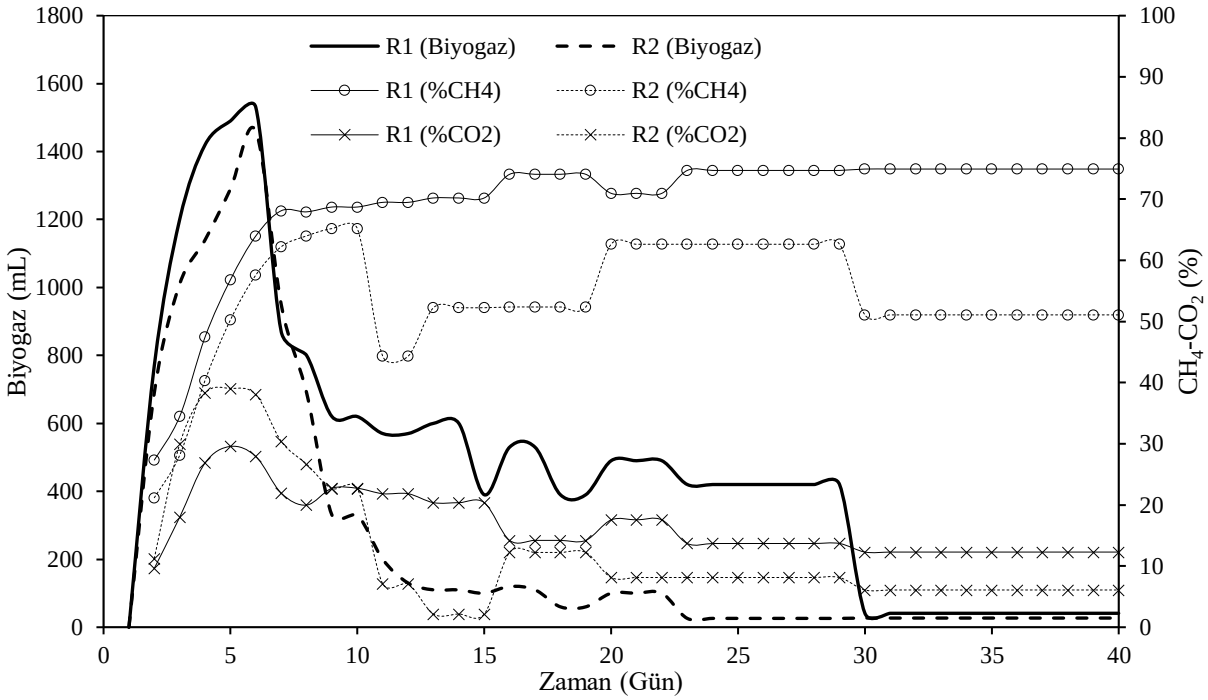


Şekil 3. İlave edilen $Ca(OH)_2$ miktarına göre susbstratın pH değerindeki değişim

Reaktörlere besleme yapıldıktan sonra 5. güne kadar biyogaz üretimi her iki reaktörde hızla artarak R1 ve R2 reaktörlerinde sırasıyla 1530 mL/gün ve 1460 mL/gün seviyelerine çıkmıştır. Aynı şekilde 5. günden 8. güne kadar her iki reaktörde de günlük biyogaz üretim miktarı hızla düşüşe geçerek R1 ve R2 rektörlerinde sırasıyla 800 mL ve 690 mL olduğu görülmüştür. 9. günden itibaren R1 reaktöründeki günlük biyogaz üretim miktarındaki düşüş yavaşlarken (620 mL/gün) R2 reaktöründe düşüş daha hızlı gerçekleşmiş ve biyogaz üretim miktarı 330 mL/gün seviyesine gerilemiştir. Biyogaz üretimi 23. günden 29. güne kadar ortalama olarak R1 ve R2 reaktöründe sırasıyla 420 mL/gün ve 26 mL/gün seviyelerinde ölçülmüştür. 30. günden 40. güne kadar R1 ve R2 reaktörlerinde ortalama biyogaz üretiminin sırasıyla 41 mL/gün ve 27 mL/gün olduğu tespit edilmiştir. Esasında çalışmanın 23-29 ve 30-40 günlerinde biyogaz üretimi düşük olduğu için 7 ve 11 günlük oluşan toplam biyogazdan günlük ortalama biyogaz değeri hesaplanmıştır. Son 7 ve 11 günlük biyogaz üretiminde ortalama biyogaz üretiminin 4. ve 6. günlerde gerçekleştiği kabul edilirse R1 ve R2 reaktörlerinde 27. günde sırasıyla 420 mL/gün ve 26 mL/gün ve 35. günde sırasıyla 41 mL/gün ve 27 mL/gün seviyelerinde olduğu düşünülebilir. Böyle bir yaklaşım ile 40. günde her iki reaktörde de biyogaz üretiminin oldukça azaldığı söylenebilir. R1 ve R2 reaktörlerinin günlük biyogaz üretimi Şekil 4'te gösterilmiştir. Genel bir değerlendirme yapıldığında R1 reaktörünün çalışmanın başından sonuna kadar R2 reaktöründen günlük olarak daha fazla biyogaz ürettiği tespit edilmiştir. R1 ve R2 reaktörlerinde ortalama biyogaz üretiminin sırasıyla 481 mL/gün ve 248 mL/gün olduğu hesaplanmıştır. Özellikle bu fark 10. gün ile 30. gün arasında ortalama yaklaşık olarak 384 mL/gün olarak gerçekleşmiştir.

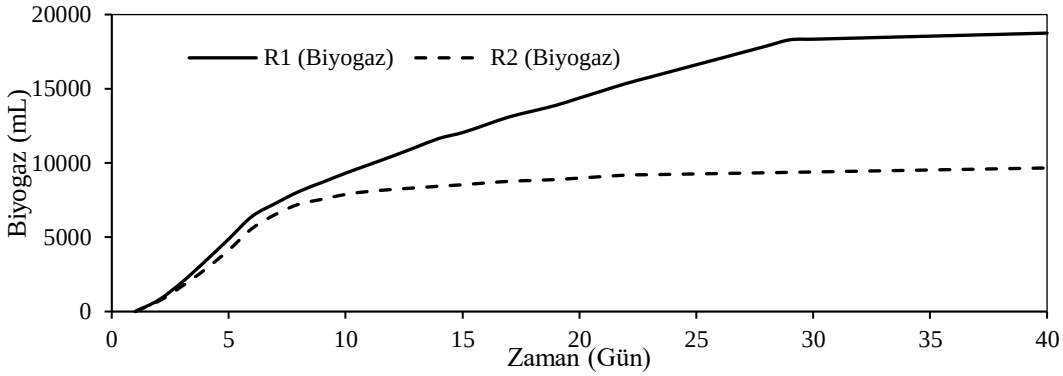
R1 reaktöründe 2. günde üretilen biyogazın CH_4 oranı %27.34 iken hızla artarak 7. günde %68.1 seviyesine çıkmıştır. 8. günden sonra CH_4 oranındaki artışlar küçük olmuş ve son ölçümde CH_4 oranının %74.92 olduğu tespit

edilmiştir. R1 reaktöründe biyogazın CO_2 oranı 2. günde %9.52 iken 5. günde %29.56 seviyesine yükselmiş daha sonraki günlerde düşüş gösteren CO_2 çalışmanın son ölçümünde %12.29 seviyelerine düşmüştür. R2 reaktörü incelendiğinde 2. günde %21.18 olan CH_4 oranı hızlı bir yükseliş ile 9. ve 10. günde %65.18 seviyelerine çıkmıştır. R2 reaktöründe biyogazın CH_4 oranı 11-12, 13-19, 20-29 ve 30-40 günleri arasında sırasıyla %44.31, %52.24, %62.62 ve %51.07 şeklinde ölçülmüştür. R2 reaktöründe biyogazın CO_2 oranı 2. günde %11.24 iken 5. günde %39.05 seviyesine çıkmış daha sonraki günlerde ise düşüşler göstererek çalışmanın sonunda %6.09 olarak ölçülmüştür. Şekil 4'te R1 ve R2 reaktörlerinde günlük üretilen biyogazın CH_4 ve CO_2 oranları gösterilmiştir. Reaktörlerdeki CH_4 oranları genel olarak değerlendirildiğinde R1 reaktörünün CH_4 oranının R2'den devamlı yüksek ve daha stabil olduğu görülmektedir. Ancak biyogazın CO_2 oranları kıyaslandığında R1 reaktörü 9. güne kadar R2 reaktörünün gerisinde iken 10. günden sonra R1 reaktörünün CO_2 oranının R2'den daha yüksek olduğu görülmektedir. Çalışmanın tamamı ele alındığında R1 ve R2 reaktörlerindeki biyogazın CH_4 - CO_2 oranlarının sırasıyla %69.5 - %16.5 ve %53.8 - %12.2 olduğu hesaplanmıştır.



Şekil 4. Kimyasal ($Ca(OH)_2$) ön işlem uygulanmış (R1) ve uygulanmamış (R2) üzüm posasının günlük biyogaz üretimi ve biyogaz kompozisyonu (%CH_4–%CO_2)

Üzüm posası atıklarından elde edilen kümülatif biyogaz hacimleri Şekil 5'te sunulmaktadır. Reaktörlerde 2. günde biyogaz üretimi başlamıştır. İlk günde substratın hidroliz ve asit oluşum safhasında olduğu düşünülmektedir. Substratın günlük biyogaz hacmi istikrarlı bir şekilde artarak 6. günde günlük yaklaşık 1500 mL'lik pik hacme ulaşmıştır. Bu durum metanojenlerin üstel büyümesine atfedilebilir (Odekanle ve ark., 2020). Bu dönemden sonra, metanojenlerin büyümesi azaldıkça günlük biyogaz üretim hacminin azalmaya başladığı görülmektedir. R1 ve R2 reaktörlerinde üretilen 39 günlük kümülatif biyogaz hacimleri, 6.15 L reaktör hacmi için sırasıyla 18751 mL ve 9669 mL olarak gerçekleşmiştir. Bu verilere göre R1 ve R2 reaktörlerinde 226 mL CH_4/gr $KOİ_{beslenen}$ ve 117 mL CH_4/gr $KOİ_{beslenen}$ değerleri tespit edilmiştir. Teorik metan üretiminin 350 mL CH_4/gr KOİ olduğu düşünüldüğünde üzüm posasının, üzüm çekirdekleri ve üzüm kabuğu gibi zor parçalanan ve bu süre içerisinde anaerobik sürece girmeyen kısımlarının bulunduğunu göstermektedir.



Şekil 5. Kimyasal ($Ca(OH)_2$) ön işlem uygulanmış (R1) ve uygulanmamış (R2) üzüm posasının kümülatif biyogaz üretimi

Görüldüğü gibi, anaerobik süreçte biyogaz üretimini arttırmada üzüm posası atıklarına yaklaşık olarak %10 oranında $Ca(OH)_2$ ilave edilerek ön işlem uygulanmasının avantajı net bir şekilde görülmektedir. Kümülatif biyogaz üretimi, ön işlem uygulanmamış substrat ile kıyaslandığında %93.9 oranında arttığı görülmektedir. Bu artış, alkali ön işleminin üzüm posasında bulunan karmaşık organik maddelerin hidrolizini kolaylaştırdığı gerçeğini ortaya koymaktadır. Ayrıca bu durum daha verimli anaerobik sindirimi sağlayarak biyo-sindirilebilirliği artırmaktadır (Li ve ark., 2012; Zheng ve ark., 2009; Zhu ve ark., 2010). Diğer araştırmacılar tarafından farklı substratlar için yapılan benzer çalışmalarda $Ca(OH)_2$ ön işleminin bir sonucu olarak biyogaz üretiminde önemli bir atışın olduğu belirtilmiştir. Gu ve ark., (2015) %8 ve %10 $Ca(OH)_2$ ilavesi ile pirinç samanının ön işleminden sonra ön işlem uygulanmayan pirinç samanına göre biyogaz üretiminde sırasıyla %34.3 ve %36.7 oranında bir atışın olduğunu bildirmişlerdir. Junoh ve ark., (2016) $Ca(OH)_2$'in ön işlem uygulanmamış gıda atığına kıyasla spesifik metan üretimini %20 oranına kadar artırabilen bir alkali kimyasal olduğunu bildirmişlerdir. Mustafa ve ark., (2018) şeker kamışı küspesi üzerine yaptıkları çalışmalarında, %8.5 $Ca(OH)_2$ ilavesi ile en yüksek kümülatif biyogaz üretiminin ön işlem görmeyen gruba kıyasla % 23.3 oranında artış gösterdiğini ifade etmişlerdir.

SONUÇ

Bu çalışmanın sonuçları, $Ca(OH)_2$ ile kimyasal ön işlem uygulamanın, üzüm posası atıklarının biyogaz üretimi ile beraber biyogazın CH_4 oranını ve anaerobik süreç verimliliğini önemli ölçüde artırabileceğini göstermektedir. $Ca(OH)_2$ ön işleminin pH kontrolüne de yardımcı olduğu görülmüştür. $Ca(OH)_2$ ile kimyasal ön işlem uygulanan ve uygulanmayan substratların beslendiği 6.15 L aktif hacimli reaktörlerde üretilen kümülatif biyogaz miktarları sırasıyla 18751 mL ve 9669 mL olarak tespit edilmiştir. Kimyasal ön işlemin uygulandığı ve uygulanmadığı reaktörlerindeki biyogazın CH_4 - CO_2 oranlarının sırasıyla %69.5 - %16.5 ve %53.8 - %12.2 olduğu hesaplanmıştır. Üzüm posasına yaklaşık %10 oranında $Ca(OH)_2$ dozlanmasının (3gr $Ca(OH)_2$/30.75 gr kuru üzüm posası) 40 günlük periyotta kümülatif biyogaz üretimini %93.9 oranında, biyogazın CH_4 içeriğini ise %15. 7 oranında artırdığı tespit edilmiştir.

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➢ *ORAL PRESENTATION*

Economic values of cave bacteria

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Abstract
With the evolution of the industry, our environment become polluted with toxic chemicals. Avoiding such problems, replacement of synthetic materials by natural products still the best solution. Therefore, microorganisms are the favorable sources due to their continuous availability and manipulation at low cost. Research for new bioactive compounds conducts scientists to the extreme environments such as caves. Caves constitute great microbiological laboratories from where microorganisms with human welfares could be isolated. These underground environments are extreme ecosystems in which their biotic factors, especially bacteria, are found to contribute to nowadays biotechnology and industries. From drug production, bioremediation, food processing to construction, these bacteria are thought to be economically the good sources of bioactive compounds used in these different processes. However, it is thought that most of the cave bacteria are uncultivated. With the improvements of molecular based methods, microbiological investigations in caves may become easier. In this present study, microbiological studies of caves around the world are summarized and the economic aspect of the bacteria inhabiting these ecosystems is highlighted by analyzing the industrial and biotechnological potential of these bacteria. This study should be helpful, in the urgent worldwide health problem caused by the development of drug resistance as well as the earth pollution by microplastics and other organic wastes.
Keywords: Bacteria, Biotechnology, Cave, Extreme environments.

INTRODUCTION

Since the dawn of time, human uses bacteria in different activities for benefic purposes such as soil fertilization (agricultural sector), food processing industries, pharmaceutical industries, and clinical purposes. In addition, bacteria are well known for their important role in organic materials decomposition, in metal reductions as well as aromatic compounds degradation such as hydrocarbons and oils. They are considerably contributing to the bioremediation of the environment, production of biogas and other bio-compounds through reusing of wastes materials. In this context, bacteria are thought to contribute to both industrial and biotechnological sectors. Thereby, the bacterial economy can be defined as the benefactions provided by bacteria in both biotechnological and industrial areas.

Caves are special environments, and their specificity is based on their abiotic factors characterized by darkness, usually low constant temperature, high humidity, low oxygen content, and absence or elemental nutrients (Tomczyk-Zak and Zielenkiewicz, 2016). Therefore, and considering the life conditions on the earth surface, cave ecosystems are placed in the extreme environments' category. These conditions constitute an interactive factor for the biologists to discover the biodiversity of cave flora, in particular the microbial flora (bacteria, archaea, and fungi).

Bacteria living in such environment are of interest in the context of their living conditions. Referred to their living ecosystems, these bacteria are known as extreme microorganisms. Even-though isolated from the same cave, every bacterium in cave has specific characterizations. These specificities depend on where they are isolated from. Some of these bacteria are heterotrophs, so they can use the organic materials transported by the water flow or other transporters such as human and animals from outside the cave to inside it (White and Culver, 2019). During their feeding, they secrete different enzymes, considering as primary metabolites, depending on the chemical

composition of the organic material. In case of a stress condition like high content of nutrient, these bacteria can secrete other metabolites known as secondary metabolites served for their protection against stresses.

Other bacterial category, called chemoautotrophic bacteria have the ability to fix the inorganic carbon (White and Culver, 2019). In such oligotrophic ecosystem, both autotrophs and heterotrophs during their metabolisms can secret substances (antimicrobial compounds) against their neighbors. In other hand, cave structures known as speleothems inside caves are of specific chemical characterization. Some of them have an attractive colored structure and some of them are constituted by different crystals colors and crystals are thought to be for biogenic origins. Usually, these colors refer to the pigment of some of the colonized bacterial biofilm (White, 2019). Otherwise, the crystals are formed through some biochemical reactions between bacteria and their environment, like ammonification by secretion of some biocatalysts (enzymes). In short, cave bacteria are more favorable economically than those living in other ecosystems due to different reasons: they need low nutrients (energy) for boosting the production of needed metabolites and the produced compounds are effective under extreme conditions like low temperature (low energy consumption).

This study aims to demonstrate the biotechnological and industrial importance of cave bacteria through driven studies as well as advances studies realized in different caves in the world.

CAVE BACTERIA IN BIOTECHNOLOGY

Biotechnology is defined as the use of living organisms, a part of them or their products, in technological areas. Most of biotechnology products are used to replace the chemical ones to avoid pollution and deterioration of the environment and preserving the green environment.

Cave bacteria are potential sources of antimicrobial and anticancer compounds

Bacteria are found everywhere but still the minority of them are known or isolated. It is thought that discovering new organisms is a synonym of new product. In this context, the drug-resistant problems may be solved by discovering new bacteria. Of course, the isolation of cave bacteria still a challenge, but with the metagenomic based methods, studies of these organisms appeared easier. Through the two methods, culture based and culture independent methods, the most bacterial phyla observed in caves are *Proteobacteria, Actinobacteria*, *Firmicutes, Bacteroidetes, Nitrospirae, Chloroflex, Planctomycetes* and *Verrucomicrobia*.

In the world, most bacteria which produce antimicrobial compounds are belonging the phylum *Actinobacteria* and most of them are strains of the genus *Streptomyces* (Banerjee et al., 2019). The abundance of these bacteria in speleothems and cave sediments show a great opportunity of multiplication of drug productions. On the earth surface, these bacterial genera can be isolated but the overuse of antibiotic in this environment can induce mutations in their genetical information material. Also, the competitivity between cave bacteria in a nutrient reduced environment remains an important hypothesis that researchers stand on to identify these microorganisms considered as great sources of bioactive compounds (Ghosha et al., 2017). Standing on this concept, bacteria belonging other phyla also promote great results for antimicrobial and anticancer production potentials.

Studies run in different caves in the world show positive results even if some of them are just genotypically reported. Strains of *Actinobacteria* and *Bacilli* classes isolated from Turkish caves showed antimicrobial effects against some bacterial pathogens like *S.aureus* and the fungi strain *Candida Albicans* (Yücel and Yamaç, 2010 ; Candiroglu and Dogruoz G, 2020). In other hand, Klusaite et al., (2016) have isolated from Kurba-Vorojina cave in Georgia bacteria belonging to *Firmicutes* phylum showing positive antibacterial effects against gram positive bacteria from the same cave ecosystem even if this activity was changed when tested against gram positive pathogenic bacteria. Some molecular methods are developed to identify the bioactive compounds in these economic bacteria difficult to be cultured or to be expressed phenotypically. Non ribosomal Peptide Synthases gene has been observed through PCR method in 58.92% of *Proteobacteria* and in 23.92% of *Actinobacteria* inhabitant the Kurba-Vorojina cave (Lukoseviciute et al., 2020). Also, Majority of Actinobacteria isolated from this cave contains both Ktosynthase α and β regions of polyketide synthases domain. Some antimicrobial active compounds produced by cave bacteria are already commercialized, i.e. Peptide A12-C, cervimycins A-D, undecyprodigiosin, and xiakemycin A (Ghosh et al., 2017). Cervimycins A-D and xiakemycin A have been also found to present anticancer activities (Ghosh et al., 2017).

Bioremediation by cave bioproducts

Bioremediation can be defined as the degradation of environmental contaminants, by using living organisms, into innocuous forms (Vidali, 2001). Microorganisms transform the contaminants compounds through reactions that are a part of their metabolic processes. The process of biodegradation of compounds is often done by multiple organisms. Both soil and water are imperatively necessary in human environments. In addition, they are susceptible to more contaminations like metals, microplastics and hydrocarbons. These toxic compounds cause soil degradation and pollution, water pollution, loss of land fertilization, and intoxication to human through the food chain.

Being extreme microorganisms, cave bacteria are thought to contain genes which can be expressed under extreme conditions like contaminated environment. Some cave bacteria isolated from sulphide-rich caves like *Acidithiobacillus spp*., are sulphur oxidizers growing at relatively low temperature (Macalady et al., 2007). They could be favorable in bioremediation systems where mesophiles or thermophiles are disadvantageous. Beolchini et al., (2017) shown the potential of *Acidithiobacillus spp*., isolated from the Frasassi cave system in Italy, to remove heavy metals from contaminated marine sediments. In addition, et Hamedi al., (2019) have isolated *Actinobacteria* like *Nocardia* sp, *Streptomyces* sp and *Micromonospora* sp, from the Hampoeil cave, which show resistant effects face to toxic metals like Pb, Ni, Cd, Cu, and Zn. Authors suggest that these microbes could be good candidates for the gray biotechnology like metal biosorption or bioaccumulation. Because, some of these species neighbors such as *Streptomyces albogriseolus* HUT6045 and *Streptomyces flavoviridis* HUT 6147 have been tested positive in biosorption, recycling and, bioaccumulation of gold, thorium and uranium (Hamedi et al., 2019 ; Akira and Takehiko, 2004 and Takehiko, 2006).

Microbially induced calcium precipitation is one of the most marked processes in cave ecosystems. Bacteria which practice this process are mostly colonize cave speleothems since they contribute tı the formation and develop the speleothems (Northup and Lavoie, 2001). Here we can cite *Bacillus* species, *Streptomyces* sp., *Sporosarcina* sp., *Acinetobacter*, and others (Seifan et al, 2016 and Li et al., 2019). In fact, this process is used in a diversity of fields like bioremediation of waste waters for calcium removing. During this biomineralization process, cations are incorporated into the calcite and they are immobilized within the CaCO3 structure, thereby their transport into the environment is slowing. These bacteria can solve the problem of the industrial waste waters like those issued from paper recycling industries (Torres-Aravema et al., 2019). Since these waters can provoke living organism's dead like metal sensitive plants and animals.

One of the major environmental problems consists of microplastics distribution. Known as non-degradable synthetic materials, microplastics remain are found everywhere even in drinking water. It is suggested that their biodegradation could be the best solution of this pollution. Studies are multiplied by using different microorganisms (mostly the bacteria) specially those isolated from microplastic contaminated areas. At the writing moment, only one study has been realized with cave bacteria isolated from sediment of Kashmir cave in Pakistan. Authors shown positive results of the polyethylene biodegradation by antibiotic-producer cave bacteria, such as *Serratia* sp. KC1-MRL, *Bacillus licheniformis* KC2-MRL, *Bacillus* sp. KC3-MRL and *Stenotrophomonas* sp. KC4- MRL, and this process was enhanced in media augmented with calcium (Jamil et al., 2017). These results should encourage the multiplication of microplastic biodegradation studies focused on cave bacteria. Also, development of facilitate technics for the application of these microbes in a large scale of plastic biodegradation is needed.

Cave bacteria potentials in bio-pigment production

From the necessity of chlorophyll in the plants and that of the hemoglobin for carried oxygen in the body, it can be said that pigments are necessarily products in life on earth (Rao et al., 2017, Britton, 1995). Pigments are used in many industrial and biotechnology fields such as food allure, home used materials, wearing clothes, medicine, etc. (Rao et al., 2017). Some synthetic dyes have been found to be cancerous even-though they were firstly approved by FDA (Food and Drug Administration) for use in pharmaceuticals, food, and cosmetic productions (Rao et al., 2017). For environmental safety and healthy, natural pigments are the favorable. Plant pigments are ambiguous since they are irregularly available. Here, the microorganisms remain the main source of natural pigments due to their availability and ease genetical modification.

Pigments of microorganisms like melanin and carotenoid consist of diverse functional substances. They are acting under stress and have protection roles since they can dispense antibiotic and anticancer effects, and they protect cells against UV emission and external chemical effects (Klusaite et al., 2016, Hamilton and Gomez 2002). In this fact, the extreme environments such as caves should be rich of such pigment producer microorganisms. Cave structures can be marked with colors consisting of pigmented microorganisms. It was reported that the highest pigment producer bacteria are the *Actinobacteria* genus *Streptomyces* (Con and Jean, 1941, Rao et al., 2017). As previously said, members of *Actinobacteria* phylum such as *Streptomyces*, *Nocardia*, *Micromonospora*, *Stretosporangium*, *Rhodococcus*, are among the mostly colonizing cave walls (Tomczyk-Zak and Zielenkiewicz, 2016) and pigment production in bacteria is more likely to be present in their members (Rao et al., 2017). For all the previous statements, cave ecosystems constitute good and rich source of natural pigments that should be investigated and exploited in the different biotechnological and industrial areas.

BACTERIAL ECONOMIC IMPORTANCE IN INDUSTRY

Enzyme richness of cave bacteria

Enzymes are biological macromolecules (proteins) produced by living organisms which bring a specific biochemical reaction acting as catalysts. Some like amylases, lipases, cellulases, proteases, pectinases, xylanase are industrially important. Amylases alone account approximately 25% of the world enzyme market (Ghosh et al., 2017). Microbial enzymes are preferred instead others for divers reasons: economic rentability, high productivity, ease of product manipulation, regular production because of the permanence of microorganisms, growing of microorganisms on low cost media, high stability and catalytic activity (Gurung et al., 2013).

Cave chemolithoautotrophic bacteria gain energy from chemical materials by secretion of extracellular enzymes. Also, when the primary producer of cave ecosystems break-down organic compounds like plant, they could secrete enzymes like pectinases and cellulases. It is found that 90% of bacteria isolated from Gumki cave, in India, have at least one enzyme potential including lipases, amylases and proteases (Bukelskis, 2019). Pecnolytic activity has been reported in *Brevundimonas* and *Bacillus* species isolated from Mandeepkol Cave in India (Beolchini et al., 2017). Another culture-dependent study shown xanthan lyase, β-glycosidase, phytase as well as other enzymes activities of bacteria isolated from Magura cave in Bulgaria (Tomova et al., 2013). Furthermore, Vitek tests of bacteria from Parsık cave in Turkey shown beta-galactosidase, lipase, arylamidase activities in gram positive and gram-negative isolates (Candiroglu and Dogruoz G, 2020). These enzymes are used in food processing industries such as fruit juice production and wine manufacturing industries which use pectinase enzymes. Cellulase and pectinase also are used in pulp and paper industries. Beta-galactosidases are used in dairy products manufacturing industries. Proteases also like arylamidase enzymes can be used for food products, like meat, fermentation (Banerjee et al., 2019).

Bio-concrete fabrication and concrete crack healing by cave bacteria through microbially induced calcite precipitation

Concrete is one of the major construction materials. Divers factors like variation on the temperature, exposure to radioactive or corrosive compounds, and natural events can impact on the concrete by causing cracks on it. These cracks affect the useful life of the concrete. To solve this problem, several mechanical and chemical methods are used. But for a safe and healthy environment as well as for economic reasons bio-concrete (addition of bacteria capable for precipitation of calcium carbonate (MICP)) fabrication remains the favorable solution (Jonker, 2011). The economic side of bacterial use in concrete fabrication is standing on the fact that bacteria continuously reproduce inside the concrete and consequently induce the concrete self-healing process.

This MICP process is done through different biochemical processes such as biomineralization, urea hydrolysis, amino acids ammonification and denitrification (Castro-Alonso et al., 2019 and Dupraz et al., 2009). From all of this, the urea hydrolysis is the most studied process. It is also reported that bacteria with urease activity show higher rate of calcite precipitation, compared with the other metabolic pathways, (approximatively 20-80%) (Castro-Alonso et al., 2019). This enzyme activity is reported in many cave isolates, especially those isolated from speleothems and karstic cave sediments.

Li et al., (2019) isolated cave urease positive *Acinetobacter* sp. SC4 which is effective in the bio-consolidation of cracks in masonry cement with high rate of reduction of water absorption (42.4% in 28 days). Similar to that,

Nurgroho et al., (2019) have isolated a calcium precipitation inducer identified as *Lysinibacillus macrolides*. In this study, the effects of this bacteria in strengthening of concrete are negligeable but their effects in repairing the developed microcracks are considerable. In addition, the effects of *Sporosarcina pasteurii* in bio-concrete fabrication and crack healing are showed in different studies like in that of (El-Mahdy and Tahwia, 2020). This bacterium has been also reported importantly from cave environment as an effective urease enzyme producer and biocalcification inducer (Omoregie et al., 2018). *Pseudomonas putida* and *Sphingomonas mucossissima* isolated from Yarık Düdeni cave in Turkey have shown promising results in the production of self-healing mortars (Şener, 2020).

CONCLUSION

Cave bacteria are extreme microorganisms with many beneficial uses. Despite the limited study of them, they are known as sources of many industrial and biotechnology tasks. They can be used in drugs fabrication, bioremediation, enzymes production, and constructions. Caves over the word are multiple, however most of them still unexplored. Some of them are not protected. The economic values of the cave living microorganisms need an improvement in the cave exploration and protection technics. Scientists, ministries, cavers and cave managers are called to work together for this challenge.

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➢ *ORAL PRESENTATION*

The effects of acrylamide and Vitamin E administration on adult female rat hippocampus and serum during pregnancy

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Abstract
The present study aimed to investigate the effects of acrylamide (AA) and vitamin E on the hippocampus tissue and serum of adult female rats during pregnancy. The present study was conducted after the approval of Inonu University ethics committee. After pregnancy was induced, pregnant rats were divided into the control, corn oil, Vitamin E, AA, Vitamin E + AA groups. AA, corn oil and vitamin E were administered to the rats in the relevant groups during pregnancy for 20 days at the same time via oral gavage. The pregnant rats were allowed to deliver normally. No administration was conducted to postnatal fetuses during breastfeeding. In the 8th postnatal week, serum and brain tissues were obtained from the female rats and hippocampus tissues were biochemically analyzed. AA group serum samples and hippocampus tissues were compared to the samples obtained from all other groups and it was found that malondialdehyde (MDA) and total oxidant status (TOS) levels increased, while glutathione (GSH) and total antioxidant status (TAS) levels decreased in the AA group. It was determined that GSH and TAS levels increased and MDA and TOS levels decreased in the vitamin E group when compared to all other groups. It was observed that vitamin E and AA administration could minimize the neurotoxic effects associated with AA administration. Exposure to AA is inevitable with the consumption of fast food today. Antioxidant/oxidant balance should be maintained to prevent AA toxicity. Based on the present study findings, daily consumption of nutrients with adequate vitamin E content could be recommended to maintain maternal and infant health against AA neurotoxicity.
Keywords: Pregnancy, acrylamide, vitamin E, neurotoxicity, blood, female rats.

1.INTRODUCTION

Acrylamide, which has a molecular weight of 71.08, is a very good water-soluble chemical. Although acrylamide exists naturally, it is a synthetic material, an α–β-ansatur carbonyl compound with very high chemical activity that finds quite common usage. Although extremely used in research laboratories, especially in molecular biology laboratories (such as electrophoresis, chromatography); it has widespread use in printing, textile, treatment of waste water, sectors that produce cosmetic products such as lotions and deodorants (Kopp and Dekont, 2009). In scientific research conducted in 2000s, it was demonstrated that high amounts of acrylamide formation was detected in food exposed to temperatures exceeding 120 C due to the interaction of monosaccharides and asparagine amino acid. This result induces extensive researches on food borne acrylamide (Exon 2006, Parzefall 2008). Studies conducted with humans demonstrated that acrylamide causes neurotoxicity (Spencer et al., 1979, Sickles and Goldstein 1985). Daily average acrylamide intake dose was reported as 0.5 mg/kg/day, but the intake dose varies based on factors such as age, gender, nutritional habits and lifestyle (Dybing et al., 2005, Bjellas et al., 2007). Acrylamide has been found to be especially neurotoxic, carcinogenic for animals and to be highly toxic on the reproductive system (FAO and WHO, 2006, LoPachin, 2004). In experimental studies conducted by administering acrylamide to pregnant animals, it was determined that acrylamide caused significant structural malformations causing brain, liver, bone growth retardation in fetus (LoPachin, 2004). Acrylamide that dissolves easily in water, passes through the placenta directly and reaches the fetus tissues and depending on the daily intake causes development disorders and tissue damage (Edwards, 1974). Daily average AA intake dose was reported as 0.5 mg/kg/day; however, the intake dose varies based on factors such as lifestyle, nutritional habits, age, and gender (El-Sayyad et al. 2011, Allam et al., 2011). Acrylamide causes oxidative stress by disrupting the oxidant/antioxidant balance in the body. Generally, acrylamide decreases the reduced glutathione (GSH) levels

and increases lipid peroxidation levels in tissues. All of these tissue damages are suggested to originate from the oxidative stress caused by acrylamide (El- Sayyad et al., 2011, Muralidhara 2014).

Vitamin E, the active form of which is a α (alpha) tocopherol, is a potent antioxidant soluble in lipids and could easily penetrate the placenta to reach the fetus (Azzi et al., 1998, Hidiroglu et al., 2001). Vitamin E, due to its strong antioxidant properties, detoxifies free radicals and prevents lipid peroxidation and oxidative stress-induced tissue damage by preventing free radicals from attacking the lipid layer of the cell (Reiter et al., 2007).

The present study aimed to investigate the effects of acrylamide (AA) and vitamin E on the hippocampus tissue and serum of adult female rats during pregnancy.

2. MATERIAL AND METHODS

Animals Ethical consent was taken before the study from Ethics Committee for Experimental Animals of Inonu University Faculty of Medicine. Forty female Wistar albino rats weighing 250 ± 20 g, bred at Inonu University Faculty of Medicine, Laboratory Animal Production and Research Centre (INFM-LAPRC), were used. The female rats were taken into special cages at 5:00 PM. To create a pregnancy in rats, one male rat was used for every two female rat. The rats were kept in the same cage until at 08:00 AM in the morning of the next day. At the end of this period, male rats were separated from the females. The female rats were examined under a microscope by taking vaginal smear and those with sperm in smear were accepted to be half-day pregnant. Female rats, of which their pregnancy could not be confirmed positive via smear were excluded from the experiment. Pregnant rats were weighted and randomly divided into five groups each containing eight rats. The rats were kept in rooms supplied with 12 h of light and 12 h of dark environment, continuously ventilated and with temperatures 21 ± 2 C for 20 days (gestation period) at INFM-LAPRC. They were fed ad libitum during the experimental period.

Rats were divided: Control group: eight pregnant rats, were administered normal saline. Corn oil group: eight pregnant rats, were administered corn oil. Vitamin E group: eight pregnant rats, were administered vitamin E, with a dose of 100 mg/kg (Naito et al., 2005) body weight, in corn oil. Acrylamide group: eight pregnant rats, were administered acrylamide, with a dose of 10 mg/kg (Tyla et al., 2000) body weight, in water. Acrylamide + vitamin E group: eight pregnant rats, were administered vitamin E, with a dose of 100 mg/kg body weight, and acrylamide, with a dose of 10 mg/kg body weight. All the applications were performed at the same time and continued from the 0th to 20th for 20 days with a volume of 1 mL by gavage. Rats were decapitated on the 20th day of the gestation and tissue samples were taken from female rats. Brain tissues preserved in deep freeze were removed and weighed on the day of the study. Phosphate buffer was added to produce a 10% homogenate and they were homogenized in ice for 1–2 min at 12,000 r/min (IKA, Germany). Supernatants were obtained by centrifuging tissue homogenates for 30 min at 5000 r/min and +4 degrees. Measurement of malondialdehyde (MDA) level MDA analysis was conducted with the method developed by (Uchiyama and Mihara, 1978). The MDA concentration was determined by measuring the supernatant that was extracted from the n-butanol phase of the pink colored product formed by the reaction between the MDA in supernatant and thiobarbituric acid at 95 C at 535 and 520 nm by spectrophotometry. Measurement of reduced glutathione (GSH) level GSH analysis was conducted according to the method described by (Ellman 1979). It was conducted by determining the GSH by reading the light intensity of the greenish color produced by the reaction between the GSH in the analysis tube and 5,50 - dithiobis 2-nitrobenzoic acid at 410-nm wavelength. Measurement of total oxidant status (TOS) level For TOS measurements, the absorbance at 530 nm was measured by mixing 500-mL reagent 1 (measurement buffer) and 75-mL serum, adjusting the ELISA to 25 C as indicated in the kit procedure. Mixing 25-mL reagent 2 (prochromogenic solution) was added and incubated for 10 min. After incubation, TOS levels were determined by measuring the absorbance at 530 nm again (Erel 2005). Measurement of total antioxidant status (TAS) level For TAS measurement, as described in the kit procedure, the ELISA was set at 25 C, and 500-mL reagent 1 (measurement buffer) and 30-mL serum were mixed and the absorbance was measured at 660 nm. Seventy-five microliter reagent 2 (colored ABTS solution) was added to the mixture and it was incubated for 10 min. TAS levels were determined by measuring the absorbance at 660 nm again after incubation (Erel 2004). Data were expressed as median, minimum and maximum values. Group comparisons performed by Kruskal-Wallis test and Conover pairwise comparison method. In all analysis significance level was considered to be 0.05.

3. RESULTS AND DISCUSSION

It was determined that acrylamide (10 mg/kg/bw), administered during pregnancy, statistically significantly increased adult fermale brain hipocampus and blood serum MDA and TOS levels, while it decreased GSH, TAS levels when compared with all other groups. In the vitamin E administered group (100 mg/kg/bw); GSH, TAS levels significantly increased statistically and TOS and MDA levels dropped to levels of the control group in comparison to all other groups ($p \leq 0.05$). A liberal amount of acrylamide (AA) is produced as a result of frying or baking foods in high temperatures, and individuals take certain amounts of AA everyday by consuming these food items. Pregnant women are also exposed to AA originating from food during pregnancy and their fetus are probably affected. Oral acrylamide intake has been observed by shifting the antioxidant / oxidant balance in favor of oxidants, leading to toxicity causing oxidative stress in the tissues and blood adult female rats. Vitamin E administration has been observed to prevent oxidative stress-induced toxicity by normalizing the antioxidant/ oxidant balance through detoxification. If it is considered that it is impossible to prevent exposure to AA toxicity in maternal and infant health, we recommend consuming sufficient amounts of fresh vegetables and fruits with high antioxidant properties, especially vitamin E, on a daily basis. For the complete discovery of the AA induced neurotoxicity mechanisms of AA in adult brains, further studies are required.

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➢ *ORAL PRESENTATION*

Spectral analysis of biodiesel and biodiesel blends produced by the Industry and petroleum based diesel fuels

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Abstract

Biodiesel is one of the most effective, alternative and renewable fuel, which can reduce the exploitation of fossil fuels, thus contributing to curbing greenhouse gases (GHG) emissions and reduction of anthropogenic global warming factors. Biodiesel and its blends have the possibility to be more widely used as alternative, eco-friendly fuel in various types of internal combustion engines. For this purpose it is important to achieve stable structural composition of functional groups of biodiesel fuel and its blends produced by the industry. The present work is focused on analysis of the structural compositions of the biodiesel and its blends made from used cooking oil (UCO) produced at a biodiesel plant in Georgia. The oil and acid composition of biodiesel fuel have been studied and identified through gas chromatographer and the functional groups of the fuel were analyzed using a Fourier IR spectrometer. The results of the research proved that structural composition of biodiesel, as well as its blends, remained stable even after blending with petroleum diesel. Biodiesel and its blends maintained high quality and met the demands of EN 14214, ASTM D6751 and EN 590 standards, thus proving that both biodiesel and its blends can be freely used in a wide range of internal combustion engines.

Keywords: biodiesel, fuel quality, trans-etherification, IR spectroscopy

INTRODUCTION

In the modern world the main trend of the fuel market development is closely connected to the promotion of alternative, renewable energy sources, especially those based on bio-resources. This trend, very likely, will remain in the list of world's fuel supply priorities within the nearest 25-30 years. This is the clear trend in energy sector development programs of the leading economies in the world - both in the USA and the EU, therefore Renewable energy will continue to play an increasingly important role in meeting the world's growing energy needs (Festus et al., 2019).

Biodiesel is one of the most effective, affordable, and carbon-neutral renewable fuel, which can reduce the exploitation of fossil fuels and contribute to curbing greenhouse gases (GHG) emissions and reduction of anthropogenic global warming factors (Antolin et al., 2002; Khan et al., 2007; Vicente et al., 2004).

Biodiesel is renewable and sustainable fuel and can significantly reduce the danger of environmental disasters. Biodegradability in both soil and water is another important advantage of biodiesel; some 89% of the carbon contained in biodiesel will be biodegraded in just 28 days. Biodiesel can sharply reduce - up to 85% - emissions of Polycyclic Aromatic Hydrocarbons, (PAHs) which are identified as carcinogenic compounds.

Today the name biodiesel is commonly given to mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats through the process of etherification. Biodiesel is a clean, alternative to common petroleum fuel, made from natural vegetable oils, and/or animal fats, i.e. from bio resources. Biodiesel is a high quality fuel which can be used in any segment of industry where petro-diesel is used, including the internal combustion engines (Khetsuriani et al., 2017)

Biodiesel production has been steadily developing during the last decades in the world, the new report, of "Befoul Markets and Technologies", estimates steady growth though 2018 and 2021 as a result of higher oil prices, new feedstock availability and advanced technologies including supercritical fluid technologies (Marchetti et al., 2007). Befoul production is projected to reach 65.7 billion gallons (25 million tons) per year by 2021. This process confirms the vision of Rudolf Diesel, the great German, scientist, engineer and inventor, who's invention of new

type of compression-ignition engine, named after him, shaped great deal the development of the whole world industry in the 20th century: *"The use of vegetable oils for engine fuels may seem insignificant today, but such oils may become, in the course of time, as important as petroleum and the coal-tar products of the present time" - Rudolf Diesel, 1912.* (Pahl G., 2005).

The name "Biodiesel" has been given to this biofuel since it is a clean burning alternative fuel, made from natural vegetable oils, and/or animal fats, i.e. bio resources. The physical and chemical characteristics of biodiesel are quite close to those of petroleum based diesel fuel, but at the same time, biodiesel is eco-friendly and carbon-neutral. In addition, since biodiesel has a closed carbon cycle, biodiesel, unlike petroleum-based products, does not add greenhouse gases (GHGs) to the atmosphere. (Antolin et al., 2002).

The present work is focused on analysis of the most important characteristics of both biodiesel and conventional (petroleum-based) diesel fuels, as well as biodiesel blends produced on industrial scales, by biodiesel plants using the method of trans-etherification. Based on its research and findings, the article gives the recommendations based on the results that biodiesel can be more widely used in diesel engines without any modification of the engines.

MATERIALS AND METHODS

All used chemicals were of analytical grade. Methanol, Ethanol, n-pentane, petroleum ether were purchased from Sigma-Aldrich and Merck (Sigma-Aldrich, ≥99.8%) and potassium hydroxide (Sigma-Aldrich, ≥85%) were used for trans-etherification reaction. Dowex ^{R} MB ion-exchange resin was used for purification of biodiesel. (Sigma-Aldrich, D2572).

The well-adopted method for producing biodiesel is the biochemical process named etherification of triglycerides (TGs), usually those derived from the plant oils. TGs react with alcohol in the presence of the acid, or base catalysts and the outcome is alkyl esters of fat acids and glycerin reactions are consecutive and reversible. From the pure chemical point of view, biodiesel is methyl ester if methanol is used in the process of etherification, and consequently, ethyl ester, if ethanol is applied. The whole process is comprised of three reversible reactions when di- and monoglycerides are created as intermediate products (Freedman et al., 1986).

There are several methods which can be used to receive biodiesel. The alternative method is to use heterogenic (solid) catalysts for the process of etherification. The heterogenic (solid) catalysts have the advantage that they could be removed from the area of reaction and used again. Process of etherification with solid catalysts is considered as the most "green" process of etherification. That process does not require neither recovery of the catalyst, nor the phase of water treatment (washing), thus ensuring the high outcome of the methyl esters, which are very close to the theoretical values (Cao et al., 2008), in addition the glycerin, it will be produced with the higher purity, with 98% and more not containing impurities (Bournay et al., 2005; Melero et al., 2009). As per the method of supercritical area of alcohol, it has only one phase for etherification, which can provide some advantages for the industry (Dermidas, 2002; Kusdiana et al., 2001; Madras et al., 2004 (Warabi, 2004).

Today, while producing biodiesel on an industrial scale, the majority of the plants still use the method based on catalyzed trans-etherification of oils from bio-resources, since it is the most economically viable process requiring only low temperatures and pressures and producing 98% conversion. The trans-etherification process is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and glycerol (Fig.1).

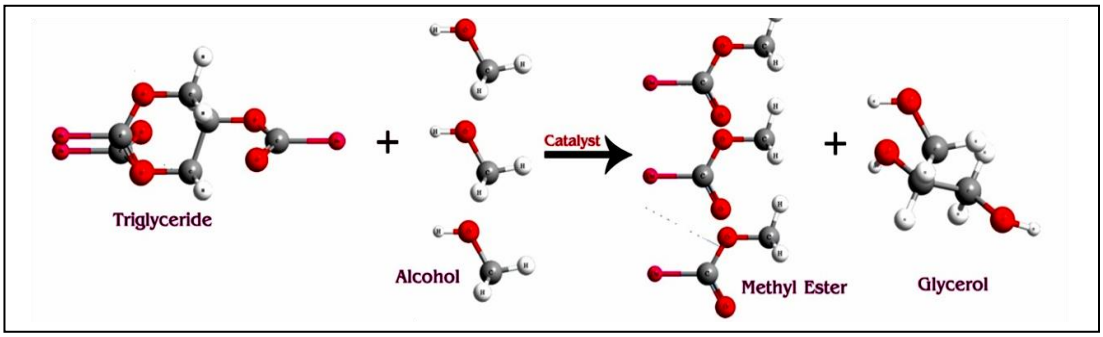

Fig.1. Trans etherification of triglycerides

Since our goal was to analyze the biodiesel fuel produced by the industry, we worked on the samples of biodiesel taken from the normal, commercial batch of biodiesel produced by a biodiesel plant operating in Tbilisi, Georgia (www.biodiesel.ge).

The plant operated by "Biodiesel Georgia LLC" produces B100 i.e.100% biodiesel. We have blended the biodiesel with conventional petroleum based diesel fuel. The blending percentage of biodiesel with petro diesel was set to 0%, 5%, 10%, 20%, and they were marked as B0, B5, B10 and B20, whereas B stands for biodiesel and the figures indicate the percentage of biodiesel in the blend. All the biodiesel blends were stored in different conical flasks and sealed with aluminum foil for further tests

The fuels have been analyzed according to the ASTM D6751, and EN 590 standards. The results of analyses and the chemical and physical characteristics are given in the Table 1.

Table 1. Fuels specifications

PROPERTY	**Biodiesel Fuel**				
	BO	B5	B10	B20	B100
Density at 15^0C, kg/m^3	834.0	834.0	835.0	840.0	890,3
Density ^{0}API	38.16	38.16	37.96	36.95	27.49
Viscosity, cCt at 40^0C	2.0	2.00	2.20	2.50	3.50
Flash point, ^{0}C	50	50	52	58	136
Acid number, mg KOH/g	0.0048	0.048	0.0048	0.0050	3.5
Cetane Number	49	49	49	49	57
Sulfur content, mg/kg	10.0	10.0	3.8	4.0	-
Ash content , %	0.016	0.016	0.016	0.018	0.022
Copper strip Corrosion, (3 hours 50^0C)	1b	1b	1b	1b	1b
Water content, ppm	200	200	200	260	492
Distillation					
%(V/V) recovered at 250 °C	65	65	65	65	-
% (V/V) recovered at 350 °C	85	85	85	85	-
95 % (V/V) recovered at	350	350	350	350	-

The oil and acid composition of biodiesel fuel has been studied and identified through gas chromatography and the functional groups of the fuel were analyzed using a Fourier IR spectrometer.

The quality of the fuel is extremely important, especially for transport sector, where diesel type internal combustion engines are widely used. The high quality of the fuel ensures high output of the engine as well as economy and long life cycle of the mechanisms. Therefore our goal was to control the top quality level of pure biodiesel B100 and its blends B5, B10 and B20.

The physical and chemical parameters of biodiesel were analyzed using *PerkinElmer SPECTRUM Version 10.4.2.* The Fig.4 shows the spectrum of B5, B10, B20, B100 and petroleum diesel, where the functional groups of the compounds have been identified.

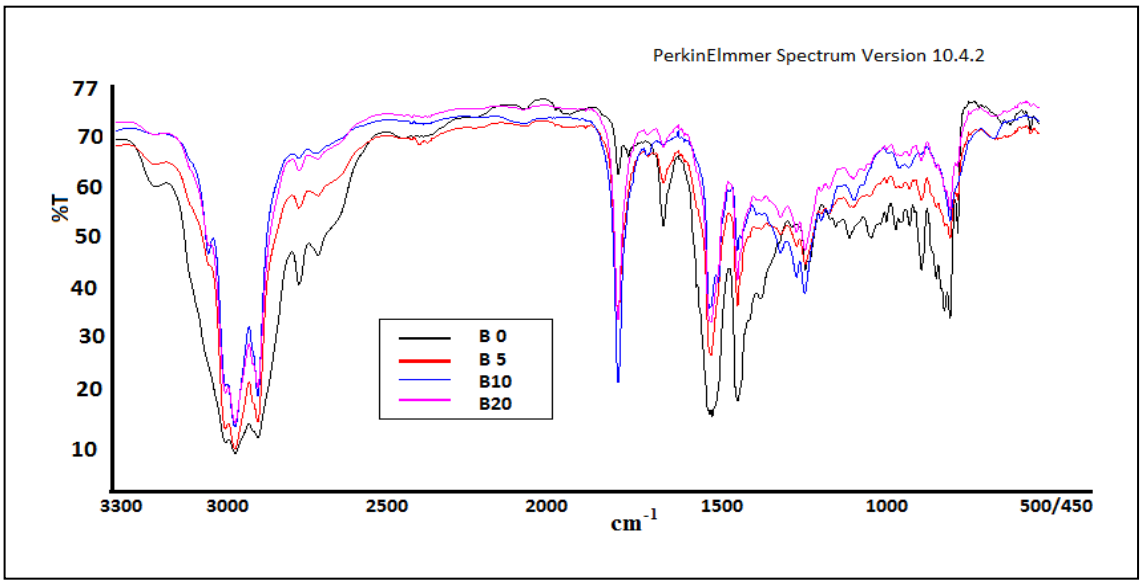

Fig. 2 - Spectral analyses of petro diesel and biodiesel blends B-5, B-10, B-20

In the Fig.2 the abbreviations stand for the biodiesel and its blends according to the internationally accepted marks, i.e. B0 – conventional diesel fuel (petroleum diesel fuel), B5 – blend of 5% biodiesel with petro diesel, B10 blend of 10% biodiesel with petro diesel, and B20 blend of 20% biodiesel with petro diesel.

RESULTS AND DISCUSSION

FTIR Analysis**.** In the infrared spectrum of biodiesel (Figure 2.), the FTIR spectrum of petroleum based diesel showed the alkane C–H bond which lies on the wave numbers from 2800 cm−1 to 3000 cm−1 and 1350 cm−1 to 1480 cm−1. Thus, it can be confirmed that both conventional diesel and biodiesel had the same functional group of C–H. However, the conventional diesel had no oxygen group, whereas biodiesel showed oxygen functional group such as C–O and C=O at 400 cm−1 to 1500 cm−1]. Therefore, the biodiesel with the existence of oxygen could be promoted as cleaner and complete combustion fuel. On the other hand, the conventional diesel without any oxygen produced more black smoke and incomplete combustion during burning.

When comparing the physical and chemical parameters of conventional biodiesel and biodiesel blends it becomes obvious that the overall quality of the biodiesel blends have been maintained and several major parameters of biodiesel even have been improved; and what is most important, the structural compositions of the functional groups remained stable .

CONCLUSIONS

The fact that biodiesel and its blends produced by the industry meet the strict quality requirements of EN 14214,, ASTM D 6751, and EN 590 standards, opens the possibilities for these biofuels to be more widely used in various sectors where petroleum diesel fuel is used, especially in internal combustion engines, in transport sector.

Biodiesel can bring many benefits to the country producer: diversification of energy supply sources, development of alternative renewable energies, launching new production plants, creating new jobs, reducing GHG gases emissions, improving the ecological conditions, reducing harmful and hazardous emissions and ultimately protecting the environment and strengthening energy independence.

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➢ *ORAL PRESENTATION*

Covid-19 hastalarının laboratuvar parametrelerinin karşılaştırılması

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Özet

Covid-19 hızlı yayılım gösteren, bulaştırıcılığı yüksek bir hastalıktır. PCR (Polimeraz Zincir Reaksiyonu) testi yapılarak covid-19 tanısı almış hastaların evdeki, servisteki ve yoğun bakımdaki kan değerlerini karşılaştırmayı amaçladık. Hastaların hemoglobin (Hb), beyaz kan hücresi (WBC), trombosit (PLT), ortalama trombosit hacmi (MPV), trombosit dağılım genişliği (PDW) ve CRP değerlerini kaydedilerek karşılaştırma yaptık. Çalışmamıza 14 kadın, 17 erkek toplam 31 Covid (+) hasta dahil edildi. Hastalardan 2 tanesi yoğun bakım takibi sırasında ex oldu. Çalışmamızda hastaların yaş ortalamaları 63.3±2.37 (min=18-max=81) idi. Hb 1 değeri Hb 2 ve Hb 3' e göre istatistiksel olarak anlamlı derecede yüksek bulundu (p: 0.011). PLT1, 2 ve 3 değerleri incelendiğinde PLT1'in düşük olmasından kaynaklı anlamlı fark elde edildi (p: 0.027). Ayrıca MPV 1 in MPV 2 ve MPV 3' ten, CRP 2'nin CRP 1 ve CRP 3' ten yüksek olmasından kaynaklanan istatistiksel olarak anlamlı fark bulundu (sırasıyla p: 0.014, p: 0.015). CRP ve WBC değerlerindeki yükselişin hastaların hastaneye yatışında etkili olabileceğini düşünmekteyiz.

Anahtar Kelimeler: Covid, CRP, hemoglobin, trombosit

Comparison of laboratory parameters of Covid-19 patients

Abstract

Covid-19 is a rapidly spreading and highly contagious disease. We aimed to compare the blood values of patients diagnosed with covid-19 by performing the PCR (Polymerase Chain Reaction) test at home, at the time of hospitalization and in the intensive care unit. We recorded the hemoglobin (Hb), white blood cell (WBC), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and CRP values of the patients and made a comparison. 14 female, 17 male, a total of 31 Covid (+) patients were included in our study. Two of the patients died during the intensive care follow-up. In our study, the mean age of the patients was 63.3 ± 2.37 (min=18-max=81). Hb 1 value was statistically significantly higher than Hb 2 and Hb 3 (p: 0.011). When PLT1, 2 and 3 values are examined; A significant difference was obtained due to low PLT1 (p: 0.027). In addition, a statistically significant difference was found due to MPV 1 being higher than MPV2 and MPV 3, CRP 2 higher than CRP 1 and CRP 3 (p: 0.014, p: 0.015, respectively). We think that the increase in CRP and WBC values may be effective in hospitalization of the patients.

Keywords: Covid, CRP, hemoglobin, platelet

GİRİŞ

Covid-19 ilk olarak Aralık 2019'da Çin'in Vuhan kentinde başlayan salgınla belirlenen, hızlı yayılım gösteren, patojenite ve bulaştırıcılığı yüksek bir enfeksiyondur. Başta akciğerler olmak üzere beyin, böbrek, karaciğer gibi birçok organı etkileyebilmektedir. En çok etkilenen ve mortalitede etkisi olan organ tutulumu akciğerdir. Hastalık esas olarak damlacık yoluyla yayılım göstermektedir. Hasta bireylerin öksürmesi, hapşırması ile ortama saçılan damlacıklar ile diğer kişilerin teması bulaşta temel yoldur. Asemptomatik kişiler de belirtileri olmasa bile virüsü

solunum yollarında taşıdığı için bulaştırıcılık açısından çok tehlikeli grubu temsil etmektedir (Lai ve ark., 2020, Zhou ve ark., 2020)).

Tüm dünyayı etkileyen bu infeksiyonun çok sayıda ölüme neden olmasının yanı sıra ekonomik, sosyal ve psikolojik birçok negatif etkisi olmaktadır. Bu nedenle hastalığın doğal seyrinin belirlenmesi, bulaşmanın engellenmesi, hastalığın yönetilmesi açısından erken teşhis, izolasyon ve tedavi çok önemlidir. Bu basamaklarda hastalığın laboratuvar indeklerinin izlenmesi yol göstericidir. Ülkemizde Covid-19 tanı, takip ve tedavisi için bir grup biyokimyasal test yapılmaktadır (Cascella ve ark., 2020, Guan ve ark., 2020). Tam kan sayımı, lenfosit sayımı, C-reaktif protein (CRP), prokalsitonin, karaciğer böbrek fonksiyon testleri, koagülasyon parametreleri, vs hastalığın tanısına destek olmasının yanı sıra tedavi takibinde de yaygın olarak kullanılmaktadır (Fan ve ark., 2020, Pascarella ve ark., 2020). Tan ve arkadaşları 2020 yılında yaptıkları çalışmada Covid-19 erken evrelerinde CRP'nin ve eritrosit sedimantasyon hızının birlikte arttığını göstermişlerdir. Luo ve arkadaşları 2020 yılında yaptıkları retrospektif bir çalışmada özellikle başvuru anındaki CRP düzeyinin hastalığın şiddetinin derecelendirilmesinde önemli olabileceğini ileri sürmektedirler.

Bu çalışmada PCR testi ile covid tanısı almış hastaların evde, serviste ve yoğun bakımdaki hemoglobin (Hb), beyaz kan hücresi (WBC), trombosit (PLT), ortalama trombosit hacmi (MPV), trombosit dağılım genişliği (PDW) ve CRP değerlerini hem kendi içinde hem de diğer hastaların değerleriyle karşılaştırmayı amaçladık.

MATERYAL VE METOD

Covid-19 pozitifliği PCR testi ile doğrulanmış 31 hastanın evde karantinada kaldığı süre içindeki, servise ilk yatış anındaki ve hastane yoğun bakıma geçiş anındaki Hb, WBC, PLT, MPV, PDW ve CRP değerleri hem kendi içinde hem de diğer hastaların değerleriyle retrospektif olarak karşılaştırıldı. Hastaların yaş, cinsiyet ve mortalite durumları da incelendi. Veri toplama aşaması sonunda elde edilen veriler bilgisayar ortamına aktarılarak analiz edildi. Analiz için SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA) paket programı kullanıldı. Verilerin normal dağılıma uygunluğu görsel (histogram ve olasılık grafikleri) ve analitik yöntemler (Kolmogrorov-Smirnov/Shapiro-Wilk testleri) kullanılarak incelendi. Sayısal verilerin özetlenmesinde; aritmetik ortalama, standart sapma, minimum ve maximum değerleri, kategorik verilerin özetlenmesinde frekans dağılımları ve yüzdelikler kullanıldı. Parametrik test varsayımlarına uymadığı için zamanla değişimin istatistiksel anlamlılığı Fridman testi kullanılarak incelendi. Gereği halinde ikişerli karşılaştırmalar Wilcoxon testi kullanılarak yapıldı ve Bonferroni düzeltmesi kullanılarak değerlendirildi. Zamanla, değişkenlerin değişimine cinsiyetin etkisi tekrarlı ölçümlerde varyans analizi kullanılarak incelendi. İstatistiksel anlamlılık için tip-1 hata düzeyi %5 olarak kabul edildi.

BULGULAR ve TARTIŞMA

Çalışmamıza 14 kadın, 17 erkek toplam 31 Covid (+) hasta dahil edildi. Hastalardan 2 tanesi yoğun bakım tedavileri sürecinde ex oldu. Çalışmamızda hastaların yaş ortalamaları 63.3±2.37 (18-81), kadın hastaların yaş ortalamaları 68.8± 3.2, erkek hastaların yaş ortalamaları 58.7±3.1 idi. Zhang ve arkadaşları'nın, 140 hastayı içeren çalışmalarında ortalama yaş 57.0 olarak bildirilmiştir ve çalışmada hastaların %50.7'si erkek ve %49.3 kadın olarak saptanmıştır. Guan ve arkadaşları'nın 1099 hasta üzerinde yaptıkları bir başka çalışmada ise hastaların %41.9'unun kadın ve ortalama yaşlarının 47 olduğu bildirilmiştir.

Hastalara ait evde ilk tanı konulduğu andaki, serviste ilk yatış anındaki ve yoğun bakımdaki laboratuvar parametreleri incelendiğinde; Hb1 ortalamaları 12.9±0.3, Hb2 ortalamaları 12.1±0.3, Hb3 ortalamaları 11.8±0.3 olarak bulundu. WBC1 ortalamaları 7.2±0.5, WBC2 ortalamaları 12.6±3.5, WBC3 ortalamaları 9.7±1 olarak bulundu. PLT1 ortalamaları 208.6±9.5, PLT2 ortalamaları 250.2±17.9, PLT3 ortalamaları 278.9±21.3 olarak bulundu. MPV1 ortalamaları 12.9±4.4, MPV2 ortalamaları 10.2±0.2, MPV3 ortalamaları 10.1±0.2 olarak bulundu. PDW1 ortalamaları 12.6±0.4, PDW2 ortalamaları 11.9±0.3, PDW3 ortalamaları 11.9±0.3 olarak bulundu. CRP1 ortalamaları 42.4±8.5, CRP2 ortalamaları 98.6±17.5, CRP3 ortalamaları 65.1±13.8 olarak bulundu.
HB1, 2 ve 3 değerleri incelendiğinde Hb 1 değerleri diğer değerlere göre yüksek olduğu için aradaki fark istatistiksel olarak anlamlı bulundu. (p:0.011). WBC1, 2 ve 3 değerleri incelendiğinde aralarında anlamlı bir fark bulunmadı (p: 0.095). PLT1, 2 ve 3 değerleri incelendiğinde PLT1'in düşük olmasından kaynaklı anlamlı fark elde edildi (0.027). MPV1, 2 ve 3 değerleri incelendiğinde MPV1 in yüksek olmasına bağlı anlamlı fark elde edildi (p: 0.014). PDW1, 2 ve 3 değerleri açısından anlamlı fark bulunamamıştır (p: 0.136). CRP1, 2 ve 3 açısından fark incelendiğinde ise CRP2 değerinin yüksek olmasından kaynaklı anlamlı bir vardı(p: 0.015) (Tablo 1).

Tablo 1: Hastalara ait evde, serviste ve yoğun bakımda elde edilen bazı laboratuvar değerleri

Değişkenler	1 (median±SD)	2 (median±SD)	3 (median±SD)	p value
Hgb (g/dL)	12.9±0.3	12.1±0.3	11.8±0.3	*0.011
WBC (10^3/mm^3)	7.2±0.5	12.6±3.5	9.7±1	0.095
PTL (10^3/mm^3)	208.6±9.5	250.2±17.9	278.9±21.3	*0.027
MPV (μm^3)	12.9±4.4	10.2±0.2	10.1±0.2	*0.014
PDW (%)	12.6±0.4	11.9±0.3	11.9±0.3	0.136
CRP (mg/dL)	42.4±8.5	98.6±17.5	65.1±13.8	*0.015

1: evde, 2: serviste, 3: yoğun bakımda *İstatistiksel olarak anlamlı $p<0.05$ level.

Huang ve arkadaşları'nın 41 hastada yaptıkları çalışmada başvuru sırasındaki kan sayımlarında %25.0 hastada lökopeni ve %5.0 hastada trombositopeni saptanmıştır (Huang ve ark., 2019). Diğer taraftan Guan ve arkadaşları, 1099 hastayı değerlendirdikleri çalışmalarında ise %33.7 hastada lökopeni ve %36.2 oranında trombositopeni varlığını bildirmişlerdir (Guan ve ark., 2019). Çalışmamızda ise başvuru anındaki değerlerinde %19 hastada lökopeni ve %9.1 hastada trombositopeni izlenmiştir. British Journal of Hematology'de yayınlanan yakın tarihli bir makalede, Jiang ve ark. 7613 katılımcıyı içeren 31 çalışmanın meta-analizinde trombositopeni ile hastanede yatan ağır Covid vakaları arasında önemli bir ilişki buldu (Jiang ve ark., 2020). Covid-19 hastalarında trombosit indekslerindeki değişim mekanizması muhtemelen çok faktörlüdür. Kemik iliği enfeksiyonuna bağlı trombosit azalması veya bağışıklık sistemi tarafından trombositlerin yıkımı olası beklentilerdir. Trombosit sayısı azaldıkça, yeni trombosit yapımı da artmaktadır. Bu değişiklikler trombosit indeksleri, MPV ve PDW'deki artışı açıklayabilir. Bir kişi düşük trombosit sayısı ve yüksek bir MPV seviyesine sahip olduğunda, kemik iliğinin hızla trombosit ürettiğini söylemek mümkündür. Bunun nedeni; eski trombositler yok edildiğinden, kemik iliği yenileriyle telafi etmeye çalışmaktadır. Bizim sonuçlarımızda da PLT1 in düşük olması ve MPV1 in yüksek olması bu verileri destekler niteliktedir.

Enfeksiyon veya doku hasarına bağlı inflamasyonda artış gösteren akut faz reaktanı CRP'nin Covid-19'da da yükseldiği izlenmiştir (Zhang ve ark., 2020). Guan ve arkadaşları, 1099 hastada %60.7; Xu ve arkadaşları 90 hastada %42.0 oranında CRP yüksekliği bildirmiştir (Guan ve ark., 2019, Xu ve ark., 2020). Çalışmamızda ilk başvuru anındaki crp düzeyi hastaların %70.1'inde yüksek (>8 mg/dL) bulundu.

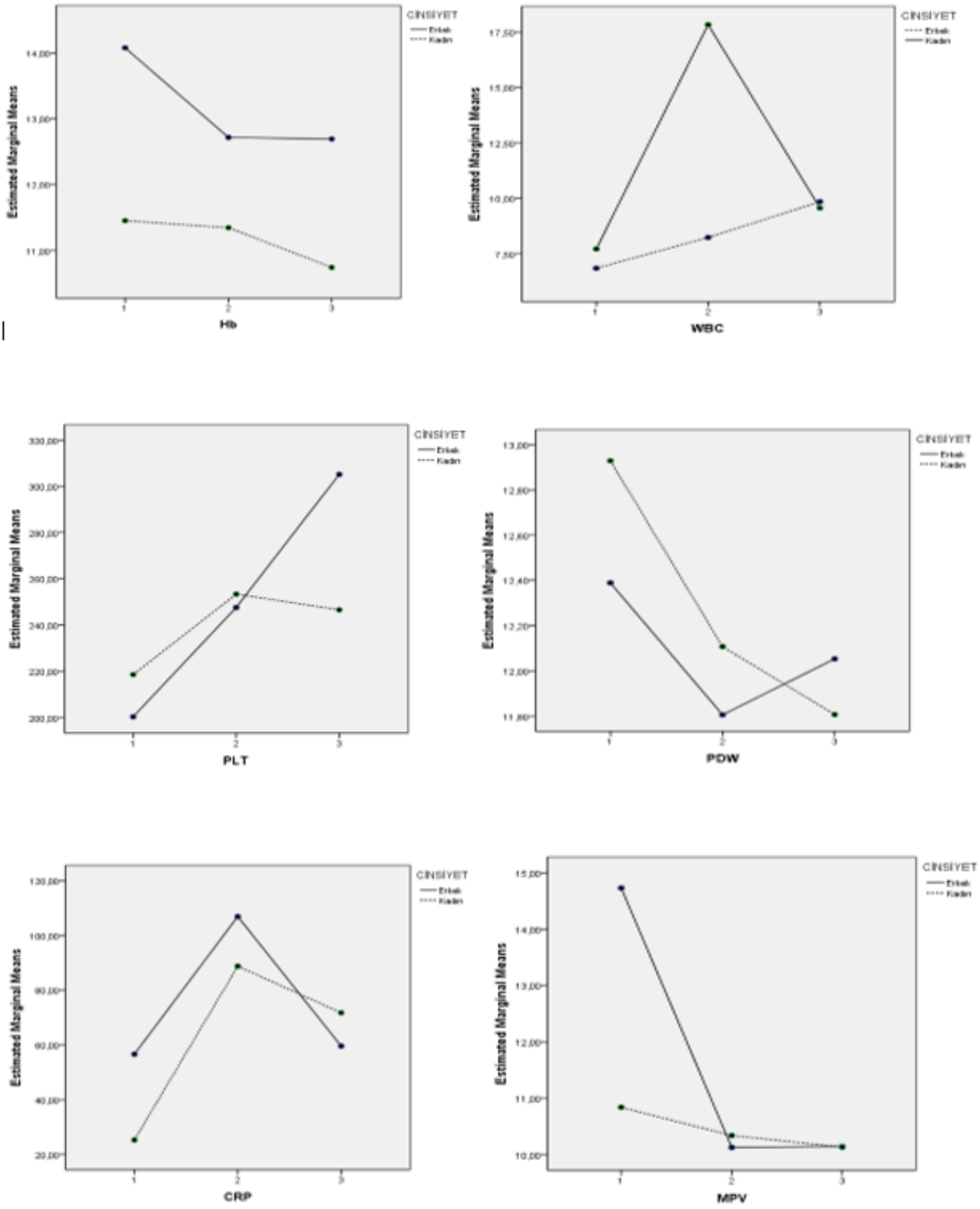
Lagadinou M ve arkadaşlarının SOFA skoruna göre hastaları 2 grup (grup 1: şiddetli Covid, grup 2: orta düzey ve ayakta tedavi edilen) yaparak gerçekleştirdikleri çalışmada PLT açısından herhangi bir farka rastlanmazken, CRP açısından şiddetli Covid grubunda CRP yüksekliği nedeniyle gruplar arası farkı anlamlı bulmuşlardır (p: 0.01) (Lagadinou ve ark., 2020).

Özer KB ve arkadaşlarının yoğun bakım ve servis hastaları arasındaki laboratuvar parametrelerini karşılaştırdıkları çalışmada, yoğun bakım ünitesinde tedavi gören hastaların CRP değerlerini servisteki hastalara göre yüksek bulduklarını bildirmişlerdir ve bu yükseklik istatistiksel açıdan önemlidir ($p <0.005$) (Özer ve ark., 2020). Bizim çalışmamızda ise CRP2 değerleri CRP1 ve CRP 3'e göre anlamlı yüksek bulunmuştur. Burada serviste takibi yapılan hastaların CRP değerlerinin yükselmesinin yoğun bakıma geçişi arttırabileceğini düşünmekteyiz.

Zamanla, bazı laboratuvar değerlerinin tekrarlı ölçümlerine cinsiyetin etkisi incelendiğinde bütün parametrelerin zamanla değişiminde cinsiyetin etkisiistatistiksel olarak mevcuttu ($p<0.005$) (Şekil 1). Her iki cinsiyette de Hb değeri açısından düşüş, PLT açısından artış gözlenmiştir. Ayrıca WBC değeri hastaların servise geçiş anında artış göstermiştir.

SONUÇ

Covid-19 tüm dünyada ve ülkemizde önemli bir sağlık sorunudur ve kan parametrelerinde değişiklikler yapabilmekte, özellikle PLT değerlerini düşürmektedir. Ayrıca CRP ve WBC değerlerindeki artışın hastaneye yatışı arttırabileceğini düşünmekteyiz. Hastaların hastanede mi evde mi takip edileceklerine, hastanın genel durumu ve laboratuvar testlerine bakılarak karar verilmelidir. Bu hastalıkla ilgili aydınlatılması gereken çok husus vardır ve hastalığın kontrol altına alınabilmesi şu aşamada aşıya bağlı görünmektedir.



Şekil 1: Zamanla, laboratuvar değerlerinin tekrarlı ölçümlerine cinsiyetin etkisi

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➢ *ORAL PRESENTATION*

Assessment of extreme rainfall measured at meteorological stations in the Büyük Menderes Basin

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Abstract

It is significant to recognize the climatic conditions and the dates and periods of many meteorological events to take the measures against the loss that may occur. In this study, the distribution of daily rainfall amounts determined at Aydın, Denizli, Dinar, Güney, Nazilli, Uşak and Yatağan meteorological stations in Büyük Menderes basin were examined and the necessary assessments were made. Perennial daily rainfall amounts of the meteorological station mentioned in the study were grouped according to rainfall amounts and repeat frequencies were determined by frequency analysis. According to frequency analyses based on perennial data, the probability of 50 mm and more rainfall on any day of the year is 40.5% in Aydın, 33.3% in Denizli, 8.1% in Dinar, 40.5% in Güney, 29.4% in Nazilli, 22.2% in Uşak and 48.6% in Yatağan. When rainfall amounts decrease to light shower from the very heavy shower the recurrence probability of occurrence frequency increases.

Keywords: Büyük Menderes Basin, Extreme rainfall, Frequency

INTRODUCTION

In recent years, natural disasters of meteorological character that affect some parts of the world are closely related to global climate changes have gained validity. Especially from an agricultural point of view, extreme rainfall has reached the amount that threatens production. Rainfall recorded at different times, amounts and high intensity had led to increased surface run-off, flooding and erosion. An important issue related to precipitation, such as the amount of precipitation and its distribution over the seasons, is the severity of precipitation, the duration and the frequency of certain amounts of precipitation. Precipitation intensity, an expression of the kinetic energy generated by precipitation, is of great importance due to its precipitation, which is effective and beneficial for plants, and its effect on soil erosion. As is known, agricultural effective and useful rainfall accounts for the time and amount of precipitation that allows water to seep into the soil and be taken by plant roots. In parallel with the increase in precipitation intensity, the water that passes into the surface stream and drains is not useful for plants. Although many factors, such as vegetation, soil structure, slope, determine the amount of erosion, the main determining element is the intensity of the precipitation. Low-intensity rainfall reduces the erosive effect of precipitation. In addition, very little surface flow occurs during this type of precipitation. However, in downpours, raindrops are larger and the resulting energy increases the wear rate of the soil (Jackson, 1979).

Heavy rains pose a great danger to the territory of Turkey. Heavy rains mean high intensity and amount of rain, especially at critical times, causing excessive surface runoff, soil erosion and flooding (Yamanlar and Nowland 1961). Rainfalls are classified as cyclonic, convection, and orographic in terms of forms of formation. Cyclonic rainfall causes more intense and effective in coastal regions, while convection rainfall occurs in Central Anatolia. However, orographic rainfall is effective in areas on mountain chains that are suitable for the rise of moisture-laden air masses. In all three ways, the extent of the damage caused by precipitation depends on many factors and can be grouped as physical, biotic, and anthropogenic effects in character. The structure of the soil, the slope of the land and the heat that

determines the development of the plants that hold the soil are important physical factors that determine the harm that precipitation will cause. The rainfall which is above the soil infiltration capacity causes very high amounts of surface runoff, posing a danger to soils.While the soils are dry, infiltration capacities may be high at first.

But with the fall of rain grains, the soil surface becomes compact. Therefore, the rate of infiltration decreases. Rain grains also make soil particles suspended in water. This suspended material also clogs the soil cavities, thus reducing the rate of infiltration and is transported by surface running water. In places where the soils are wet, less of the rainwater is absorbed by the soil and the surface flow occurs faster, resulting in an increase in the rate of erosion.

The biotic factor that may affect the degree of damage that may occur is vegetation, which can be evaluated in four separate forms. Following below;

1. Vegetation holds rain grains and reduces the amount of rainwater which causes surface flow.
2. Vegetation reduces the erosion rate caused by surface flow.
3. Plant roots increase the infiltration capacity of the soil.
4. Due to the transpiration of vegetation, the soil dries faster and therefore the infiltration capacity of the soil increases.

The human factor related to people's rightly or wrongly use of land can be defined as agricultural systems, animal breeding methods, and attitudes towards forest resources. The approach is shown by people on the issues mentioned above significantly affects the extent of the damage. For this reason, agricultural planning in agricultural areas should be such that in the most likely months of harmful rains, as much of the land as possible should be covered with vegetation.

The general distribution of extreme rainfall in Turkey depends on the climate and physical structure. The areas that suffer the greatest damage are coastal areas and the outward-facing faces of the mountains. For example, in 1995, more than 400.0 mm of catastrophic rain fell in 24 hours in Zonguldak. On 25 October 1930, more than 100 people died and many houses were destroyed with the overflow of Melez Stream in Izmir. In 1920, 80 houses were destroyed in the 28 people lost their lives in Çay district with the overflow of Bornova stream. In addition, 560 decares of agricultural land was damaged.On November 4, 1995, 124 kg of rainfall per square meter fell in 6 hours in Izmir Çiğli. This amount was determined to be the most rainfall seen in Izmir and its surroundings in the last 60 years, and 61 people died in the flood, 322 buildings were destroyed, up to 10 thousand buildings were damaged by the spreading waters.

As can be seen from the above examples, harmful rainfall is not only effective in agricultural areas, but also effective in loss of life and property. Despite all the sophistication of the XXI. century, all the material and technological capabilities and various measures taken, from developed states such as America to underdeveloped Asian countries such as Bangladesh, flooding caused by excessive rainfall can become catastrophes that cause significant material damage. However, it is a clear fact that the loss of life in these disasters increases and decreases according to the material State of the country and the degree of development of social consciousness.

According to the available meteorological data, it is estimated that extreme rainfall would be effective in the Büyük Menderes Basin in the next years. For this reason, it is important to know the frequency and periods of repetition of extreme rainfall in terms of taking measures against the damage that may occur.

MATERIAL AND METHOD

The distribution of daily rainfall amounts determined at Aydın, Denizli, Dinar, Güney, Nazilli, Uşak, and Yatağan meteorological stations in Büyük Menderes basin was examined and assessments were made. For this purpose, taking into account 20-year (Güney and Yatağan 16-year) data of meteorological stations, in order to examine the daily rainfall intensity in more detail, rainfall amounts were classified according to 3 groups: 25.1-50, 50.1-100 and more than 100.1 mm. Daily rainfall between 25.1-50.0 mm is expressed as light showers, daily rainfall between 50.1-100.0 mm is expressed as heavy showers, and daily rainfall between 100.1 mm and above is expressed as very heavy showers. A large amount of rain that falls in a fairly short time is usually called a downpour. Heavy rains, which continue from a few minutes to half an hour, produce actual showers. However, showers can continue for 24 hours. Here, 24-hour rainfall is taken into account to determine whether stations show a character of downpours (Ardel et al., 1965). According to these 3 groups, frequency analyses were performed by determining the frequency of repeated daily rainfall.

RESULTS AND DISCUSSION

Harmful rains that pose a great danger to the soil of Turkey are the rainfalls that cause excessive surface runoff, soil erosion and flooding, especially at critical times. For this reason, it is important to determine the frequency of repetition of these rains. In this study, 20-year (Güney and Yatağan 16-year) data of meteorological stations in the Büyük Menderes basin were taken into account and daily rainfall intensities were classified according to 3 groups: 25.1-50, 50.1-100 and more than 100.1 mm. According to these three groups, the frequency of repeated daily rainfall was determined and given in charts.

When the charts were examined, very heavy downpours of more than 100.1 mm were seen at stations in the research area only once. Heavy showers were usually seen in November and December although, in December, January and February were found to occur in light downpours.

According to frequency analyses based on perennial data, the probability of 50.1 mm and more rainfall on any day of the year is 40.5% in Aydın, 33.3% in Denizli, 8.1% in Dinar, 40.5% in the South, 29.4% in Nazilli, 22.2% in Uşak and 48.6% in Yatağan. When rainfall amounts decrease to light shower from the very heavy shower the recurrence probability of occurrence frequency increases.

Rainfall amounts falling in the research area vary depending on the characteristics of the meterological stations, with an annual average of 448.3 (Dinar) to 638.6 mm (Yatağan). According to the general atmosphere circulation, the winter and transition seasons in the region are rainy, and the summer season is dry. But there are big differences between the amount of precipitation according to the years. For example, 359.2 mm of rainfall was detected in 1972 against 954.7 mm of rainfall determined in 1967 in Aydın. Likewise, 977.6 mm of rainfall was recorded in Yatağan in 1981, while 388.9 mm of rainfall was recorded in 1992.

In general, heavy rainfalls occur more frequently in the coastal regions of the Büyük Menderes basin than in the inner regions and during the winter months. On the other hand, extreme precipitation is less frequent in inner regions and shows a homogeneous distribution between winter and spring months. Extreme rains have been observed in Denizli and Nazilli during the summer months.

Table 1. Repetition frequency of daily rainfall in Aydın, Denizli and Dinar

Station Name	Aydın			Denizli			Dinar		
Observation time	1976-1996 (20 Years)			1976-1996 (20 Years)			1980-1996 (16 Years)		
Precipitation classes (mm)	25.1-50	50.1-100	100.1->	25.1-50	50.1-100	100.1->	20.1-50	50.1-100	100.1->
January	16	3	-	10	2	-	1	-	-
February	10	3	-	10	1	-	-	-	-
March	9	1	-	6	-	-	3	-	-
April	10	-	-	4	-	-	2	-	-
May	4	-	-	5	-	-	1	-	-
June	2	-	-	2	-	-	1	-	-
July	-	-	-	1	1	1	2	-	-
August	-	-	-	-	-	-	1	-	-
September	1	-	-	2	-	-	-	-	-
October	4	1	-	4	-	-	3	-	-
November	15	4	-	11	1	-	5	-	-
December	20	4	-	10	4	-	1	-	-

Table 2. Repetition frequency of daily rainfall in Güney, Nazilli, Uşak and Yatağan

Station Name	Güney			Nazilli			Uşak			Yatağan		
Observation time	1976-1996 (20 Years)			1976-1996 (20 Years)			1976-1996 (20 Years)			1980-1996 (16 Years)		
Precipitation classes (mm)	25.1-50	50.1-100	100.1->	25.1-50	50.1-100	100.1->	25.1-50	50.1-100	100.1->	25.1-50	50.1-100	100.1->
January	6	1	-	24	1	-	5	-	-	14	-	-
February	7	1	-	10	-	-	3	1	-	13	1	-
March	9	-	-	6	1	-	1	-	-	7	2	-
April	3	1	-	5	-	-	5	-	-	1	-	-
May	2	2	-	3	1	-	2	-	-	-	1	-
June	5	-	-	-	1	-	1	-	-	5	-	-
July	2	-	-	2	-	-	2	-	-	1	-	-
August	-	-	-	1	-	-	1	-	-	2	-	-
September	2	-	-	-	-	-	-	-	-	1	-	-
October	3	-	-	4	1	-	2	-	-	5	-	-
November	13	-	-	18	2	-	7	-	-	12	7	-
December	11	3	-	21	5	-	5	-	-	14	3	-

CONCLUSION

Rainfall amounts falling in the research area vary depending on the characteristics of the stations, with an annual average of 448.3 (Dinar) to 638.6 mm (Yatağan). According to the general atmosphere circulation, the winter and transition seasons in the region are rainy, and the summer season is dry. However, there are big differences between the amount of precipitation according to the years. For example, 359.2 mm of rainfall was detected in 1972 against 954.7 mm of rainfall determined in 1967 in Aydın. Likewise, 977.6 mm of rainfall was recorded in Yatağan in 1981, while 388.9 mm of rainfall was recorded in 1992.

According to frequency analyses based on perennial data, the probability of 50 mm and more rainfall on any day of the year is 40.5% in Aydın, 33.3% in Denizli, 8.1% in Dinar, 40.5% in Guney, 29.4% in Nazilli, 22.2% in Uşak and 48.6% in Yatağan. As the amount of precipitation falls, the probability of it happening increases.

In addition to the spatial differences in the amount of precipitation falling in the Büyük Menderes Basin, which is located between the middle rainy regions of Turkey with a total annual rainfall of 600.0- 750.0 mm, great changes can be observed over time. Especially extreme rainfall, which causes that, also greatly affects the average values.

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➢ *ORAL PRESENTATION*

İyonik Sıvıların Akciğer Kanseri Hücreleri Üzerindeki Antiproliferatif ve Antimetastatik Etkilerin *In Vitro* Koşullarda İncelenmesi

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Özet

İyonik sıvılar (İS'ler) 100°C' nin altındaki sıcaklıklarda sıvı kalabilen tuzlar olarak tanımlanır. Çok düşük buhar basıncı, yanıcı olmayan, yüksek kimyasal ve termal kararlılık, yüksek iyonik iletkenlik ve geniş elektrokimyasal pencere içerebilen benzersiz özellikleri nedeniyle, İS'ler çeşitli uygulamalar için kullanılmıştır.

Kanser, yaygın sağlık problemlerinden biridir ve Dünya genelinde kanser nedenli ölüm oranı oldukça yüksektir. Kolorektal kanser de günümüzde insanlardaki kanser ölümlerinin başında yer almaktadır. Kanser tedavisinde klasik kemoterapötiklerin kullanılmasının yanı sıra, tedavi etkinliğinin arttırılması için yeni ilaç hedefleri araştırılmakta ve yeni moleküller tanımlanmaya çalışılmaktadır. Kullanılan ajanların kanser hücreleri üzerindeki etkisinin belirlenmesinde ve kanser tedavisi için aday molekül olarak kullanılmasında hücre hatlarında *in vitro* olarak yapılan araştırmalar büyük öneme sahiptir.

Çalışmada, sentezlenen maddelerin *in vitro* koşullarda akciğer kanseri (A-549) hücre hattı üzerindeki antiproliferatif özelliği MTT analiziyle belirlenmiştir. Bu analiz, 8 farklı konsantrasyonda (1.56µg/ml - 200µg/ml) 3 tekrar olarak çalışılıp söz konusu maddeler ile 48 saat süresince muamele edilmiştir. Bunlara ek olarak, A-549 hücre hattı migrasyon analizi için 6 well kültür pleytlerine alınmıştır. Hücreler %80-90 konflensiye geldiğinde yara modelleri oluşturulup, ardından etkin konsantrasyonlara göre hazırlanan maddeler yara modelleri üzerine eklenerek 24. ve 48. saat için görüntüleri alınarak % migrasyon alanı hesaplanmıştır.

Anahtar Kelimeler: İyonik sıvılar, Akciğer kanseri, Antiproliferatif etki, Antimetastatik etki

In Vitro Conditions Of Its Antiproliferative And Antimetastatic Effects Of Ionic Fluids On Lung Cancer Cell

Abstract

Ionic liquids (ILs) are defined as salts that can remain liquid at temperatures below 100 °C. Because of their unique properties, which can include very low vapor pressure and non-flammable, high chemically and thermallystability, high ionic conductivity and wide electrochemical window, ILs have been used for various applications.

Cancer is one of the common health problems and the death rate caused by cancer worldwide is quite high. Lungl cancer is one of the leading cancer deaths in humans today. In addition to using classical chemotherapeutics in cancer treatment, new drug targets are being investigated and new molecules are tried to be defined in order to increase the effectiveness of the treatment. In vitro research in cell lines is of great importance in determining the effect of the agents used on cancer cells and using them as candidate molecules for cancer treatment.

In the study, the antiproliferative properties of the synthesized substances on the lung cancer (A-549) cell line under in vitro conditions were determined by MTT analysis. This analysis was studied in 8 different concentrations (1.56µg / ml - 200µg / ml) in 3 replicates and treated with these substances for 48 hours. Additionally, the A-549

cell line was placed in 6 well culture plates for migration analysis. When the cells reached %80-90 confluency, wound models were created, then materials prepared according to effective concentrations were added on to the wound models, and their images were taken for the 24th and 48th hours and the% migration area was calculated.
Keywords: Ionic liquids, Lung cancer, Antiproliferative effect, Antimetastatic effect

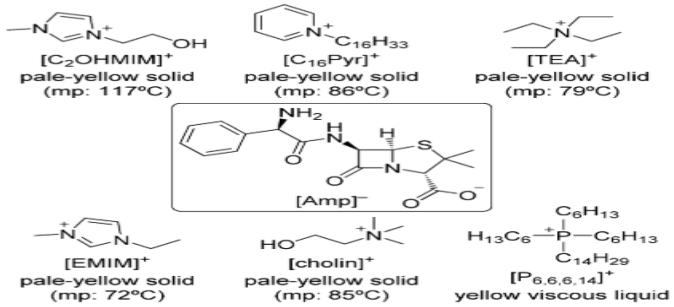
1.GİRİŞ

Kanser yaygın sağlık problemlerinden biridir ve kanser tedavisinde kayda değer gelişmeler sağlanmış olsa da dünya genelinde kanser nedenli ölüm oranı oldukça yüksektir. Uzun yıllardır kanser kaynaklı ölümleri önlemek veya azaltmak amacıyla özellikle tedavi amaçlı olarak kullanılabilecek etkin, değerli kimyasal moleküllerin dizaynı ve sentezi konusunda yoğun çalışmalar yapılmıştır. Fakat kanserin çok çeşitli tiplerinin olması nedeniyle hala tedavi amaçlı kullanılabilir yeni kimyasal moleküllerin geliştirilmesine ihtiyaç duyulmaktadır. Kanserden meydana gelen ölümlerin artması ve üretilen moleküllerin büyük bir kısmının çeşitli sebeplerle kullanımının uygun olmaması nedeniyle, antikanser potansiyele sahip yeni moleküllere duyulan ihtiyaç her geçen gün artmaktadır (Pors ve ark., 2005).

Kansere karşı alternatif ve etkili tedavilerin araştırılması, ilaç endüstrisinin güncel bir hedefidir. İlaç endüstrisinin şu anda karşılaştığı birçok zorluk arasında, yenilikçi ve etkili tedaviler geliştirme ihtiyacı da bulunmaktadır. Günümüzde bilim insanları sadece yeni kimyasal bileşikler sentezlemekle yetinmemekte, aynı zamanda ekonomik değere sahip, hedefe yönelik ve insanlığa faydalı olabilecek bileşiklerin üretimi konusunda çaba sarf etmektedirler. Dolayısıyla, geçmişte olduğu gibi günümüzde de kanser kemoterapisi kimyacılar için önemli bir araştırma alanı olmaya devam etmektedir (Johnson ve ark., 1997). Bu sayede her geçen gün yeni kemoterapötik bileşikler sentezlenmekte ve biyolojik aktiviteleri araştırılmaktadır.

Kanser tedavisinde klasik kemoterapötiklerin kullanılmasının yanı sıra, tedavi etkinliğinin arttırılması için yeni ilaç hedefleri araştırılmakta ve yeni moleküller tanımlanmaya çalışılmaktadır. Kullanılan ajanların kanser hücreleri üzerindeki etkisinin belirlenmesinde ve kanser tedavisi için aday molekül olarak kullanılmasında hücre hatlarında *in vitro* olarak yapılan araştırmalar büyük öneme sahiptir. Maddelerin biyokimyasal ve moleküler etki mekanizmasının anlaşılması hedefe yönelik geliştirilecek yeni ilaç araştırılmaları açısından oldukça önemlidir.
İyonik sıvılar (IS'ler) son zamanlarda ilaç endüstrisindeki araştırmacılar tarafından yeni terapötik ajanlar arayışlarında olan bir araştırma konusu olarak ortaya çıkmıştır. İyonik sıvılar genellikle, erime noktaları 100 ° C'nin altında olan organik tuzlar olarak tanımlanırlar. (Torimoto T. ve ark., 2011) IS'ler yüksek iyonik iletkenlik, zor tutuşan, uçuculuğu düşük ve yüksek sulu çözünürlük ile karakterize edilir (Kumar SJ ve ark., 2017).

IS alanında yapılan gelişmeler ve uygulamaları, üç kuşak halinde gruplaşmalarına olanak sağlamıştır. Birinci nesil IS'ler, erime noktası, yoğunluk, viskozite, ısıl kararlılık, iletkenlik ve hidrofobiklik gibi fiziksel özelliklerine dayanır. (Ferraz R ve ark., 2011) İkinci nesil üyeler fiziksel ve kimyasal özellikleri (kimyasal reaktivite, koordinasyon ve çözme) ile karakterize edilir. Daha yakın zamanda, üçüncü nesil IS'ler, biyolojik aktiviteye sahip olmaları için üretimlerinde aktif farmasötik terkip maddelerinin kullanılmasını içerir (Hough, W ve ark., 2007-Smiglak ve ark., 2007).



Şekil 1: Ampisilin tuzları formunda potansiyel antitümör ajanların örnekleri (Ref. [18] 'den modifikasyonlarla uyarlanmıştır).

Üçüncü nesil IS'ler oda sıcaklığında hem kimyasal hem de termal olarak stabil olmaları çok önemli avantaj sağlamaktadır. IS'ler diğer bir yönü ile de kimyasal çözücülerde çok nadir olarak çözünüp, genel olarak suda çözünmeleri de iyonik sıvılara özgünlük katmaktadır.

2.MATERYAL VE METOD

3.1. Hücre Hatlarının Kültüre Edilmesi, Pasajlanması ve Stoklanması

Önerilen çalışmada kullanılacak olan akciğer kanseri (A549) hücre hattı Gaziosmanpaşa Üniversitesi, Mühendislik ve Doğa Bilimleri Fakültesi, Biyomühendislik Bölümü, Hayvan Hücre ve Doku Kültürü Laboratuvarı'nda oluşturulan hücre stoklarından kullanılacaktır. Stoktan kullanılacak hücrelerin kültür ve stoklanmasına ilişkin yöntem basamakları aşağıda özetlenmiştir.

- A-549 hücreleri, DMEM HG besi ortamı, %10 (v/v) inaktif fetal sığır serumu (FBS) ve %1 (v/v) L-glutamin içeren besi ortamı ile sağlanacaktır.
- Sıvı azot (-196 °C) içerisinde saklanan kryotüpler çıkartılır çıkartılmaz, vakit kaybetmeden 37 ˚C' deki su banyosu içerisinde çözdürülür. İçeriği çözünen kryotüp biyogüvenlik kabinine alındıktan sonra üzerine hazırlanan besi ortamı damla damla eklenerek, tüp içeriği dikkatlice toplanıp santrifüj tüpüne aktarılır. Hücreler 800 rpm (devir/dakika)'de 4 °C'de 5 dk. santrifüjlenir. Santrifügasyon sonrasında oluşan üst faz (süpernatant) atılarak, pellet üzerine taze besi ortamı eklenerek homojenize edilir. Homojenize edilen hücreler 37 °C'de, %5 CO_2 içeren %95 nemli ortamda inkübe edilir. Hücrelerin morfolojileri ters-faz mikroskop ile incelenir ve hücrelerin besi ortamı iki günde bir değiştirilir. %70-80 konfluensiye ulaşan hücreler ise pasajlanarak çoğaltılır.
- Uygun etiketleme yapıldıktan sonra kültür kabı 37ºC' de, %5 CO_2 içeren inkübatörde inkübasyona bırakılır.
- Hücrelerin pasajlanması için üzerinde bulunan kullanılmış besi ortamı uzaklaştırıldıktan sonra 37 °C'de ısıtılmış olan, steril Ca^{+2} ve Mg^{+2} içermeyen fosfat tamponu ile hücrelerin yüzeyi yıkanır. Daha sonra hücrelerin üzerine 37 °C' de ısıtılmış tripsin-EDTA (2,5 g/L tripsin, 0,5 mM EDTA) çözeltisi eklenerek 37 °C'de 5 dk bekletilerek hücrelerin yüzeyden ve birbirlerinden ayrılması sağlanır.
- Ayrılan hücrelerin üzerine serum içeren besi ortamı ilave edilerek süspansiyon homojenize edilir ve tüpler, 800 rpm (devir/dakika)'de 4 °C'de 5 dk. santrifüjlenir. Santrifügasyon sonrasında üst faz (süpernatant) atıldıktan sonra, pellet üzerine taze besi ortamı eklenir ve hücreler homojenize edilir. Hücre süspansiyonundan, hücrelerin pasaj oranına göre seyreltilerek, yeni hücre kültür kaplarına aktarılır ve hücreler 37 °C'de, %5 CO_2 içeren %95 nemli ortamda inkübe edilir.
- Çalışmanın devamlılığının sağlanması için hücreler stoklanır. Bunun için tripsinize edildikten sonra santrifüj edilen hücre pelleti üzerine hazırlanan dondurma ortamı (%90 (v/v) FBS, %10 (v/v) dimetilsülfoksit, DMSO) eklenir ve hücreler kriyotüplere paylaştırılır. Kriyotüpler önce -80 °C bir gece bekletildikten sonra daha uzun süre saklanması için -196 °C'ye (sıvı azot) kaldırılır.

3.2. İyonik Sıvıların Kanser Hücre Hatları Üzerindeki Anti-Proliferatif Etkilerinin Belirlenmesi

İlgili bölümde belirtilen laboratuvar stoklarından çözdürülerek kültive edilen hücre hatları 5×10^4 hücre/ ml konsantrasyonunda olacak şekilde üç tekrarlı (n=3) olarak 96 gözlü kültür kaplarına alınır. Hücreler %5 CO_2 içeren 37 °C'deki nemli inkübatörde 24 saat boyunca inkübe edilir. 24 saatlik inkübasyonun ardından hücreler üzerine sentezlenen bileşikler faklı konsantrasyonlar da eklenir. Hücreler ilgili bileşenler ile birlikte 24 v e 48 saat inkübe edilir. Bu sürelerin sonunda MTT (3-(4,5- dimetilthiazol-2-yl)-2,5-difeniltetrazolyum bromid, sarı tetrazolyum tuzu) ile canlılık testi uygulanır. MTT'nin mitokondriyal suksinat dehidrogenaz enzimi ile indirgenerek MTT-formazan kristallerine dönüşmesi temeline dayanan MTT testi, aslen mitokondriyal aktiviteyi ölçmesine rağmen dolaylı yoldan hücre canlılığının belirlenmesinde sıkça kullanılan bir yöntemdir. MTT testi için, pleytteki kullanılmış besi ortamı çekilir ve hücrelerin üzerine %10 MTT (5 mg/mL konsantrasyonda, PBS içerisinde hazırlanmış) içeren besi ortamı eklenir. Hücreler karanlıkta 37 ℃' de %5 CO_2'li inkübatörde 3 saat süre ile inkübe edilir. 3 saat inkübasyonun sonunda MTT içeren ortam çekilir ve hücrelerin üzerine DMSO eklenerek oluşan formazan kristallerinin çözünmesi sağlanır. Formazan kristalleri çözündükten sonra pleyt, pleyt okuyucuda 570 nm dalga boyunda okutularak absorbans değerleri kaydedilir. Her bir örneğin absorbans değeri, o deney setine ait hücre kontrolün absorbans değerine oranlanıp % canlılık oranları belirlenir ve grafiklendirilir.

3.3. Hücre Göçü Analizi ile Antimetastatik Etkilerin Belirlenmesi

Bir önceki basamakta ayrıntılı olarak açıklanan MTT analizinde hesaplanan yüzde canlılık değerleri dikkate alınarak hücre göçü analizi gerçekleştirilecektir. Bu analizde genel geçerlilik gören, zaman ve maliyet açısından oldukça avantajlı bir yöntem olan in vitro çizik testi kullanılacaktır. Böylece, ilgili maddelerin metastaz üzerine etkileri modellenmiş olacaktır. Bu testin ayrıntıları aşağıda özetlenmiştir:

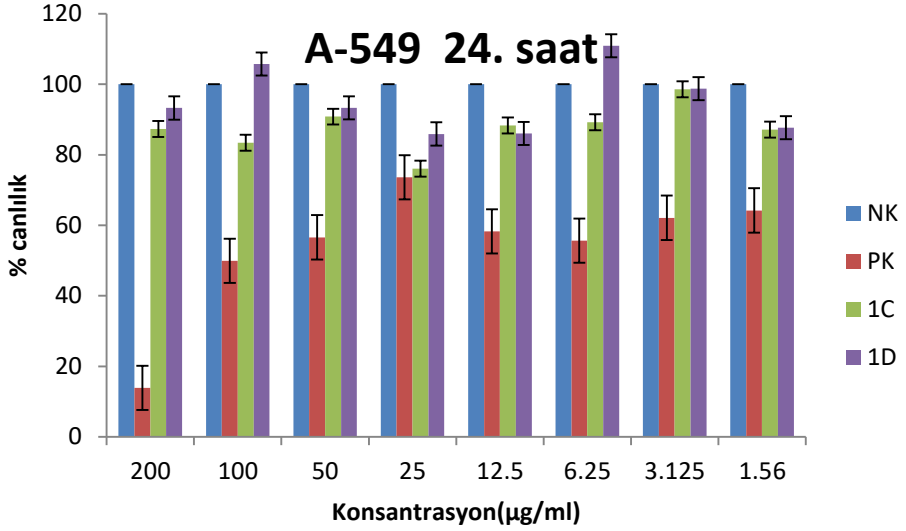
- A-549 hücreleri 6 gözlü kültür plakalarına ekilecek ve hücrelerin %80-90 konfluensiye gelmesi beklenecektir.
- Belirtilen konfluensideki hücrelerin üzerinde steril P200 pipet ucu ile bir 'çizik' oluşturulacak in vitro çizik testi için hücreler sentezlenen bileşikleri içeren besi ortamı ile inkübasyona bırakılacaktır.
- Hücreler ilgili bileşenlerle 48 saat kültüre edilecek ve her 24 saatte bir inverted mikroskop altında fotoğraflanacaktır.

48 saat sonunda görüntüler, Olympus mikroskop yazılımı aracığıyla analiz edilecek ve yara kapanma oranları alansal olarak hesaplanacaktır. Elde edilen kantitatif değerler negatif kontrolle oranlanacak ve % migrasyon alanı tespit edilecektir.

4.BULGULAR ve TARTIŞMA

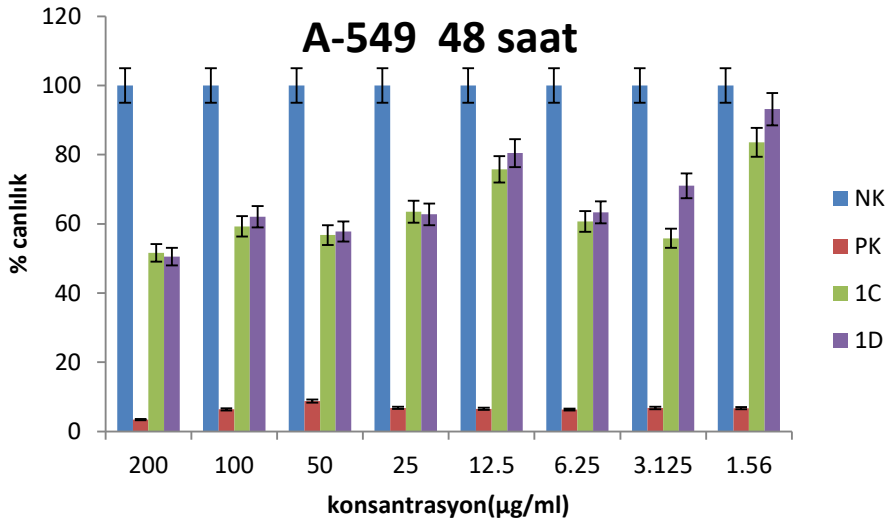
4.1. İyonik Sıvıların Akciğer Kanseri Hücre Hattı Üzerindeki Anti-Proliferatif Etkilerinin Değerlendirilmesi

Elde edilen absorbans değerleri dikkate alınarak, maddelerin yüzde canlılık grafikleri oluşturulmuştur (Şekil 2-3).



Şekil 2. Akciğer kanseri (A-549) hücre hattı üzerinde dietilentriamin türevli iyonik sıvıların 24. saatteki antiproliferatif etkileri

Bazı konsantrasyonlarda (100-6.25µg/ml) 1D maddesinin hücre canlılığını arttırdığı görülmektedir. 3.125 µg/ml konsantrasyonda her iki madde de negatif kontrolle aynı proliferasyona sahip olduğu görülmektedir. Diğer maddeler için 24. saatte çok zayıf antiproliferatif etkisi olduğu görülmektedir.
24. saatte her iki madde karşılaştırıldığında zayıfta olsa 1C maddesinin antiproliferatif özelliği olduğu söylenebilir.

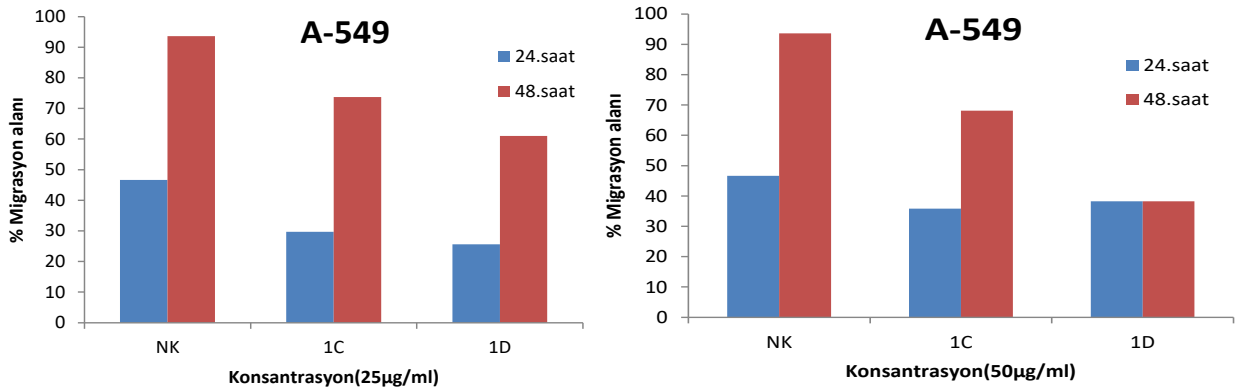


Şekil 3. Akciğer kanseri (A-549) hücre hattı üzerinde dietilentriamin türevli iyonik sıvıların 48. saatteki antiproliferatif etkileri

En düşük konsantrasyon hariç tüm konsantrasyonlarda her iki maddenin de antiproliferatif özelliği söz konusudur. 50 µg/ml ve 25 µg/ml maddeler hem pozitif hem de negatif kontrolle karşılaştırınca maddeler için en etkili dozun bu iki konsantrasyon olduğu görülmektedir. Fakat maddeler sadece negatif kontrolle kıyaslandığında en yüksek dozda hücrelerin canlılığı yarı yarıya azalmaktadır. 48. Saatte 1C maddesinin en etkili olduğu görülmektedir.

4.2. İyonik Sıvıların Akciğer Kanseri Hücre Hattı Üzerindeki Anti-Metastatik Etkilerinin Değerlendirilmesi

Elde edilen migrasyon alanları dikkate alınarak, maddelerin A-549 hücreleri üzerindeki 24 ve 48. saatteki hücre göçü ile ilgili veriler yer almaktadır (Şekil 3).

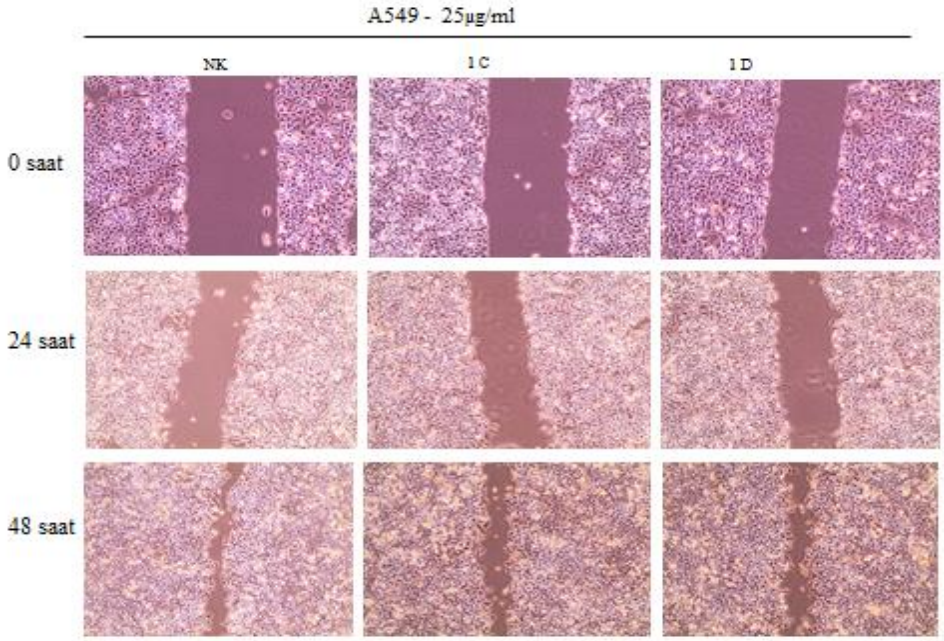


(a) (b)

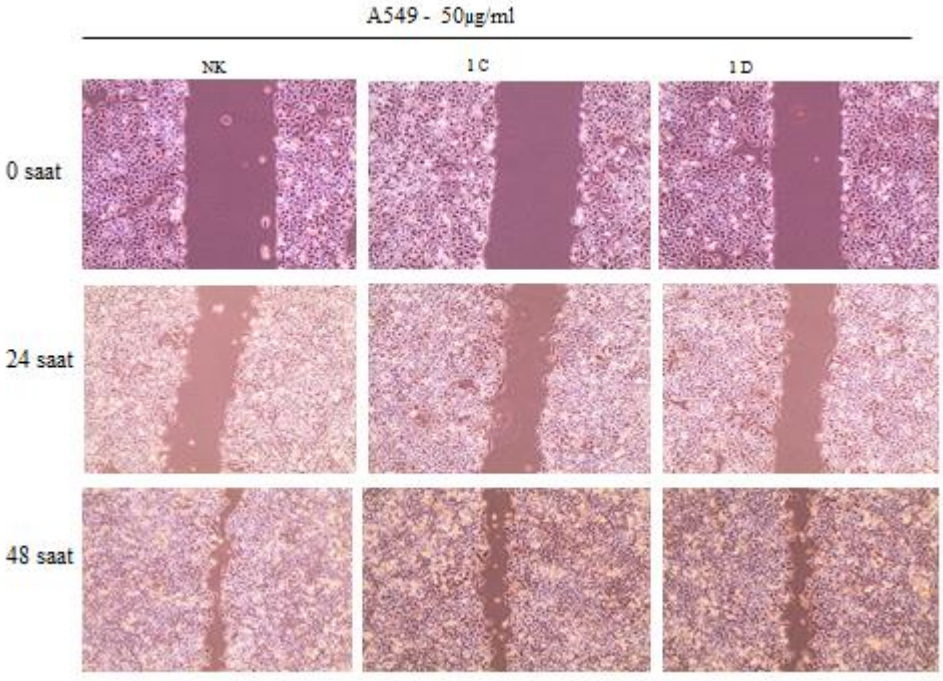
Şekil 3: a) A549 hücre hattı üzerinde bileşiklerin 25 µg/ml konsantrasyondaki migrasyon alanı. **(b)** A549 hücre hattı üzerinde bileşiklerin 50 µg/ml konsantrasyondaki migrasyon alanı.

Hücreler üzerinde oluşturulan yara modelleri 25µg/ml konsantrasyonda her iki madde de negatif kontrolle karşılaştırılınca hücre göçünü inhibe etmiştir. 50 µg/ml konsantrasyonda ise 24. saatte negatif kontrole oranla her iki madde de hücre göçünü çok az inhibe ettiği görülmektedir. Fakat 48. Saat için 1D maddesi oldukça iyi bir şekilde hücre göçünü inhibe etmiştir.

Yara modeli oluşturulan A-549 hücrelerinin 0, 24 ve 48. saat görüntüleri verilmiştir. (Şekil 5-6)



Şekil 5: Yara modeli oluşturulan A-549 hücrelerinin 25µg/ml konsantrasyonda 0, 24 ve 48. saat görüntüleri



Şekil 6: Yara modeli oluşturulan A-549 hücrelerinin 50µg/ml konsantrasyonda 0, 24 ve 48. saat görüntüleri

SONUÇ

Yapılan in vitro çalışmalar sonunda, dietilentriamin türevli iyonik sıvıların, akciğer kanseri üzerinde antiproliferatif özelliği değerlendirildiğinde, maddelerin kronik etkisinin akut etkisinden daha iyi olduğu

görülmekte olup akciğer kanseri için etkili maddenin 1C ve düşük konsantrasyonda(25 µg/ml) daha etkili olduğu görülmektedir.

TEŞEKKÜR

Gaziosmanpaşa Üniversitesi Bilimsel Araştırma Projeleri tarafından 2020/70 nolu proje kapsamında olup, mali desteğinden dolayı Gaziosmanpaşa Üniversitesi Bilimsel Araştırma Birimine teşekkür ederiz.

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➢ *ORAL PRESENTATION*

Immunomodulatory potential for active fraction from *Phaleria macrocapa*

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Abstract

Background: Immunomodulators are materials can modification immune system response to threaten agent, making it standby to defense against any dangerous.The immunomodulatory effect of fractions compound from *Phaleria macrocapa* is investigated in this work.
Methods: *P. macrocapa* ethanol extract was fractionated by using different solvents. The ethanol extract and effective isolated fraction were used to investigate the potential immunomodulatory effect of *P. macrocapa* fraction with 1µg/ml LPS and 100µg/ml on RAW 246.7 cells by detecting intracellular INF-γ, IL-6, and IL-8 expressions using flowcytometry.
Results: The intracellular expression of the INF-γ, IL-6 and IL-8 showed significant $P \leq 0.05$ increase in the stimulation RAW264.7 cell with *P. macrocarpa* Fraction1,for INF-γ, IL-6, IL-8 intracellular expression.
Conclusions: The results of this study obviously indicate that *P. macrocarpa* has immunomodulatory effects through the stimulation of INF-γ, IL-6, and IL-8 expressions thus could use this naturally active fraction as natural drug in the treatment of immunosuppressed cases to enhance immune response.

Keywords: *Phaleria macrocapa,* Immunomodulatory, INF-γ, IL-6, and IL-8

INTRODUCTION

The immune system is a remarkably developed defense system found inside vertebrates to guard them from attacking factors of illness. It is capable of producing a diverse range of molecules and cells which are able to distinguish and reduce the unlimited changes of external and unwanted agents. The modulation of the immune system refers to some alteration in the immune response, which could include stimulation, amplification and expression or inactivation of the immune response. Therefore, the immunomodulator is a substance used for its effect on the immune system. There are commonly two kinds of immunomodulators based on their effects, immunostimulators and immunosuppressers. They have the ability to mount an immune response or defence against pathogens or tumors (Saroj et al., 2012). In different parts of the world, plant extracts have been widely investigated for the possibility of immunomodulation. Many of the studies have demonstrated the isolation of potential bioactive molecules (Alamgir & Uddin, 2010). For example, *Acorus calamus* rhizome extract inhibited the growth of several cell lines for humans and mice. It also inhibited the production of nitric oxide (NO), interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α). In addition, it can cause down-regulation expression of the CD25 marker (Mehrotra et al., 2003) and many plant derived compounds like sterols, sterolins, polysaccharides, alkaloids, flavonoids, lectin and glycoprotein are used as immunomodulators (Kolm et al., 2007). Modulation of the immune response to reduce diseases has long been an attraction for researchers. There have been many studies lately on ethnomedicinal plants as immunomodulatory agents. Immunopharmacology is a relatively new division of pharmacology, which aims to search for immunomodulators. The possible usage of immunomodulators for clinical medicine can comprise reconstruction for immune deficiency, for example, treatment of the suppression of normal or exaggerated immune roles like treatment of autoimmune diseases and AIDS. An important source of immunomodulators is medicinal plants and their active components. Therefore, the improvement of drugs from natural compounds for their immunomodulation and anti-tumor potential is an interesting project (Alamgir & Uddin, 2010). This study was aimed to investigate immunomodulatory potential for active fraction of P. macrocarpa.

MATERIALS AND METHODS

Fractionation of crude extracts

One gram of ethanol crude extract was dissolved in 5 ml of methanol and subjugated to column chromatography fractionation in glass columns at 3.0 x 50 cm (Kontes Scientific Glassware, Vineland, NJ, USA) packed with silica gel G60, 70 - 230 mesh (Merck, Darmstadt, Germany) and linked with an EYEL-L4 pump (Tokyo Rikaki, Tokyo, Japan). A gradient concentration of solvent that differed in polarity (25 ml each time of five different concentrations; 20, 40, 60, 80 and 100 %) was used to elute the crude extract. The solvents used were hexane, ethyl acetate, methanol, acetonitrile and water in order to increase the polarity, starting with lower polarity and ending with high polar solvent. The yield fractions were collected in a clean class tube and gathered based on the similarity of the solvents used. Solvents were evaporated from yield fraction under reduced pressure using a centrifuge evaporator. The all fractions were tested for their effect *in vitro* on the RAW 264.7 cell line.

Immunomodulatory effect *in vitro*

A murine macrophage cell line RAW 264.7 obtained from the American Type Culture collection (ATCC, Rockville, MD) was cultured in DMEM and supplemented with 4500g glucose/L, 110mg sodium pyruvate/L and 1% penicillin–streptomycin (Sigma-Aldrich, UK) and 10% heated-in activated, endotoxin level less than < 0.1 EU/ml fetal bovine serum (FBS) (BIOWEST, France). The cells were cultured at 37◦C in a 5% CO2 atmosphere incubator (NuAire, Plymouth, MN, USA). For the experiments, the cells were sub cultured when they reached 80% confluence, then the cell suspension was diluted to 5×10^5 cell/ml (Guan et al., 2011). One hour before the experiments, 1ml fresh medium (37°C) was changed in the 96-well plates (Coster, Corning,NY). In this experiment the cells were cultured for 24h.

Proliferation assay

For detecting the effect of plant extracts on RAW264.7 cell proliferation, the assay was performed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (MTT) assay (Merck, Germany). The MTT test measures the ability of the cells to transform MTT to formazan. The cells were plated in 96-well tissue culture plates at a density of $5x10^5$ cells / ml, 5000cell/ well in complete DMEM medium and incubated in triplicate in a 96-well plate in a final volume of 100µl for 24h at 37°C and 5% CO2 (Gauley & Pisetsky, 2010). Cells were stimulated with plant all fractions were at a final concentration (25, 50, 100, 200, 400, 800, and 1000) µg/ml and incubated for 24h at 37° C and 5% CO2, then 10µl from 5mg/ml in phosphate buffered saline MTT solution was added to each well. After 4h of incubation at 37° C the spent media and MTT were aspirated before the addition of 100 µl of dimethyl sulfide (DMSO) (Fisher Scientific, UK) to dissolve the yellow MTT tetrazolium salt produced metabolically and change to purple MTT formazan salt. The amount of MTT formazan salt produced is proportional to the amount of viable cells. The cell proliferation rate is determined by measuring the absorbance at a wavelength of 570 nm with the microplate reader (Groesdonk et al., 2006; Guan et al., 2011).

Stimulation for intracellular cytokine production

To evaluate the ability of plant all fractions extracts in the stimulation of the RAW 264.7 cell to produce cytokine, RAW264.7 cells (1×10^6 cells /ml, 6 ml /well) were incubated in complete DMEM medium for 24 h at 37°C in 5% CO2 .Then the cells were stimulated with LPS (1 µg/ml) (Escherichia coli 055: B5, Difco, Detroit, MI,USA), (1 µl/ml) brefeldin A (BD GolgiPlugTM) and (100 µg/ml) of P.macrocarpa fraction1 100 µg/ml (P.m F1) incubated for 6 h . Before activation with LPS (1 µg/ml), the medium was removed and the cells were washed with 5 ml of PBS and replenished with a complete medium. Cells stimulated with LPS (1µg/ml) alone were used as control and after the incubation period, cells were washed twice with PBS and resuspended in 0.5 ml of staining buffer PBS containing (1% FBS and 0.09% (w/v) sodium azide).

Flow cytometer immunostaining of intracellular cytokines

Cells were re-suspended with a density of 10^7 cells/ ml, 100µl of the suspension cells were aliquoted into tubes for staining. To reduce nonspecific immunofluorescent staining, suspension cells were pre-incubated with mouse BD Fc BlockTM purified anti mouse CD16/CD32 mAb 2.4G2 (BD Fc Block™; Cat.No.553141) (1 µg/ 10^6 cells in 100 µl) at 4°C for 15 minutes. The cells were then fixed /permeabilized with 250 µl for 20min at 4°C of fixation/ permeabilization solution (BD Cytofix/Cytoperm plus fixation/ permeabilization, BD Golgi Plug™ protein transport inhibitor, Cat.No.555028). Cells were washed two times in 1ml of 1× BD Perm/WashTM buffer, 250xg and 5min at 4°C. Fixed/permeabilized cells were re-suspended in 50µl of BD Perm/WashTM buffer and incubated

at 4° C for 30 minutes in the dark with a fluorochrome-conjugated anticytokine antibody: PE Anti-Mouse IL-6 (≤ 0.25 µg/million cells, BD Cat. No: 554401), FITC rat anti-mouse IFN-γ (≤ 0.5 µg/ /million cells, BD Cat. No: 554411) and APC rat anti-mouse IL-8 (≤ 0.5 µg/million cells, BD Cat. No: FAB2164A). After incubation, cells were washed two times with 1×BD Perm/WashTM buffer (1 ml/wash) and resuspended in Staining Buffer prior to the flow cytometric analysis.

Flow cytometric analysis

The samples were analyzed with gated on the two-dimensional forward and side scatter of the flow cytometer and fluorescence intensity was analyzed with at least 10,000 cells collected for each sample. The cytokine analysis was carried out with a FACS Canto II flow cytometer and FACSDiva version 6.1.3 software (BD Biosciences).

RESULTS and DISCUSSION

Proliferation effect on the RAW264.7 cell

In order to research the immunomodulatory effect of the plant fractions on the RAW264.7 cell line and choose the best one for applying in the investigation on the effect of the intracellular expression of cytokines, we investigated the effect of five fractions for plants on the proliferation of the RAW264.7 cell line. The isolated fractions appeared to have a different effect on RAW264.7 cell proliferation. Results showed a significant ($P \leq 0.05$) increase in the viability of the cell after treatment for a 24h incubation period with P.macrocarpa (F1 and F5) in a dose dependent manner and a significant decrease in viability of the cell ($P \leq 0.05$) after treatment with P.macrocarpa (F2, F3 and F4) in a dose dependent manner (Figure 1).

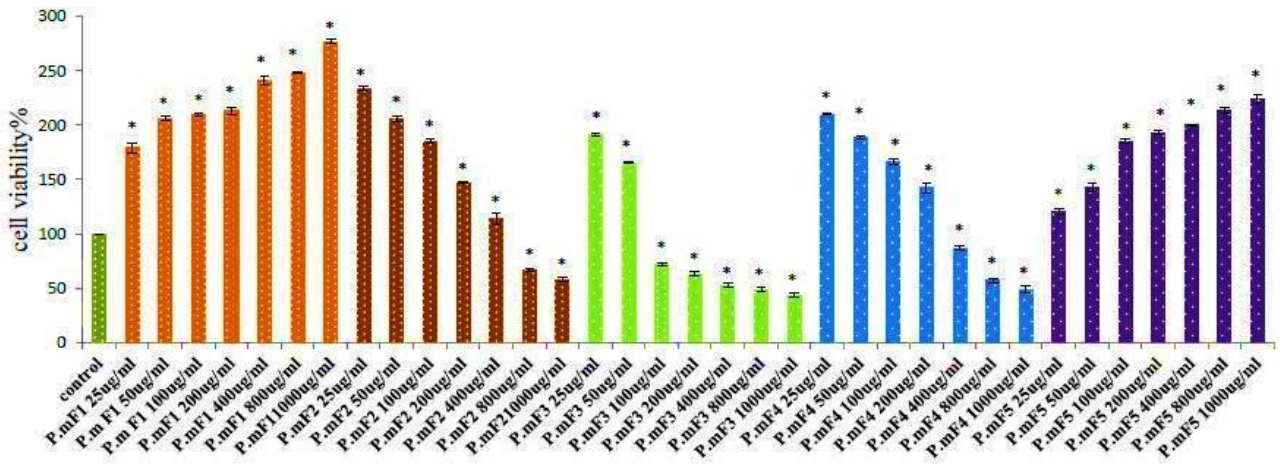
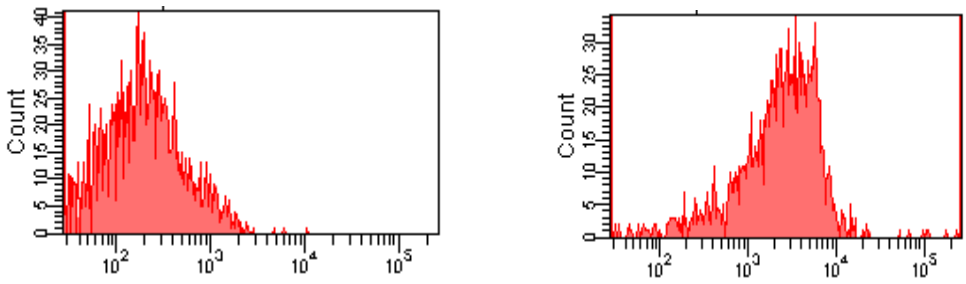


Figure 1: Cell viability percentage of RAW264.7 cell treated groups with P. macrocarpa fractions (F1, F2, F3, F4 and F5) compared to control (untreated group). Each value is presented as mean percent ± S.D.*significantly different versus control group, ($P \leq 0.05$).

In vitro stimulation for intracellular cytokines production

Stimulation of the production of (IFN-γ, IL-6 and IL-8) was measured intracellularly by flow cytometry on the stimulated RAW264.7 macrophage cell with 1µg/ml LPS and 100µg/ml from P. macrocarpa F1.The intracellular expression of the IFN-γ showed significant ($P \leq 0.05$) increase in the stimulation of the RAW264.7 cell with P. macrocarpa F1, at a mean percent ± SD (22.90 ± 5.1)% compared to control RAW264.7 cell stimulation with LPS alone (1.13±0.92)% (Figure 2).

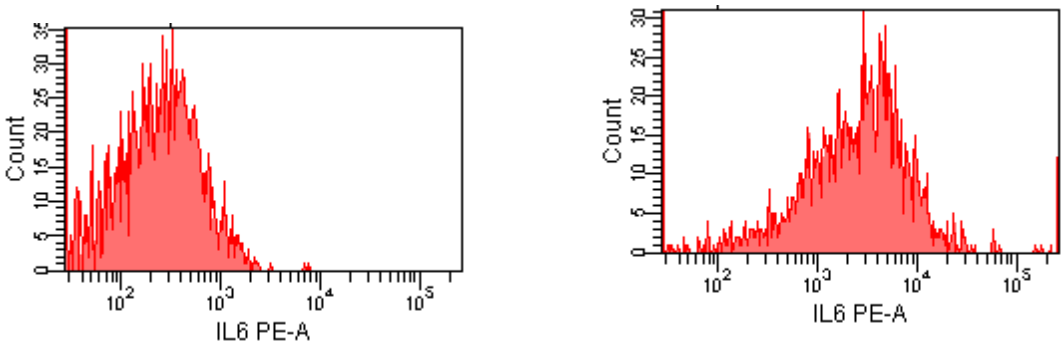
In addition, results showed a significant increase in intracellular expression of IL-6 and IL-8 ($P \leq 0.05$) in the stimulation of the RAW264.7 cell with P. macrocarpa F1. In (Figure 3) the results show mean percent ± SD for IL-6 intracellular expression (14.0 ± 4.65)% and (Figure 4) shows mean percent ± SD for IL-8 intracellular expression (14.60 ± 2.8)% compared to control, RAW264.7 cell stimulation with LPS alone for IL-6 (0.43± 0.32)% and for IL-8 (0.77± 0.7)%.



A: LPS 1µg/ml (Control) (1.13±0.92)

B: LPS1µg/ml + P.macrocarpa F1 100µg/ml (22.90 ± 5.1)*%

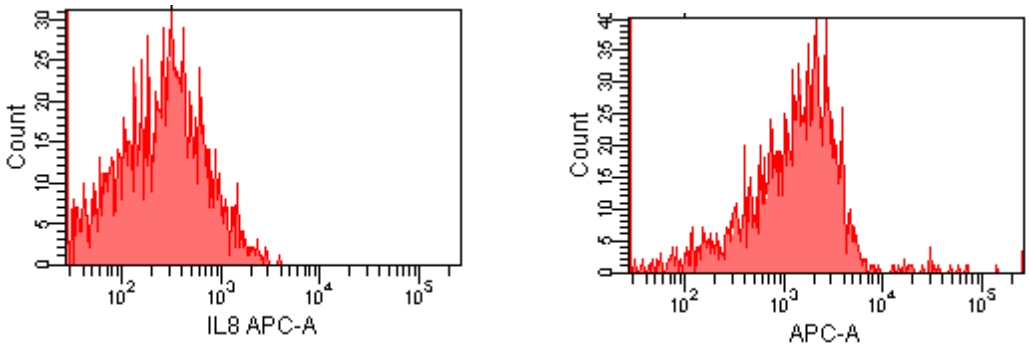
Figure 2: Intracellular expression of IFN-γ on RAW264.7 macrophage cell. Flow cytometry analysis was used to assess the intracellular expression of IFN-γ. The figures show the expression percent of IFN-γ on RAW264.7 cell stimulated with (A)1µg/ml LPS alone as a control. (B) LPS1µg/ml + P.macrocarpa F1 100µg/ml. The number represents the mean percent of the cell ±SD.*Significant ($P \leq 0.05$) versus control.



A: LPS 1µg/ml (Control) (0.43± 0.32)%

B: LPS1µg/ml + P.macrocarpa F1 100µg/ml (14.0 ± 4.65)* %

Figure 3: Intracellular expression of IL-6 on the RAW264.7 macrophage cell. Flow cytometry analysis was used to assess the intracellular expression of IL-6. The figures show the expression percent of IL-6 on RAW264.7 cell stimulated with (A) 1µg/ml LPS alone as a control. (B) LPS1µg/ml + P.macrocarpa F1 100µg/ml. The number represents the mean percent of the cell ±SD.*Significant ($P \leq 0.05$) versus control.



A: LPS 1µg/ml (Control) (0.77± 0.7)%

B: LPS1µg/ml + *P.macrocarpa* F1 100µg/ml (14.60 ± 2.8)*%

Figure 4: Intracellular expression of IL-8 on the RAW264.7 macrophage cell. Flow cytometry analysis was used to assess the intracellular expression of IL-8. The figures show the expression percent of IL-8 on RAW264.7 cell stimulated with (A) 1µg/ml LPS alone as a control. (B) LPS1µg/ml + P.macrocarpa F1 100µg/ml. The number represents the mean percent of the cell ±SD.*Significant ($P \leq 0.05$) versus control.

DISCUSSION

Modulation of the immune response to reduce diseases has long been of interest. There are many recent studies on ethnomedicinal plants as immunomodulatory agents. A relatively new and developing
branch of pharmacology is immunopharmacology which aims to search for immunomodulators. The probable use of immunomodulators in clinical medicine comprises the reconstruction of immune deficiency .There has been much research on plant extracts in different parts of the world for their possible immunomodulatory properties. Some of these studies have demonstrated the isolation of potential bioactive molecules (Alamgir & Uddin, 2010). Shosaiko-to, a Japanese herb, possesses many ethopharmacological effects and these effects are via the modulation of numerous host immune responses. Researchers have suggested that its effect in hepatitis B might be because of its ability to stimulate INFs and activate natural killer cell activity (Borchers et al., 2000). Macrophages are the first line of defense in innate immunity against microbial infection, professional in phagocytes engulf, kill microorganisms and present antigens for exciting adaptive immune responses (Girotti et al., 2004). Macrophages play a main function in tissue remodeling through development, wound healing and tissue homeostasis. In addition, it is essential to innate immunity and pathology of tissue injury and inflammation (Robert et al., 2011). Through phagocytosis, macrophages secrete cytokines as interleukins, TNF-α, IFNs and inflammatory mediators like nitric oxide (Stojanovic et al., 2011).

The results of the study showed that there was an immunomodulatory effect of P.macrocarpa fraction by increasing the RAW 264.7 macrophage cells proliferation in a dose dependent manner and significant inducing of the intracellular expression of cytokines IFN-γ, IL-6 and IL-8. These findings clearly indicate the significant immunomodulatory effect as immunostimulators.These findings are valuable and point to both isolated fractions from P.macrocarpa as very appropriate candidates for modulation of macrophage function and inducing the immune system. It is important to note from the literature that immunomodulatory action plays a crucial role in antitumor activity (Abu et al., 2014).

Therefore, the active isolated fractions from P.macrocarpa are potential candidates for antitumor efficacy. Preliminary studies on the antiproliferative efficiency of P.macrocarpa support this hypothesis (Tungpradit er al., 2010).

Our results clearly revealed that treatment with fractions could enhance the immune response and stimulate the production of essential mediator cytokines such as INF-γ, IL-6 and IL-8; these play an important and main role in the acute and chronic inflammatory response. Stimulation of acute phase protein synthesis by the liver is through IL-6, and acts as a growth factor for mature B cells and stimulates their final maturation into antibody producing plasma cells; this involves T cell activation and differentiation and its effect in the induction of Il-2 receptor expression and in addition its role in acute phase response, chronic inflammation, autoimmunity, fibrogenesis and endothelial cell dysfunction (Barnes et al., 2011).

IFN-γ effects are identified in murine kupffer cells, stimulated macrophages and share in the development of Th1 cells. Besides this, the cellular effects include up-regulation of pathogen recognition, antigen processing and presentation, antiviral case, inhibition of cellular proliferat ion, effect on apoptosis and immunomodulation (Schroder et al., 2004). IL-8 has potential as a neutrophil chemotactic factor. Many types can produce a large amount of IL-8 in response to avariety of stimuli such as proinflammatory cytokines, microorganisms and their products and environmental alteration including hypoxia and hyperoxia. IL-8 is a main mediator in neutrophil mediated acute inflammation and has a varied range of actions on different types of cells which include endothelial cells, fibroblasts, monocytes, lymphocytes and neutrophils. These functions suggest that IL-8 has an important function in different pathological disorders such as chronic inflammation and cancer (Mukaida, 2003).

Immunosuppression is one of the major problems in chemotherapy and radiotherapy. Thus, it is important to find a new antitumor drug that can enhance immune response. Activated macrophages play a crucial role in the immune system against tumor growth through their ability in pathogenesis, synthesis, and release of nitric oxide and H2O2 that are believed to be cytotoxic against particular tumors (Alonso-Castro et al., 2012). Besides improved immune cell function, natural killer cells play an important role in immune surveillance by the secretion of cytokines such as IFN-γ (Brutkiewicz & Sriram, 2002). Kadsura marmorata polysaccharide could stimulate Th1 cells to production IL-2, IFN-γ, and TNF-α. In turn, Th1 response can support protective immunity against intracellular infections such as bacteria, viruses and protozoa against cancer cells (Wang et al., 2013).

CONCLUSION

From our outcomes and previous studies, we conclude that we can employ medicinal plants and their isolated compounds as immunomodulators to treat chronic inflammation diseases, autoimmune diseases or any other immune disorder disease and as antitumor agents.

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➢ *ORAL PRESENTATION*

Alabalık (*Oncorhynchus mykiss*) seminal plazmasının koç spermasının 5ºC sıcaklıkta kısa süreli olarak saklanması üzerine etkisi

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Özet

Bu çalışmada, farklı dozlarda alabalık seminal plazması ilave edilmiş Tris bazlı sulandırıcının koç spermasının 5°C'ta kısa sureli saklanması üzerindeki etkisinin değerlendirilmesi amaçlandı. Çalışma amacıyla alınan ejakülatlar pooling yapılarak dört eşit hacme bölündü ve %5, %10 ve %15 alabalık seminal plazması (ASP) ilave edilmiş (%5 ASP, %10 ASP ve %15 ASP sırasıyla) ve edilmemiş (kontrol) sulandırıcılarla tek aşamalı olarak sulandırıldı. Sulandırılmış spermanın sıcaklığı 5°C'a düştükten sonra 0, 24, 48 ve 72. saatlerde; sperm motilite, plazma membran fonksiyonel bütünük [hipoozmotik şişme testi (HOST)] ve akrozomal hasar [FITC-Pisum sativum agglutinin (PSA-FITC)] muayeneleri yapıldı. Akrozom yönünden değerlendirildiğinde; saklama süresi boyunca gruplar arasında istatistiksel bir fark tespit edilmedi (P>0.05). Spermanın kısa süreli olarak saklanmasının 72. saatinde alabalık seminal plazması katılan gruplarda motilite ve plazma membran fonksiyonel bütünlüğünün kontrol grubuna göre daha yüksek olduğu belirlendi. Çalışmanın sonuçları, koç spermasının 5 ºC'ta saklama esnasında %10 ve %15 ASP ilavesinin yararlı olduğunu göstermektedir.

Anahtar Kelimeler: koç sperması, alabalık seminal plazması

Effect of rainbow trout's (*Oncorhynchus mykiss*) seminal plasma on liquid storage of ram semen at 5ºC

Abstract

The aim of the study was to evaluate different rainbow trout's seminal plasma (RTS) doses in Tris based extenders for liquid storage of ram semen. Pooled semen was divided into four equal volumes and diluted in a single-step dilution method with rainbow trout's seminal plasma supplemented (5% RTS, 10% RTS and 15% RTS respectively) groups and RTS-free (control) extenders. Semen samples were assessed for sperm motility, plasma membrane functional integrity using hypo-osmotic swelling test (HOST), damaged acrosome using FITC-Pisum sativum agglutinin (PSA-FITC) at 0, 24, 48 and 72h of storage at 5 °C. When evaluated in terms of acrosomes, there were no statistical differences among the control and 5% RTS, 10 % RTS and 15% RTS during the experiment (P>0.05). At the end of the 72nd hour, it was found that the motility and plasma membrane functional integrity of the ram semen was higher in the groups that contained RTS than the control group. The results show that the addition of specially 10% and 15% RTS is beneficial to the preservation of ram semen during liquid storage at 5 ºC.

Keywords: ram sperm, rainbow trout seminal plasma

GİRİŞ

Islah çalışmalarının etkin bir şekilde yapılabilmesi için kaliteli damızlık erkek hayvanın genetik materyalinin yaygın bir şekilde sahaya aktarabilmesi gerekmektedir. Ancak bu durum, damızlık bir erkek hayvandan alınan sperma ile fazla sayıda dişinin tohumlanması ve bu genetik materyalin uygun şekilde transportu ile olasıdır. Bu nedenle spermanın geniş bir alana aktarılabilmesi ve istenilen süre boyunca canlı ve fertil olarak saklanabilmesi için spermatozoon metabolizmasının geri dönüşümlü (reverzible) olarak baskılanması gerekmektedir (Maxwell ve Watson, 1996). Spermanın metabolizmasının baskılanması ve daha uzun süre saklanması sıcaklığının 5˚C'a düşürülerek kısa süreli saklama ya da spermanın dondurularak uzun süreli saklanması (kriyoprezervasyon) ile

olasıdır (Hafez, 1993; Salamon ve Maxwell, 2000). Koç spermasının biyokimyasal yapısı (yüksek oranda çoklu doymamış yağ asidi içeren plazma membranı) nedeniyle dondurma-eritme prosedürü sonunda akrozom başta olmak üzere morfolojik bozukluk sayısındaki artış ve viabilitedeki azalma donmuş koç sperması ile yapılan servikal tohumlamalarda fertilite oranının düşük olmasına neden olarak araştırmaların alternatif yöntem olarak koç spermasının 5ºC'ta kısa süreli saklanması üzerinde yoğunlaştırmıştır.

Günümüzde halen koç spermasının 5ºC'ta kısa süreli saklanması ve dondurma-eritme sonrası fertilizasyon yeteneğini optimize etmek amacıyla sperma sulandırıcılarına farklı katkı maddeleri eklenerek çok sayıda çalışma gerçekleştirilmektedir. Bu çalışmalarda; spesifik antioksidanlar, vitaminler, kriyoprotektanlar, seminal plazma gibi çeşitli etken maddeleri sulandırıcıya eklenerek yüksek oranda motilite, plazma membran bütünlüğü, akrozomal bütünlük, DNA bütünlük oranları elde edilmesi amaçlanmıştır (Arando ve ark., 2019; Banday ve ark., 2017; Bucak ve ark., 2013; Üstüner ve ark., 2016; Üstüner ve ark., 2018).

Üstüner ve ark. (2016) ve Gökçe, 2019 koç spermasının dondurulmasında alabalık seminal plazması (ASP) eklenmesinin dondurma-eritme sonrası spermatolojik parametreler üzerindeki etkisini değerlendirmiştir. Söz konusu araştırmalarda, farklı oranlarda ASP eklenen spermaların dondurma-eritme sonrası spermatolojik parametereleri ASP eklenmeyen kontrol gruplarına göre daha yüksek oranda korunduğu bildirilmiştir. Araştırmacılar, ASP'nin koç spermasının dondurulması üzerinde gözlemlenen bu olumlu etkisinin ASP'nın biyokimyasal ve antioksidan içeriğinden kaynaklandığı görüşünde olduklarını bildirmişlerdir.

Alabalık seminal plazmasında izole edilen bir çok spesifik proteinlerin genel işlevlerine bakıldığında, spermatozoa için canlılığını sürdürebileceği mikro bir çevre olmasının yanı sıra, spermatozoayı ortamda bulunan metabolik atık ve lipit peroksidasyonun zararlı etkilerine kaşı koruduğu sonucu çıkarılmaktadır (Nynca ve ark., 2017). Konu ile ilgili yapılan diğer bir araştırmada, serbest aminoasitlerin büyük bir bölümünün antioksidan etki gösterdiği, alabalık seminal plazması içerisinde yoğun arjinin, glutamik asit, isolöysin, löysin, metiyonin ve prolin, spermatozoa içerisinde ise sistein, arjinin ve metiyonin bulunduğu saptanmıştır (Lahnsteiner, 2009). Spermanın 5ºC'ta kısa süreli saklanması sırasında, spermatozoon motilitesinde ve membran bütünlüğünde kayıplar ve DNA kırıklı spermatozoa oranında artışlar, bunların sonucu olarak fertilite oranlarında düşmeler yaşanmaktadır (Bucak ve ark., 2007). Spermatozoonda meydana gelen bu tarz değişiklikler, spermatozoon membran fosfolipitlerinde gelişen lipit peroksidasyonu ve bunun sonucu olarak ortaya çıkan serbest oksijen radikallerin ROS: süperoksit anyonu, hidrojen peroksit, hidroksi radikali) oluşturduğu yıkımlara bağlı olabilmektedir (Bucak ve ark., 2007).

Bu çalışmanın amacı; koç spermasının 5ºC sıcaklıkta 72 saat saklanması esnasında sulandırıcıya katılan farklı dozlarda (%5, %10 ve %15) alabalık seminal plazmasının motilite, sperma membran fonsiyonel bütünlüğü ve akrozom hasarı üzerine etkisinin belirlenmesidir.

MATERYAL VE METOD

Bursa Uludağ Üniversitesi Veteriner Fakültesi Hayvan Sağlığı ve Hayvansal Üretim ve Uygulama Merkezi Koyunculuk Ünitesinde aynı bakım ve besleme koşullarında barındırılan 2–4 yaşlarında 4 baş Kıvırcık ırkı koç kullanıldı. Kullanılan koçlar, deney süreci boyunca kuru çayır otu ve hayvan başı günlük 1 kg konsantre yemden oluşan rasyon ile beslenirken; koçlara yalama taşı ile su ad-libitum olarak verildi.

Alabalık seminal plazmalarının temini:

Çalışmada kullanılacak olan alabalık seminal plazmasının temini amacıyla, alabalıkların üreme mevsimi içerisinde, erkek alabalıklardan sperma sağım yöntemi ile alındı. Sperma sağım işlemi öncesinde erkek balıklar, 100 mg/lt yoğunlukta MS-222 bulunan su dolu kaba konularak anestezi sağlandı. Anestezi altına alınan balıklar sudan çıkarılıp havlu ile kurulanmalarının ardından abdominal basınç ile sperma sağımı gerçekleştirildi. Alınan spermalar 10,000 x g'de 10 dakika boyunca santrifüj edildi ve süpernatant kısmı alınarak 10,000 xg'de 10 dakika ikinci bir santrifüj işlemi uygulandı. Ardından elde edilen süpernatant 1,5 ml hacimli santrifüj tüplerine bölünerek sulandırıcı hazırlanacağı güne kadar -20 ˚C'ta saklandı. Buzdolabında saklanan alabalık seminal plazmaları, çalışma günü oda sıcaklığında eritilerek çalışma gruplarına uygun şekilde sperma sulandırıcılarına eklendi.

Spermanın alınması ve sulandırılması:

Koçlardan sperma, aşım mevsimi dışında gün aşırı olmak üzere toplam 5 seferde ve elektroejakülatör (Ruakura koç probu, Hamilton, Yeni Zelanda) yöntemi ile alındı. Toplanan ejakülatlar, 28-32 °C'a ayarlanmış su banyosuna

yerleştirilerek spermatolojik muayenelerin yapılacağı androloji laboratuvaruna taşındı. İlk olarak faz kontrast mikroskop (Olympus BX51-TF - Olympus Optical Co., Ltd., Japonya) ile mass aktivite ve motilite muayeneleri yapıldı.

En az 0,5 ml hacim, +++ mass aktivite, >%75 motilite ve $1x10^9$ spermatozoa/mL yoğunluğa sahip ejakulatlar çalışmada kullanıldı. Bireysel farklılıkları elimine etmek amacıyla çalışma gününde seçilmiş olan ejakülatlar birleştirildi (pooling) ve dört eşit hacime bölündü. Her grup final konsantrasyonu yaklaşık $500x10^6$ (spermatozoa/mL) olacak şekilde kontrol (ASP içermeyen) ve farklı oranlarda (%5, %10 ve %15) ASP ihtiva eden Tris-sitrik asit-fruktoz-trehaloz (%20 yumurta sarısı) sulandırıcısı ile tek aşamalı olarak sulandırıldı. Sperma grupları, bir su banyosunun içerisine konarak suyun sıcaklığı kademeli olarak 60 dakikada 5˚C'a düşürülerek, 0.saat kabul edildi. Soğutulmuş sperma örnekleri 5˚C'ta 72 saat boyunca saklandı.

Spermanın değerlendirilmesi:

5C'ta saklanan sperma örneklerinden motilite, plazma membran fonksiyonel bütünlüğü (hypo-osmotic swelling test [HOST] ve akrozom hasarını (FITC conjugated Pisum-sativum agglutinin [FITC-PSA]) belirlemek için 0, 24, 48 ve 72. saatlerde numuneler alındı.

Fluorescein lectin staining assay (FITC conjugated Pisum-sativum agglutinin [FITC-PSA]): FITC-PSA akrozom yapısının değerlendirmek amacıyla kullanıldı (Üstüner ve ark., 2018). Her preparatta en az 200 adet spermatozoa florasan mikroskopta (Olympus BX51) sayıldı.

The hypo-osmotic swelling test (HOST): Sperm membran fonksiyonel bütünlüğü hypo-osmotic swelling test ile belirlendi. Bu değerlendirme şekline göre kıvrık ve kalınlaşan kuyruğu olan spermatozoonlar membran fonksiyonel bütünlüğü bakımından pozitif olarak değerlendirildi. Her çalışma grubundan sperma örnekleri (10 µL) 100 µL 100 mOsM hipo-osmotik solüsyonda (Bucak ve ark.,2007) 37 C'ta 60 dakika boyunca inkübe edildiler. İnkübasyon sonrası, solüsyondan bir damla lama alınarak üzerine lamel kapatıldı. Faz kontrast mikroskopta (Olympus BX51) en az 200 hücre sayılarak kuyruğu kalınlaşmış ve kıvrık olanlar pozitif olarak kabul edildi.

İstatistiksel analiz: Çalışmadan elde edilen tüm veriler SPSS (Windows için SPSS 23.0; SPSS, Chicago, IL, ABD) kullanılarak analiz edildi. Veriler ortalama ± standart hata olarak sunuldu. Normallik testi olarak Shapiro Wilk testi kullanıldı. Elde edilen sperm parametrelerinin ortalamaları Kruskal Wallis testi ve ardından Mann Whitney U testi kullanılarak analiz edildi.

BULGULAR

Sperma örneklerinin 0, 24, 48 ve 72. saatlerdeki motilite, plazma membran fonksiyonel bütünlüğü ve akrozom hasarı Tablo 1 ve Tablo 2'de sunulmuştur.

Tablo 1. 0 ve 24. saatlerdeki spermatolojik parametrelerin ortalama±standart hata ($\bar{x}$±S$\bar{x}$) değerleri (n=5)

Gruplar	0.saat			24.saat		
	Motilite (%)	HOST (%)	Hasarlı akrozom oranı (%)	Motilite (%)	HOST (%)	Hasarlı akrozom oranı (%)
Kontrol	74.00±1.87^a	80.60±2.01^a	13.40±1.20^a	56.00±1.87^a	78.60±3.98^a	15.80±2.22^a
%5 ASP	78.00±1.22^a	87.80±1.31^b	12.80±0.86^a	61.00±1.87^{ab}	77.60±3.82^a	11.70±1.09^a
%10 ASP	77.00±2.54^a	77.00±2.54^b	11.70±1.62^a	66.00±1.87^b	85.20±2.57^a	10.80±0.96^a
%15 ASP	79.00±1.87^a	88.40±0.60^b	11.50±2.56^a	66.00±1.00^b	83.40±1.53^a	15.70±2.65^a

a ve b: Her muhafaza saati için aynı sütunda farklı harfleri taşıyan sulandırıcı ortalamaları arasında istatistiksel olarak fark vardır ($p<0.05$).

Tablo 2. 48 ve 72. saatlerdeki spermatolojik parametrelerin ortalama±standart hata (x̄±Sx̄) değerleri(n=5)

Gruplar	48.saat			72.saat		
	Motilite (%)	HOST (%)	Hasarlı akrozom oranı (%)	Motilite (%)	HOST (%)	Hasarlı akrozom oranı (%)
Kontrol	45.00±1.58[a]	73.20±4.36[a]	19.00±2.02[a]	34.00±7.96[a]	67.80±3.89[a]	21.60±1.12[a]
%5 ASP	49.00±1.00[a]	78.60±3.10[a]	16.60±1.93[a]	41.00±11.97[a]	74.80±2.98[a]	17.50±1.89[a]
%10 ASP	53.00±2.54[a]	79.20±3.69[a]	18.80±1.52[a]	48.00±8.74[a]	76.00±2.07[a]	20.40±1.50[a]
%15 ASP	55.00±4.47[a]	78.60±3.74[a]	16.40±0.81[a]	52.00±7.17[a]	75.10±1.84[a]	19.90±2.32[a]

a ve b: Her muhafaza saati için aynı sütunda farklı harfleri taşıyan sulandırıcı ortalamaları arasında istatistiksel olarak fark vardır ($p<0.05$).

Motilite: Tüm çalışma grupları değerlendirildiğinde; kısa süreli saklama yönteminin spermanın motilitesi üzerinde negatif etkisi olduğu gözlemlendi. 0. saatte çalışma grupları arasında motilite bakımından istatistiksel bir fark gözlemlenmezken ($P>0.05$), 24. saatte en düşük motilitenin kontrol grubunda olduğu ($P<0.05$) ve 72.saat sonunda ise istatistiksel bir fark gözlemlenmese de en düşük motilitenin (%34,0) kontrol grubunda ve en yüksek motilitenin %15ASP grubunda olduğu tespit edildi.

Plazma membran bütünlüğü: Plazma membran bütünlüğü yönünden bakıldığında; 0, 24 ve 48.saatlerin kendi içinde değerlendirildiğinde HOST sonuçlarının birbirine yakın olarak gözlemlendiği fakat 72.saatin sonuna gelindiğinde istatistiksel bir fark olmasada en düşük membran fonksiyonel bütünlüğünün kontrol grubunda gözlemlenerek motilite sonuçları ile paralellik gösterdiği tespit edildi.

Akrozom hasarı: Akrozom hasarı yönünden gruplar arasında istatisistiksel bir fark tespit edilmedi.

TARTIŞMA

Koç spemasının kısa süreli saklanması, koç spermasının dondurulmasının spermatozoa üzerindeki olumsuz etkisi nedeniyle alternatif bir yöntem olarak kullanılmaktadır (Maxwell ve Salamon, 1993). Bununla birlikte kısa süreli saklama esnasında zaman geçtikçe spermatozoa metabolizmasının devam etmesi ve soğuk şoku nedeniyle spermatozoa üzerinde bazı negatif etkiler gözlemlenmektedir (Maxwell ve Salamon, 1993). Buna bağlı olarak çalışmamızda 72.saatte elde edilen motilite değerlerinin 0.saate göre daha düşük olduğu gözlemlenmiştir.

Spermatozoa motilitesi sperma değerlendirilmesinde kullanılan temel muayene şeklidir. Spermanın serviksten geçebilme ve zona pellusidayı penetre etme yeteneğinin göstergesidir. Sunulan çalışmada 0. saatte elde edilen motilite değerleri Dai ve ark. (2018) ile benzer, Gökçe ve ark. (2017)'dan yüksek olduğu gözlemlenmiştir. Çalışmamızın 72. saatteki motilite değerleri aynı araştırmacılarla kıyaslandığında; Gökçe ve ark. (2017) ile benzerlik gösterdiği fakat Dai ve ark. (2018)'dan ise daha düşük motiliteye sahip olduğu tespit edilmiştir. Koç spermasının kısa süreli saklanması sonrasında motilitede gözlemlenen varyasyonların sebebi kullanılan koçun ırkı, coğrafi bölge veya kullanılan sulandırıcı farklılığı olabilir. Çalışmamızda istatiksel fark olmasa da 72. saatin sonunda %15 ASP grubundan elde edilen %52,0 motilite değerinin kontrol grubundan (%34) yüksek olması ASP içeriğinin protein içeriği yönünden zengin olmasıyla ilişkili koruyucu etkisine bağlanabilir. ASP içerisindeki cathepsin D ve M, calpain, sitosolik nonspesifik dipeptidaz, proteozom ve antifiriz protein (type-4 ice-structuring protein [LS-12]) vb. proteinler ile ilişkilendirmek olasıdır. Söz konusu proteinler içerisinde özellikle LS-12'nin balık spermasının soğuk su koşullarında zarar görmesini engellediği bilinmekte olup memeli spermasında bulunmaması göze çarpmaktadır (Nynca ve ark., 2017; Zhao ve ark., 1998). Yukarıda söz edilen diğer proteinlerin ise motilitenin uyarılması ve hasarlı spermatozoonların metabolik atıklarının elimine edilmesinde önemli olduğu bilinmektedir (Nynca ve ark., 2014).

Spermatozoon membranının fonksiyonel bütünlüğünü belirleyen HOS-test sonuçlarının yüksek olması (%50≤), motilite ve in vivo/vitro fertilite parametreleriyle yakından ilişkilendirilmektedir (Jeyendran ve ark., 1984). Çalışmamızda plazma membran fonksiyonel bütünlüğü motilite ile paralellik göstererek 72. saatin sonunda en düşük kontrol grubunda en yüksek ise ASP ilave edilen gruplarda elde edildi ($P>0.05$). ASP'nın plazma mebran bütünlüğünü başarılı olarak koruyor olmasını alabalık seminal plazması içerisindeki proteinlerin, spermatozoon membranına tutunarak membran fosfolipit yapısı ve kompozisyonunu düzenlemesi ile açıklamak olasıdır (Lahnsteiner ve ark., 2004; Lahnsteiner, 2009; Shaliutina-Kolešová ve ark., 2016). Gomme ve ark. (2005), alabalık

seminal plazmasında içerisinde yüksek oranda bulunan transferrin ve albumin, spermatozoon membranını oksidatif stresten ve sitotoksik bileşiklerin olumsuz etkilerinden koruduğunu bildirmektedir. Çalışmamızın 72. saat ASP içeren gruplarının plazma membran fonkiyon bütünlüğü Varışlı ve ark. (2018)'dan yüksek Dai ve ark. (2018)'nın kontrol, 0.05, 0,1 ve 0,2 melatonin içeren grupları ile benzer olduğu gözlemlenmektedir.

Akrozom bütünlüğü, içerdiği enzimler nedeniyle spermanın fertilizasyonu açısından önemli rol oynamaktadır.ASP ilave edilen gruplar ve kontrol grubu arasında akrozom hasarı yönünden istatistiksel fark tespit edilmemiştir. Spermanın dondurulması yada kısa süreli saklanması sırasında şekillenen soğuk şoku ve reaktif oksijen türlerinin akrozom bütünlüğü üzerinde negatif etkisi bulunmaktadır. Sunulan çalışmada da 72.saatin sonunda akrozom kayıplarının 0. saate göre artmasının sebebi belirtilen nedenler olabilir. Sunulan çalışmanın 24. saate kadar akrozom hasarının Dai ve ark (2018) ve Bucak ve ark.(2007) ile uyumluluk gösterdiği gözlemlenmektedir. 24. sattten sonra Dai ve ark. (2018)'nın akrozom hasarındaki artış ve Varışlı ve ark. (2018)'nın 72.saat çalışmasındaki akrozom hasarının sunulan çalışmadan daha düşük olması ırk, bireysel farklılık ve sulandırıcı içeriğinin değişkenliğine bağlanabilir.

SONUÇ

Spermanın 5ºC'ta kısa süreli olarak saklanmasının 72. saatinde alabalık seminal plazması katılan gruplarda motilite ve plazma membran fonksiyonel bütünlüğünün kontrol grubuna göre daha yüksek olması, geliştirilen sulandırıcının alternatif bir sulandırıcı olarak kullanılabileceğini göstermektedir. Sunulan çalışmada 24.saatin sonunda %10 ve %15 ASP içeren gruplarda motilitenin diğer gruplara göre istatistiksel olarak yüksek olması, sahada suni tohumlama uygulamalarının başarısı için önemli bir detay oluşturmaktadır. Ayrıca bu kısa süreli saklanan spermaların suni tohumlamada kullanılması ve verilerin fertilite sonuçları ile desteklenmesi çalışmayı olumlu yönde destekleyecektir.

TEŞEKKÜR

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➢ *ORAL PRESENTATION*

Ratlarda İndometazin İle Oluşturulan Mide Ülseri Üzerine Chrysin'in Etkilerinin Araştırılması

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Özet

Bu çalışmanın amacı indometazin kaynaklı mide ülseri üzerine chrysin'in etkilerinin araştırılmasıdır. Çalışmada 42 Sprague Dawley cinsi erkek rat, her grupta 7 rat olacak şekilde 6 gruba ayrıldı. 1. Grup (Kontrol); Oral yolla serum fizyolojik verildi. 2. Grup (İndometazin); Mide ülseri sağlamak için 25 mg/kg tek doz oral indometazin verildi. 3. Grup (Referans); Ülser tedavisinde kullanılan Ulcuran 30 mg/kg dozunda verildikten 30 dk sonra 25 mg/kg indometazin oral yolla verildi. 4. Grup (İndometazin+Chrysin25); 25 mg/kg dozunda chrysin verildikten 30 dk sonra 25 mg/kg indometazin oral yolla verildi. 5. Grup (İndometazin+Chrysin50); 50 mg/kg dozunda chrysin verildikten 30 dk sonra 25 mg/kg indometazin oral yolla verildi. 6. Grup (İndometazin+Chrysin100); 100 mg/kg dozunda chrysin verildikten 30 dk sonra 25 mg/kg indometazin oral yolla verildi. Kontrol ve referans grubu ile kıyaslandığında, indometazin grubunda SOD, KAT, GPx ve arginaz aktiviteleri ile GSH düzeyinin düştüğü, MDA seviyesinin arttığı ($p<0.05$) tespit edilmiştir. Ayrıca indometazin grubunda iNOS, TNF-α, IL-1β, NFκB, MAPK-14, MPO ve 8-OHdG düzeyleri artarken, COX-2 aktivitesi ile PGE_2 seviyesi azaldığı belirlenmiştir. Diğer taraftan indometazin ile birlikte verilen chrysin'in üç dozundan özellikle 50 ve 100 mg/kg dozları ülseratif ratlardaki bu değerleri yükseltmiştir ($p<0.05$).Bu çalışmada indometazin kaynaklı mide ülseri üzerine 50 ve 100 mg/kg chrysin dozlarının daha etkili olduğu bulunmuştur.

Anahtar Kelimeler: Chrysin, İndometazin, İnflamasyon, Oksidatif Stres, Mide Ülseri.

Investigation of the Effects of Chrysin on Indomethacin-induced Stomach Ulcer in Rats

Abstract

The aim of this study was to investigate the effects of chrysin on indomethacin-induced stomach ulcer.42 Sprague Dawley male rats were used in the study. The rats were divided into 6 groups of 7 in each group. Group 1 (Control); Oral saline was administered. Group 2 (Indomethacin); A single oral dose of 25 mg/kg indomethacin was administered to provide gastric ulcers. Group 3 (Reference); Ulcuran used in the treatment of ulcer was given 30 mg/kg dose and then 25 mg/kg indomethacin was administered orally 30 minutes later. Group 4 (Indomethacin+Chrysin25); 25 mg/kg indomethacin was administered orally 30 minutes after chrysin was given at 25 mg/kg dose. Group 5 (Indomethacin+Chrysin50); 25 mg/kg Indomethacin was administered orally 30 minutes after chrysin was given at 50 mg/kg dose. Group 6 (Indomethacin+Chrysin100); 25 mg/kg Indomethacin was administered orally 30 minutes after chrysin was given at 100 mg/kg dose. Compared with the control and reference groups, SOD, KAT, GPx and arginase activities and GSH levels decreased and MDA levels increased ($p<0.05$) in the indomethacin group. In addition, levels of iNOS, TNF-α, IL-1β, NFκB, MAPK-14, MPO and 8-OHdG were increased in the indomethacin group while COX-2 activity and PGE_2 levels were decreased. On the other hand, three doses of chrysin given with indomethacin, especially doses of 50 and 100 mg/kg increased these values in ulcerative rats ($p<0.05$). In this study, doses of 50 and 100 mg/kg chrysin were found to be more effective on indomethacin-induced stomach ulcer.

Keywords: Chrysin, Indomethacin, Inflammation, Oxidative Stress, Stomach Ulcer.

GİRİŞ

Mide ülserasyonu, midenin aşırı asit ve pepsin aktivitesine maruz kalması sonucu mukozal epitelde meydana gelen iyi huylu bir lezyondur. (Khazaei ve Salehi, 2006; Sabiu ve ark., 2015; Abebaw ve ark., 2017) Ülser oluşumunda gastrointerstinal mukozayı koruyan ve mukozanın hidrolitik ve proteolitik sindirimine neden olan faktörler arasında meydana gelen dengenin bozulması önemli rol oynamaktadır. (Güneş ve ark., 2013; Silen ve ark., 1981; Desai ve ark., 1997) Gastrik ülser hastalığının nedenleri arasında *Helicobacter pylori* enfeksiyonu, steroidal olmayan anti-inflamatuar ajanlar (NSAİİ'ler) ve tümörler bulunur.[6] NSAİİ, stres ve çeşitli çevresel faktörlerle birlikte oluşan oksidatif hasarda ülser oluşmasının edinsel faktörlerinden biri olarak bilinmektedir. (Güneş ve ark., 2013; Silen ve ark., 1981; Mertz ve Walsh, 1991) Gastrik mukoza normalde koruyucu faktörlerle hasar veren faktörlere cevap verir.(Silen ve ark., 1981; Jiang ve ark., 2005; Magni ve ark., 1982)

Geleneksel olarak, steroidal olmayan antiinflamatuar ilaçlar (NSAİİ'ler), spor yaralanmaları kas-iskelet rahatsızlığı olan hastalarda, tendon ve ligament yangılarında, ayrıca ameliyat sonrası analjezik amaçlı olarak kullanılmaktadır. (Su ve Connor, 2013) İndometazin ilk sentezlenen NSAİİ'lerden biridir. İndometazin, lipoksijenaz ve siklooksijenazı etkileyerek araşidonik asit metabolizmasını inhibe eder. Bundan dolayı anti-inflamatuar bir ilaç olarak kullanılır.(Hilário ve ark., 2006; Zhang ve ark., 2014) İndometazin gastrointestinal toksisitesini gastrik asit sekresyonunda artış gibi çeşitli mekanizmalarla sağlamaktadır. İndometazin PGE2 sentezinin inhibisyonu, serbest radikallerin üretimi, gastrik nitrik oksit seviyesinin azaltılması ve aktifleştirilmiş nötrofillerin invazyonu, gastrik hücrelerde apoptosisin indüklenmesine neden olur ve mukozal hücre rejenerasyonunu etkiler.(Matsui ve ark., 2011) Ayrıca indometazin nötrofillerin gastrik endotele doğrudan bağlanmasını teşvik eder ve bu da kılcal damarları tıkanması ile kan akışında azalmaya yol açarak ülserasyona neden olur.(Shim ve Kim, 2016)

Son zamanlarda popüler fitokimyasallardan olan Flavonoidler insan sağlığı üzerinde potansiyel olarak yararlı etkiye sahip bitkilerden elde edilen kimyasallardır. (Manach ve ark., 1997; De Lira Mota ve ark., 2009) Flavonoidlerin gastrointestinal sistemde anti-spazmodik, (Lima ve ark., 2005) anti-sekretuvar, anti-diyarel (Carlo ve ark., 1993) ve anti-ülser (La Casa ve ark., 2000) olarak etki ettiği bildirilmiştir. Doğal flavonoid bir bileşik olan chrysin, antioksidan savunma mekanizmalarını destekleyerek serbest radikallerin eliminasyonunu sağlar. (Sultana ve ark., 2012; Sathiavelu ve ark., 2009) Ayrıca chrysin lipit peroksidasyon seviyelerini de azaltmaktadır. (Anand ve ark., 2012) Chrysin'in, hem in vitro hem de in vivo olarak, Tümör Nekroz Faktörü-α (TNF- α), interlukin-6 (IL-6), interlukin-1β (IL-1β) ve nitrik oksit (NO) dahil olmak üzere çeşitli sitokinleri inhibe ederek anti-inflamatuar etkiye sahip olduğu da bildirilmiştir. (Cho ve ark., 2004; Harasstani ve ark., 2010)

Bu amaçla sunulan çalışmada ratlarda indometazin ile oluşturulan mide ülseri üzerine chyrsinin etkilerinin araştırılması amaçlanmıştır.

Materyal ve Metot

Kullanılan Deney Hayvanları

Bu çalışmada Atatürk Üniversitesi Deneysel Araştırma ve Uygulama Merkezinde (ATADEM) üretilen 240-270 g ağırlığındaki 42 adet Sprague Dawley cinsi erkek rat kullanıldı. Gruplara ayrılan ratlara yem ve su *ad libitum* verilerek ortamlarına uyum sağlamaları sağlandı. Çalışma, Atatürk Üniversitesi Hayvan Deneyleri Yerel Etik Kurul Başkanlığının 26.10.2017 tarihli (toplantı sayısı:11) ve karar no:134 izni ile belgelendirildi.

Deneysel Uygulamalar

Tüm ratlar her grupta 7 rat olacak şekilde 6 gruba ayrıldı. Çalışmada (Guidobono ve ark., 1997)' nın ülser oluşturma modeli kullanıldı.

1. Grup: Kontrol (Sağlıklı); Hiçbir ilaç uygulaması yapılmadı, sadece oral olarak serum fizyolojik (SF) verildi. **2. Grup:** İndometazin (İnd); Mide ülseri sağlamak üzere 25 mg/kg tek doz oral olarak verildi. **3. Grup:** Referans; Ülser tedavisinde kullanılan Ulcuran 30 mg/kg dozunda verildikten 30 dk sonra 25 mg/kg indometazin oral olarak verildi. **4. Grup:** İnd+CH25; Chrysin 25 mg/kg dozunda verildikten 30 dk sonra 25 mg/kg indometazin oral olarak verildi. **5. Grup:** İnd+CH50; Chrysin 50 mg/kg dozunda verildikten 30 dk sonra 25 mg/kg indometazin oral olarak verildi. **6.Grup:** İnd+CH100; Chrysin 100 mg/kg dozunda verildikten 30 dk sonra 25 mg/kg indometazin oral olarak verildi (Halici ve ark., 2016; Almasaudi ve ark., 2017; Pushpavalli ve ark., 2010).

Biyokimyasal Analizler

Mide dokusunda bir lipid peroksidasyon ürünü (LPO) olan malondialdehitin (MDA) ölçümü Placer ve ark., (1966) bildirilen yönteme göre ölçülmüştür. Glutatyon (GSH) düzeyleri Sedlak ve Lindsay (1968) tarafından bildirilen yönteme göre ölçülmüştür. Glutatyon peroksidaz (GPx) aktivitesinin ölçümü Matkovics (1988) tarafından bildirilen yönteme göre ölçülmüştür. Hazırlanan mide dokusu homojenatındaki süperoksit dismutaz (SOD) aktivitesinin ölçümü, Sun ve ark., (1988) metoduna göre ölçülmüştür. Mide dokusundaki katalaz (KAT) aktivitesi Aebi (1983) metoduna göre ölçülmüştür. Numunelerdeki protein konsantrasyonu, Lowry ve ark., (1951) metoduna göre belirlendi. Mide dokusu arginaz aktivitesi Pesce ve Kaplan (1987) metoduna göre belirlendi. Biyokimyasal analizler ELISA Plate Reader (Bio-Tek, Winooski, VT, ABD) ile yapılmıştır.

İstatistiksel Analizler

Bu çalışmanın istatistiksel analizleri SPSS 20.0 yazılım programı kullanılarak yapılmıştır. Bütün ölçümlerde istatistiksel farklılıklar ve önem seviyeleri "One-way Analysis of Variance (ANOVA)" testi ile belirlenmiş ve çoklu karşılaştırmalarda Tukey testi uygulanmıştır. $p<0.05$ seviyesindeki sonuçlar önemli kabul edilmiştir.

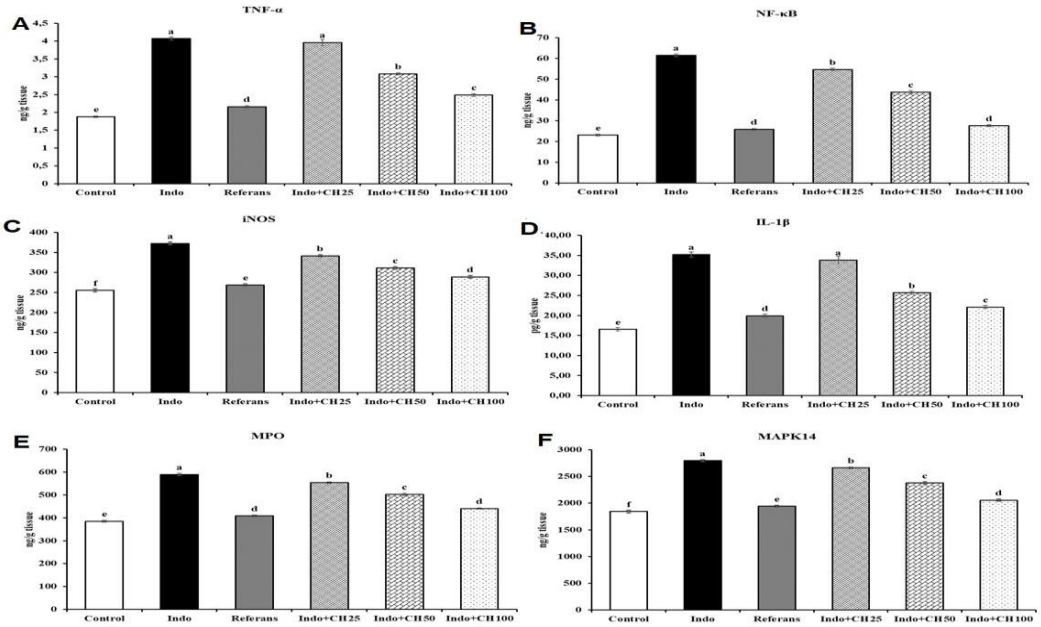
BULGULAR

Çalışmada gruplara ait mide dokusu SOD, KAT, GPx, Arginaz aktiviteleri ile MDA ve GSH Düzeyleri tablo 1'de verilmiştir.

Tablo1. Mide dokusu SOD, KAT, GPx, Arginaz aktiviteleri ile MDA ve GSH Düzeyleri

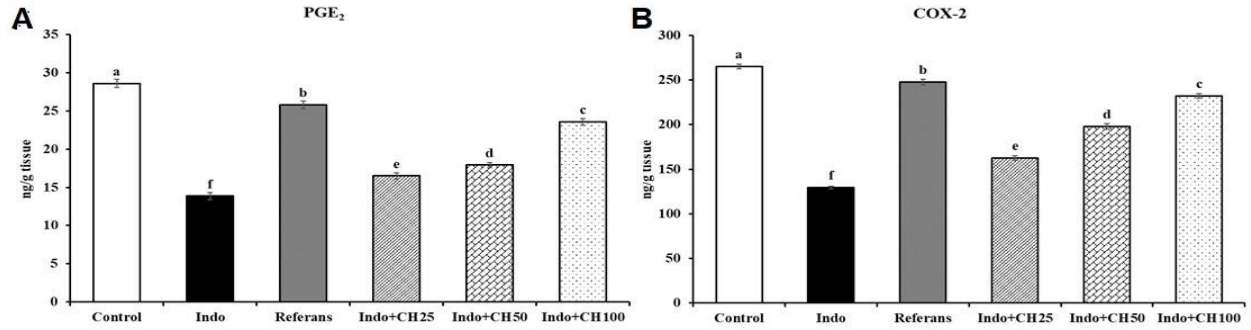
Gruplar/Parametreler	Kontrol	İndo	Referans	İndo+CHR25	İndo+CHR50	İndo+CHR100
MDA (nmol/g doku)	19.44±0.55^{f}	53.09±0.94^{a}	22.43±0.77^{e}	49.99±0.73^{b}	32.94±0.79^{c}	25.44±0.43^{d}
GSH (nmol/g doku)	2.09±0.03^{a}	0.78±0.01^{e}	1.88±0.05^{b}	0.87±0.03^{e}	1.49±0.03^{d}	1.75±0.03^{c}
SOD (U/g protein)	10.31±0.24^{a}	3.29±0.07^{f}	9.46±0.23^{b}	4.41±0.0^{9e}	6.05±0.08^{d}	8.51±0.21^{c}
KAT (katal/g protein)	7.93±0.11^{a}	2.79±0.06^{f}	7.38±0.06^{b}	4.25±0.06^{e}	5.49±0.05^{d}	6.68±0.07^{c}
GPX (U/g protein)	12.27±0.28^{a}	5.11±0.11^{d}	11.68±0.28^{a}	5.19±0.24^{d}	7.16±0.25^{c}	9.83±0.24^{b}
Arginaz (U/g doku)	0,72±0,02^{a}	0,23±0,01^{e}	0,59±0,02^{b}	0,28±0,01^{d}	0,41±0,01^{c}	0,59±0,02^{b}

Çalışmada gruplara ait mide dokusu TNF-α, NF-κB, iNOS, IL-1β, MPO ve MAPK14 Aktivitesi Şekil 1'de verilmiştir.



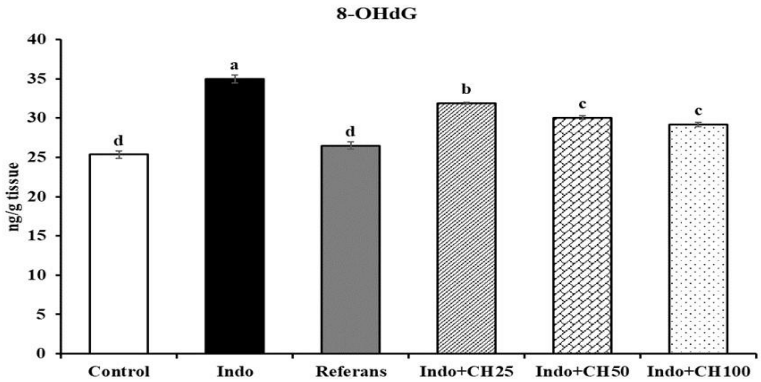
Şekil 1. Mide dokusu TNF-α, NF-κB, iNOS, IL-1β, MPO ve MAPK14 Aktivitesi

Çalışmada gruplara ait mide dokusu PGE_2 ve COX-2 Aktiviteleri Şekil 2'de verilmiştir.



Şekil 2. PGE_2 ve COX-2 Aktiviteleri

Çalışmada gruplara ait mide dokusu 8-OHdG Seviyesi Şekil 3'de verilmiştir.



Şekil 3. 8-OHdG Seviyesi

TARTIŞMA

Mide ülseri, dünya nüfusunun %5-10'unda görülen günümüzün en yaygın hastalıklarından biridir. (Lauret ve ark., 2015) Ülser, duedonal ve gastrik ülserleri kapsayan kompleks bir hastalıktır. (Pesce ve Kaplan, 1987; Sumbul ve ark., 2011) Gastrik ülser, temel olarak midenin dış kısmındaki koruyucu epitel tabakadan başlayarak,

iç kısımdaki endotel tabaka, düz kaslar ve bağ dokuyu kapsayan mide duvarındaki derin bir yaradır. (Fahmy ve ark., 2015)

Flavonoidler, insan sağlığı için hayati öneme sahip olan bitkilerin sekonder metabolitleridir ve genellikle şekerler ile konjuge halde bulunurlar. (Samarghandian ve ark., 2017)

Sabiu ve ark., (2016) ratlarda indometazin ile gastrik ülser oluşturmuşlar ve indometazinin mide dokusu MDA düzeylerinde önemli düzeyde artışa neden olduğunu tespit etmişlerdir. Çalışmada da literatürlere benzer şekilde indometazin ile oluşturulmuş mide ülserinde doku MDA düzeylerinde önemli düzeyde artış saptanmış ve chrysinin özellikle 50 ve 100 mg' lık dozlarının MDA düzeylerini azaltmada etkili olduğu görülmüştür.

Sunulan çalışmada indometazin grubu ratlarda mide dokusu GSH düzeyinde azalma tespit edilmiştir. Yapılan çalışmalarda chrysinin GSH seviyelerini yükselttiği bildirilmiş olup, mevcut çalışmada da chrysinin GSH seviyelerini artırdığı ve böylece ratların mide dokusunda meydana gelen ülser alanlarının doza bağlı olarak azaldığı tespit edilmiştir. (Khan ve ark., 2012; Khan ve ark., 2012)

Farklı kimyasal ajanlarla yapılan toksikasyon modellerinde chyrsinin GPx aktivitesini arttırarak antioksidan sistemi güçlendirdiği rapor edilmiştir. (Koriem ve ark., 2015; Rehman ve ark., 2013) Sunulan çalışmada da indometazinin GPx aktivitesini büyük oranda azalttığı ve indometazin ile birlikte verilen chrysinin 50 mg ve 100 mg uygulanan dozlarında GPx aktivitesinin yükseldiği tespit edilmiş ve elde edilen bulgular literatürler ile uyum göstermiştir.

Pineda ve ark., (2018) da indometazin ile oluşturdukları ülser modelinde antioksidan enzim aktivitelerinde bir düşüş olduğunu ve özellikle SOD aktivitesinin belirgin şekilde azaldığını bildirmişlerdir. Mevcut çalışmada yapılan çalışmalara benzer sonuçlar elde edilmiş olup, indometazin grubunda SOD aktivitesi kontrol ve referans gruplarına göre önemli ölçüde azalmış, tedavi amaçlı verilen chrysinin 25 mg'lık dozundan başlayarak 100 mg'lık dozuna kadar enzim aktivitesinde yükselme izlenmiştir.

Koriem ve ark., (2015)'ları tarafından yapılan bir çalışmada, ratlarda indometazin ile gastrik ülser oluşturulmuş ve bu ratların mide dokusu KAT aktivitelerinde önemli ölçüde azalma gözlenmiştir. Yaptığımız çalışmada da literatürlere benzer şekilde indometazin grubunda katalaz aktivitesinin, kontrol ve referans gruplarına göre önemli derecede azaldığı, destekleyici tedavi olarak verilen chrysinin tüm dozlarında KAT aktivitesinde artış olduğu ve en fazla artışın 100 mg'lık dozda görüldüğü tespit edildi. Sunulan çalışmada indometazin grubundaki hayvanların mide dokusunda arginaz aktivitesinin düştüğü, uygulanan chrysin ile birlikte aktivitenin arttığı ve bu artışın 50 ve 100 mg'lık dozlarda etkin olduğu tespit edildi.

Antonisamy ve ark., (2016) indometazin ile gastrik ülser oluşturduğu ratlarda pro-inflamatuar protein olan TNF-α düzeylerini incelemişler, indometazinin mide dokusunda bu düzeyleri kontrol grubuna göre artırdığını tespit etmişlerdir. Sunulan çalışmada da literatürlere benzer şekilde indometazin grubunda TNF-α nın yükseldiği görülmüştür.

Carrasco ve ark., (2016) indometazin ile oluşturdukları gastrik hasarın moleküler mekanizmasını incelemişler ve NF-κB düzeylerinin arttığını, artan bu düzeyinde proinflamatuar sitokin sentezini hızlandırdığını tespit ederek NF-κB'nin inflamatuar yanıtın gelişmesinde önemli bir rol oynadığını bildirmişlerdir. Çeşitli flavanoidler NF-κB sentez yolağını baskılayarak anti-inflamatuar etki oluşturmaktadırlar. Chrysin, NF-κB'nin güçlü bir antagonisti olarak hareket eder ve bu şekilde iNOS üretimini azaltır. (Lawrence ve ark., 2009)

Zheng ve ark., (2016) ratlarda indometazin ile gastrik mukozal hasar oluşturmuşlar ve iNOS seviyelerinin indometazin grubunda arttığını tespit etmişlerdir. İndometazin uygulanan gruptaki elde ettiğimiz veriler neticesinde iNOS aktivitesinin kontrol ile referans grubuna göre yükselmesi konu ile ilgili yapılmış olan benzer çalışmalarla uyum göstermiş, (Antonisamy ve ark., 2016) chrysinin artan iNOS aktivitesini azalttığı belirlenmiştir. iNOS aktivitesindeki bu azalmanın chrysinin NF-κB aktivasyonunu baskılamasının bir sonucu olduğu düşünülmüştür.

Yapılan çalışmalarda, indometazinin COX-2 aktivitesini azaltarak PGE_2 seviyelerinde düşüşe neden olduğu ve bunun sonucunda da gastrik ülserin şekillendiği bildirilmiştir. (Antonisamy ve ark., 2014; Lee ve ark., 2017) Mevcut çalışmada da benzer sonuçlar elde edilmiş olup, indometazin grubunda COX-2 aktivitesi ile PGE_2 seviyelerinde önemli bir azalma gözlenmiş ve bu azalmanın muhtemel nedeninin de indometazinin COX inhibitörü olmasından kaynaklanabileceği şeklinde yorumlanmıştır. Çeşitli doku hasar modellerinde tedavi amaçlı kullanılan chrysinin COX-2 aktivitesi ile PGE_2 seviyelerinde azalmaya neden olduğu yapılan çalışmalarla tespit edilmiştir. (Zeinali ve ark., 2017; Mantawy ve ark., 2014) Literatürlerin aksine elde ettiğimiz verilerde chriysin uygulaması ile birlikte COX-2 aktivitesi ve PGE_2 düzeylerinde bir artış gözlenmiş ve bununda chrysinin, indometazinin COX-2 üzerine olan inhibitör etkisini ortadan kaldırarak başardığı kanısına varılmıştır.

Katary ve ark., (2017) gastrik ülserli ratlarda yaptıkları bir çalışmada inceledikleri IL-1β düzeylerinin, indometazin ile ülser oluşturulmuş olan hayvanlarda arttığını belirtmişlerdir. Yao ve ark., (2014) farklı kimyasal ajanlar ile ratlarda inflamasyon ve oksidatif stresi indüklemişler, tedavi amacıyla verdikleri chrysinin IL-1β seviyelerinde azalmaya neden olduğunu rapor etmişlerdir. Literatüre uyumlu olarak sunulan çalışmada da indometazin grubundaki ratlarda IL-1β seviyelerinin kontrol grubuna göre önemli derecede yükseldiği, chrysinin 50 ve 100 mg'lık dozlarının IL-1β seviyelerini düşürmesine rağmen 25 mg'lık dozunun etki etmediği tespit edildi.

Sinha ve ark., (2015) yaptıkları bir çalışmada, indometazin grubunda MPO aktivitesinin kontrol grubuna göre önemli oranda arttığını ve flavanoid uygulamasının ise artmış olan bu aktiviteyi azalttığını bildirmişlerdir. Mevcut çalışmada da benzer sonuçlar elde edilmiş olup, indometazin grubunda MPO aktivitesinin önemli ölçüde arttığı, uygulanan chrysinin üç farklı dozunda da MPO aktivitesinde belirgin şekilde azalma sağladığı saptanmış, en etkili dozun 100 mg olduğu belirlenmiştir.

Ülserli dokuda oksidatif stresteki artışın MAPK14 aktivitesinde artışa neden olduğu Yadav ve ark., (2012) tarafından rapor edilmiştir. Chrysin, MAPK14 aktivitesini azaltmaktadır. (Zeinali ve ark., 2017; Khan ve ark., 2012) Sunulan çalışmada da benzer şekilde indometazin grubunda MAPK14 aktivitesinin arttığı ve kontrol ile referans gruplarının değerlerinin üzerinde olduğu, chrysin tüm doz uygulamalarında MAPK14 aktivitesinde önemli derecede azalma olduğu ve literatür bilgilerinin elde ettiğimiz verileri desteklediği görülmüştür.

Bolajoko ve ark., (2017) mide ülserli hastalarda yaptıkları çalışmada, 8-OHdG seviyelerinin yüksek olduğunu belirtmişlerdir. Benzer şekilde indometazin kaynaklı gastrik ülserli ratlarda yapılan çalışmalarda da 8-OHdG seviyelerinde yükselme gözlenmiştir. (Bicer ve ark.,2016)

Flavonoidler, antioksidan özellikleri sayesinde, serbest radikal düzeylerini azaltarak DNA'yı bu radikallerin etkisinden korumaktadır. (Benzer ve ark., 2018; Kandemir ve ark., 2017) Elde ettiğimiz veriler literatür bilgiler ile karşılaştırıldığında indometazin grubu 8-OHdG düzeylerinde kontrol ile referans grubuna göre önemli ölçüde artış gözlenmiş, chrysinin üç farklı dozununda 8-OHdG seviyelerini düşürerek DNA hasarını en aza indirgediği tespit edilmiştir. Chrysinin 50 ve 100 mg'lık dozlarının etkisinin hemen hemen aynı olduğu ve 25 mg lık dozdan daha etkili oldukları saptanmıştır.

SONUÇ

Elde edilen veriler ışığında, chrysin maddesinin 25 mg'lık doz uygulamasının mide ülserini tedavi etmede yetersiz kaldığı, doz artışı ile (50 ve 100 mg) mide ülserleri alanlarında önemli derecede azalma sağlandığı, chrysinin en etkili dozunun 100 mg olduğu tespit edildi. Chrysinin indometazin kaynaklı oluşan ülserlerin tedavisinde antioksidan, anti-apoptotik ve anti-inflamatuar etkilerinden dolayı kullanılmasının yararlı olacağı kanaatine varıldı.

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➢ *ORAL PRESENTATION*

Polielektrolit içeren dekante edilmiş aerobik arıtma havuzu çamurunun toprak düzenleyici olarak kullanıma uygun hale getirilmesi

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Özet

Ülkemizde 2019 yılına kadar aktif arıtma çamurunun tarımda kullanılması gerekli şartlar dahilinde sınırlandırılmaktaydı. Fakat son yıllarda izlenilen sıfır atık projeleri kapsamında bu sınırlamalar kaldırılarak aktif çamurun tarımda kullanılması tamamen serbest bırakıldı. Teknolojinin çok gelişmesine rağmen günümüzde biyolojik arıtma tesislerinde elde edilen aktif çamurun kuru maddesi %10-15 arası gelmektedir. Kuru maddenin düşük olması bertaraf firmasına gönderilen çamurun su ağırlıklı olmasına ve işletme maliyetlerinin çok fazla artmasına neden olmaktadır. Bu maliyeti azaltmak adına birçok firma arıtma çamurunu dekante işlemi sırasında dekantöre çamurun topaklaşmasını sağlayan Polielektrolit beslemesi yapmaktadır. Bu çalışmada amaç, polielektrolit kullanılarak dekante edilen aktif arıtma çamurunun toprak düzenleyici olarak uygun hale getirilmesi ve bu sayede bertaraf edilmeyerek tarıma ve ülke ekonomisine katkı sağlaması ile bertaraf sırasında oluşan karbon salınımının da önüne geçilmesidir. Bu amaçla, arıtma çamuru(A.Ç.) Düzce Pakmaya Fabrikasının biyolojik arıtma havuzlarından polielektrolit (zetag) kullanılarak dekante edilerek elde edilmiş ve yine Düzce Pakmaya fabrikasında hammadde olarak kullanılan şeker melasının stabilize edilmesi sırasında oluşan üretim çamuru (melas çamuru-Ü.Ç.) ile farklı oranlarda harmanlanarak kompost elde edilmiştir.

Elde edilen kompostlar, Düzce iline bağlı Döngelli köyünde kurulan sera da domates fideleri üzerinde toprakaltı gübre olarak kullanılmış ve domateslerin gelişimleri fiziki olarak takip edilmiştir. Çiçek mevsiminde yaprak numunelerinden klorofil analizleri yapılarak meyve verebilme potansiyelleri kıyaslanmıştır. Sonuç olarak kontrol grubuna göre kompost kullanılan fidelerin %8-20 arası meyve verimi artışı gözlemlenmiştir Elde edilen kompostların analizlerinde yoğun ağır metale rastlanmamış olup değerlerin yasal sınırlar içinde olduğu görülmüştür.

Anahtar Kelimeler: Arıtma çamuru, polielektrolit, kompost, toprak düzenleyici

Making the decanted aerobic treatment pool sludge containing polyelectrolyte suitable for use as a soil conditioner

Abstract

Until 2019, active treatment sludge in our country was limited to the conditions required to be used in agriculture. However, within the scope of the zero waste project monitored in recent years, the use of activated sludge in agriculture was completely released by removing these limitations. Despite the great development of technology, dry matter of activated sludge obtained in biological treatment plants today comes between 10-15%. The low dry matter causes the sludge sent to the disposal firm to be water-weighted and the operating costs to increase. In order to reduce this cost, many companies supply Polyelectrolyte, which enables the sludge to agglomerate to the decanter during the decanting process. The aim of the project is to make the active treatment sludge decanted using polyelectrolyte suitable as a soil conditioner, thus preventing carbon emission during its disposal by contributing to agriculture and the country's economy.

The treated sludge, which is the subject of our study, was decanted from the biological treatment pools of Duzce Pakmaya Factory using polyelectrolyte (zetag) and blended with the production sludge (molasses sludge) formed during the stabilization of sugar molasses, which is also used as a raw material in the Duzce Pakmaya factory, and compost was obtained.

The composts obtained were used as underground fertilizers on tomato seedlings in the greenhouse established in Döngelli village of Düzce province and the development of the tomatoes was physically followed. Chlorophyll analysis was performed on leaf samples during the flower season and their fruit-bearing potentials were compared. As a result, an 8-20% increase in fruit yield of seedlings using compost compared to the control group was observed. In the analysis of the obtained compost, no heavy metal was found and the values were found to be within legal limits.

Keywords: Polyelectrolyte, Compost, Sewage sludge, Soil conditioner

GİRİŞ

Arıtma çamuru, atık su arıtımı sonucu oluşan sıvı ya da yarı katı halde, kokulu, uygulanan arıtma işlemine bağlı olarak ağırlıkça %0.25-12 katı madde içeren bir çeşit katı atıktır (Durak,2005). Arıtma çamurları, makro ve mikro besin elementleri ve eser elementler gibi birçok yararlı bileşiklerin yanında, organik kirleticileri, mikroorganizmaları ve parazit yumurtalarını da içerebilmektedir (Alloway ve Jackson, 1991). Arıtım sonucu ortaya çıkan çamurlardaki makro ve mikro besin elementlerinin bu atığa faydalı bir gübre; organik maddelerin ise iyi bir toprak ıslah edici özellik vermesi nedeniyle, çoğu otorite bu ürünlerin tarımda kullanımını desteklemekte ve birçok ülkede uygulamaları yaygınlaşmaktadır (D. Strauch,1991, Düring ve Gäth,2002).
Arıtma tesisi sayısının artması, oluşan çamurun nasıl yok edileceği konusunda daha fazla araştırmaya ve uygulamaya yönelik çalışmalar ortaya çıkartmaktadır. Türkiye'de arıtma çamurlarının kullanımı konusunda yeterli bilgi birikiminin ve araştırma bulgusunun olmaması sebebiyle artıma tesisi işletmecilerinin çöp depolama alanlarına çamuru dökmekte ve ayrıca çamuru arazide kullanmak isteyen çiftçilerin bilinçsiz kullanımına sunmaktadır. Büyük bir organik madde kaynağı olan arıtma çamurlarının daha bilinçli kullanımı söz konusu olduğu takdirde çevre kirliliğinin önüne geçilebildiği gibi ülke ekonomisine de katkı sağlanmış olacaktır (Angın ve Yağanoğlu, 2009)
Ülkemizde yıllık evsel arıtma çamuru miktarının 1,38 milyon ton olduğu tahmin edilmektedir. Oluşan arıtma çamurlarının büyük bir kısmı katı atık depolama sahalarında ya da arazide depolanmak sureti ile bertaraf edilmektedir (Aksu, 2008). Son yıllarda bu bertaraf yöntemlerine ek olarak kuru maddesi kısmen daha yüksek (%12-20) ve yanmaya uygun olan arıtma çamurları bertaraf lisansı olan çimento firmalarına gönderilerek yakma sonucu ısı enerjisine çevrilip ilgili tesisin enerji kaynağı olarak kullanılmaktadır. Bu yöntemin arıtma sahibi firmaya hem bertaraf hem navlun masrafı olarak yansıması işletme maliyetlerini arttırmaktadır.
Türkiye'de özellikle de İç Anadolu Bölgesindeki toprakların organik madde bakımından yetersiz olmasından dolayı verimliliği arttırmak amacıyla bu çalışma yapılmaktadır. Çalışma da hem toprak verimliliği hem de arıtma çamuru bertaraftı hedeflenmektedir.

1.Kompost ve kompostlama

Kompost, biyokimyasal olarak ayrışabilir çok çeşitli organik maddelerin organizmalar tarafından stabilize edilmiş mineralize olmuş ürünlerdir. Kompostlama, mikroorganizma adı verilen ve çoğunluğu gözle görülmeyen canlıların, ortamın oksijenini kullanarak çöp-atık içerisindeki organik maddeleri biyokimyasal yollarla ayrıştırmasıdır. Bu olayın gerçekleşebilmesi için çöp-atık kütlesindeki su içeriğinin %45-60 dolaylarında olması gerekmektedir.(Erdin, 1980;Alyanak,1986).

Organik atıkların havalı şartlarda mikrobiyal parçalanmaya (çürümeye) tabi tutularak bitki besin elementleri ihtiva eden, organik madde bakımından zengin, sağlık yönünden zararsız olan, humus görünümünde stabil haldeki son ürününe kompost adı verilir.(Erdin 1981)
Ayrışma sırasında mikroorganizmaların metabolik faaliyetleri sonucu oluşan antibiyotikler de patojen organizmalara öldürücü etki yapmakta ve onları elimine etmektedir. Ayrıca ortam sıcaklığının 70˚C'ye kadar çıkması da pastörizasyon etkisi yapmaktadır. Ancak mikroorganizmaların rahat faaliyet gösterebilmeleri, yeterince besin maddelerine ulaşabilmeleri, oksijen alabilmeleri için homojen bir dağılımın gerçekleştirilmesi gerekmektedir. Bu ya dinamik sistemlerde sürekli karıştırmakla olur, ya da statik sistemlerde olduğu gibi zaman zaman aktarmak ve böylece de karışımı gerçekleştirmekle olur. Dinamik ve statik sistemlerin dışında her ikisinin kombinasyonundan oluşan dinamik / statik sistemler de vardır.

Kompost ürünlerinin nitelendirilmesi konusunda kesin bir standartlaşma yoktur. Her araştırmacı kendine göre bazı kompost tanımları yapmış ve niteleyici koşullar önermiştir

1.1 Kompostlamanın Avantajları

Biyokatılar aşağıdaki nedenlerden dolayı önem kazanmıştır.
•Katı atıklar için uygun depolama alanlarının olmaması,
•Kompost ekonomisi, depolama alanı katı atık sahası çöp boşaltma fiyatlarının artışına bağlı olarak daha faydalı olması
•Eyalet, yerel ve federal kademelerde yeniden kullanımının yararının gittikçe önem kazanması
•Kompost ürünün depolanmasının, taşımasının ve kullanılmasının diğer yöntemlere göre daha kolay olması
•Kompostun toprağı fosfor, azot, potasyum ve organik karbon içeriği bakımından zenginleştirmesi,kompostlaştırmanın avantajları arasında sıralanmaktadır (EPA, 2002)

Bizim çalışmamızda kullanmayı tercih ettiğimiz yöntem açık yığın kompostlaştırma tekniğidir. İlk denemeleri düşük kütlelerde deneyecek olsak da amacımız yüksek miktarda üretilen arıtma çamurunu işlemek olduğu için en uygun ve maliyeti düşük olan yöntem budur. Bu amaçla yapılan araştırmalar incelendiğinde bu tekniği en iyi izleyebileceğimiz deneysel çalışmanın açık varil kompostlaştırma yöntemiyle mümkün olduğu tespit edilmiş ve yöntem uygulanmıştır.

MATERYAL VE METOD

Pakmaya Düzce fabrikasının arıtma tesisinden polielektrolit kullanılarak dekante edilen arıtma çamurundan ve üretim çamurundan alınan numunelerin birbirleriyle farklı oranlarda karıştırılması sonucu 5 farklı karışım elde edilmiştir. Bunlar;

1.karışım;5kg A.Ç+5kg Ü.Ç (K-1)
2.karışım;10kg A.Ç+5kg Ü.Ç (K-2)
3.karışım;15kg A.Ç+5kg Ü.Ç (K-3)
4.karışım;5kg A.Ç+10kg Ü.Ç (K-4)
5.karışım;5kg A.Ç+15kg Ü.Ç (K-5)

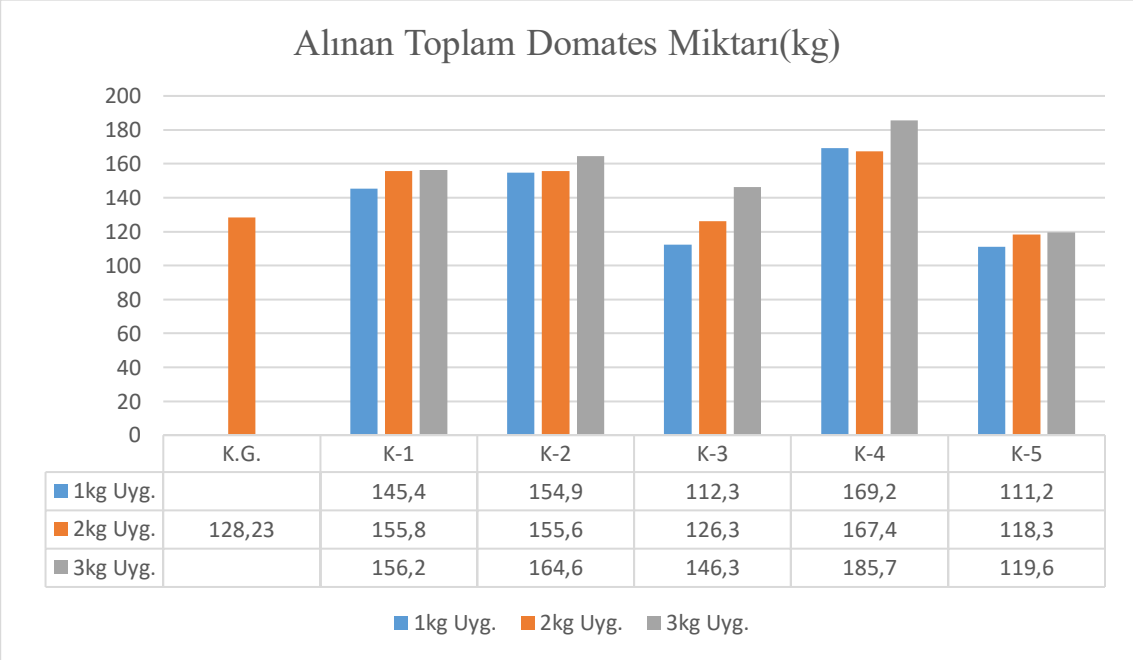
Standart sapmanın hesaplanabilmesi amacıyla aynı karışımlardan 3'er adet hazırlanmış ve toplam da 15 farklı kompost elde edilmiştir. 50lt hacime sahip yarım varillere ilgili karışımlar doldurularak karıştırılmış ve üzerleri ışık geçirgenliğine sahip naylonla kapatılarak kompostlamaya bırakılmıştır. Hergün 10 dk bu naylon kaldırılarak kompostların havalanması sağlanmış ve bu esnada metan gazı ve sıcaklık ölçümü yapılarak kompostlaşmanın optimum koşulları öğrenilmiştir. Her gün metan gazı oluşumu ve sıcaklık ölçümü yapılan numunelerin sıcaklığı bir noktadan sonra sabitlenmiş ve düşmeye başlamıştır. Metan gazı oluşumu da son bulduğunda kompostlamanın sona erdiği kanısına varılıp işleme son verilmiştir.
Fide yetiştirilmesinde 5 farklı kompost türünün 3 denemesi kullanılmıştır. Her deneme artan miktarlarda 1kg/pafta ,2kg/pafta ve 3kg/pafta olarak toprağa uygulanacağı için sera 46 eş paftaya ayrılmıştır. Her paftaya 3 eş boyda domates fidesi ekilmiştir. 46. pafta kompost eklenmeyen kontrol grubuna ait paftadır.
Oluşturulan paftalar arasında kompostların etkilerinin dağılmaması için damlama sulama sistemi tercih edilmiştir. Sulama sistemi tamamlandıktan sonra domates fidelerinin ekim işlemi gerçekleştirilmiştir. Fideler ekildikten sonra ilk boy ölçümleri yapılmış ve kayıt altına alınmıştır. İlk ekim işlemiyle son hasat arasında geçen yaklaşık 4 aylık süreç boyunca toplanan her domates meyvesi tartılıp kaydedilmiştir. Her paftanın verimi kaydedilerek kontrol grubuyla karşılaştırılması yapılmıştır. Ayrıca ilk çiçek mevsiminde klorofil analizi yapılması için yaprak numunesi alınıp toprak ve kompost numuneleriyle birlikte ilgili kuruluşa(Dü-Bit) gönderilmiştir.

Tablo 1. Klorofil analiz sonuçları

ÖRNEK	Paftaya 1 kg	Paftaya 2 kg	Paftaya 3 kg
Kontrol Grubu	0.788 (0.009)		
K-1	0.706 (0.073)	0.557 (0.059)	0.657 (0.053)
K-2	0.989 (0.034)	0.769 (0.14)	0.894 (0.045)
K-3	0.875 (0.057)	0.540 (0.051)	0.479 (0.039)
K-4	0.822 (0.081)	0.810 (0.008)	0.969 (0.19)
K-5	0.822 (0.031)	0.743 (0.038)	0.510 (0.019)

Tablo 2. Toprak ve Kompost içerik analizi sonuçları

Parametre	Toprak	K-1	K-2	K-3	K-4	K-5
Fosfor(g kg^{-1})	0.1	5.2(0.02)	7.9(0.07)	2.3(0.4)	4.9(0.4)	5.9(0.8)
Potasyum(g kg^{-1})	0.9	99.6(5.6)	102.6(8.6)	72.3(4.9)	84.1(9.0)	89.809(1.7)
Kalsiyum(g kg^{-1})	9.7	12.0(0.3)	18.3(0.6)	8.9(0.3)	14.8(3.3)	18.8(4.3)
Magnezyum(g kg^{-1})	1.9	1.3(0.5)	1.8(0.2)	0.4(0.09)	0.7(0.2)	0.2(0.01)
Demir(g kg^{-1})	0.1	5.1(0.1)	5.8(0.09)	6.3(0.2)	2.5(2.2)	5.7(0.3)
Bakır(g kg^{-1})	19.6	1.1(0.2)	1.3(0.1)	0.7(0.09)	1.1(0.2)	0.9(0.1)
Çinko(mg kg^{-1})	19.8	196.5(69.9)	253.5(16.4)	131(77)	216.1(40.2)	231(5.2)
Mangan(mg kg^{-1})	360.1	168.9(43,7)	155.1(3)	30.78(3.9)	341.1(13.4)	181.3(66.3)
Krom(mg kg^{-1})	0.03	51.5(8.8)	82.5(20.9)	23.1(2.3)	90.3(11.1)	111.3(12.1)
pH	7.04	6.92(0.01)	7.02(0.4)	7.44(0.08)	7.25(0.11)	7.3(0.3)
EC(mS cm^{-1})	1548	16.7(1.1)	15.8(0.5)	13.29(0.13)	16.3(0.7)	15.3(0.1)



Şekil 1. Yetiştirilen toplam domates miktarları(kg)

BULGULAR ve TARTIŞMA

Üretilen kompostların artan miktarlarda sera domatesi yetiştiriciliğinde toprak düzenleyici olarak kullanımı sonucu bitki verimlerine ilişkin sonuçlar değerlendirildiğinde; kontrol grubuna kıyasla artan dozlarda kompost kullanımının en yüksek verimlere ulaştığı gözlemlenmiştir. Arıtma çamuru (A.Ç.) içeriğindeki besin elementlerinden ve organik maddeden bitkinin olumlu şekilde yararlandığı tespit edilmiştir. Meyve verimliliği açısından özellikle K-2 ve K-4 ün yüksek doz kullanımı en iyi sonucu vermiştir. Maksimum bitki boyları incelendiğinde ise K-1 ve K-2 nin yüksek dozda kullanımı en iyi sonucu almamızı sağlamıştır.

B. Ravindran'ın arıtma çamurunun farklı kompostlarını kullanarak domates yetiştiriciliğinde kullandığı çalışmasında, kullanılan kompostların pH değerleri incelendiğinde 6.6-6.9 arası ölçüldüğü görülmüştür. Bu çalışmada üretilen kompost pH'ları ise 6.9-7.4 arası değişkenlik göstermiştir. Bu açıdan her iki çalışmada da elde edilen kompostlar toprak pH'ına zarar vermeyecek sınırlar dahilinde olduğu gözlemlenmiştir.

Arıtma çamurlarının toprağa artan miktarlarda uygulamalarıyla domates bitkisinin gelişimi genel olarak pozitif yönde artmıştır. Bu konuda yapılan çalışmalarda (Kadunc ve ark., 1994; Paulraj ve Ramulu, 1994; Tirmizi ve ark., 1996. Gomez ve ark., 1993; Pinemonti ve ark., 1997; Topcuoğlu ve ark. 2001) domates ve diğer test bitkilerine uygulanan arıtma çamurunun bitkide mineral madde içeriğini arttırdığını belirlemişlerdir.

Benzer bir çalışma 2 yıl yinelemeli olarak Topçuoğlu B. tarafından 2002-2003 yıllarında yapılmıştır. Farklı arıtma çamurlarının artan miktarlarda kullanılmasıyla gerçekleştirilen bu çalışmada ilk yıl olumlu yönde gelişme gösteren domates bitkilerinin 2.yıl aynı etkiyi göstermemiş olması arıtma çamurlarının toprakta tuzluluğu arttırıcı etkisine dayandırılmış ve toprakta ağır metal birikiminin insan sağlığı için izin verilen miktarların üzerinde olduğu tespit edilmiştir. Kullanılan arıtma çamurlarının analizleri incelendiğinde yaptığımız çalışma için üretilen kompostlara oranla ağır metal miktarının fazla olduğu anlaşılmaktadır.

Sortino et al. (2014) tarafından domates türünde 30, 145 ve 500 kg/ha olarak uygulanan arıtma çamurunda büyüme ve verimde maksimum değerlere 145 kg/ha dozunda ulaşılmış ve dozlar arasında önemli bir artış bulunduğu saptanmıştır ki bu miktar yaptığımız araştırmada en iyi etkiyi veren dozun %1 ine denk gelmektedir.

Yan-chao et al. (2014), *Lolium perenne* türünü kullandıkları araştırmalarında en iyi bitki büyümesinin en yüksek dozlar olan 150 ton/ha ve 300 ton/ha çamur uygulamasından elde ettiklerini bildirmişlerdir. Araştırmada kullanılan doz miktarı yaptığımız çalışmada uygulanan doz miktarlarıyla örtüşmektedir. Buradan farklı bitki türleri için farklı dozların araştırılması ve en iyi etkiyi elde edecek dozun bulunması gerekliliği ortaya çıkmaktadır.

Hülya Akat, Gülbin Çetinkale Demirkan (2013,2014) tarafından yürütülen çalışmada, Gökova-Akyaka Atık Su Arıtma Tesisi"nden elde edilen arıtma çamurunun artan dozlardaki uygulamalarının *Limonium sinuatum* 'Compindi White' çeşidinde bitki verimi ve çiçek kalitesine ilişkin bulgular değerlendirilmiştir. Kontrol dozuna (D1) kıyasla arıtma çamurunun uygulandığı dozların en yüksek değerlere ulaştığı gözlemlenmiştir. Arıtma çamurunun içeriğindeki besin elementlerinden ve organik maddeden bitkinin olumlu şekilde yararlandığı tespit edilmiştir. Özellikle %50 (D3) ve %75 (D4) dozlarından en yüksek değerler elde edilmiştir. Gerçekleştirdiğimiz çalışmada bitki meyve veriminin %20 ye kadar yükseldiği tespit edilerek artan dozların meyve verimine olumlu etkisi saptanmıştır.

Ümmügülsüm Günay ve Şükrü Dursun,(2018) arıtma çamuru ve zirai atıkların kompostlanarak tarım arazilerinde kullanımı üzerine yaptıkları çalışmada arıtma çamurlarının kompostlanması sonucu araziye uygulanmasının olumlu etkileri tespit edilmiştir. Kompostlama işleminin, tarımsal alanları korumak amacıyla yapılan çevre dostu sürdürülebilir bir proses olduğu, kompostlama sonrası arıtma çamurunun karbon emisyonları azalıp ve araziye uygun hale geldiği bildirilmiştir. Bu sayede farklı organik atıklar tarımda kullanılabilmektedir. Bu kullanım ile toprağın su ve besin maddesi tutma kapasitesi, pH'sı gibi birçok parametre değeri bakımından iyileştirilebilmektir. Ürettiğimiz kompostların pH değerlerinin tarımda uygulanabilirlik sınırları dahilinde olduğu tespit edilmişdir.

Arıtma çamurunun tek başına kompostlaştırılmadan ve %50 oranında toprağa karışımının bitki gelişimini ve çiçek verimini olumsuz etkilediği, bunun arıtma çamurunun yüksek pH ve EC değerlerinden kaynaklanabileceği bildirilmiştir (Tariq ve ark. 2012). Şakayık (Xue ve Huang 2013), Krizantem (Wraga ve Zawadzińska 2007), sardunya (Zawadzinska ve Salachna 2014) ve begonvil (De Lucia ve ark. 2013) bitkilerinde yapılan çalışmalarda, arıtma çamuru uygulaması ile kontrol grubuna göre verim artışları izlenmiş, ancak belirli dozun üzerinde arıtma

çamurunun olumsuz etkileri gözlenmiştir. Araştırmamızda K-2 ve K-4 gruplarının en yüksek dozda uygulanması ile en iyi verim elde edilmiştir.

Singh ve Agrawal (2009) bamya bitkisiyle yaptıkları çalışmada %0, %20 ve %40 oranlarında arıtma çamuru kullanmışlardır. Araştırmacılar yüksek dozda arıtma çamuru uygulamasının klorofil içeriğini olumsuz yönde etkilediğini, düşük dozda ise artırdığını; bamya için %20'lik bir arıtma çamuru uygulamasının iyi bir verim ve bitki gelişimi için, gübrelemeye alternatif bir seçenek olduğunu bildirmişlerdir. Kumar ve Chopra (2014) %20, %40, %60, %80 ve %100 oranında arıtma çamuru karıştırılmış toprakta yetiştirdikleri fasulye bitkilerinde, klorofil içeriğinin %60 seviyesine kadar arttığını, daha yüksek oranlarda ise azaldığı sonucuna ulaşmışlardır. Araştırmacılar bu düşüşün nedeni olarak, yüksek ağır metal içeriği ve tuzluluk artışına bağlamışlardır. Sera domatesleri üzerine yaptığımız araştırmada klorofil miktarının tüm kompost gruplarında paftaya 1 kg uygulanmasında arttığı, 2 ve 3 kg uygulanmasında ise genel olarak azaldığı tespit edilmiştir.

Lorenzo ve arkadaşlarının gül bitkisiyle yaptıkları çalışmada farklı EC değerlerinde (1.2-3 mS cm^{-1}) bitki besin maddelerinin alınımı izlenmiş, 3mScm^{-1} uygulamasında alınan toplam N, P ve K miktarının azaldığı bildirilmiştir. Pakmaya fabrikasının A.Ç ve Ü.Ç'ları kullanılarak elde edilen kompostlar ham melasın menşeine bağlı olarak farklı oranlarda N,P ve K içerebilmektedir. Bu sebeple araştırmamız sonucu elde edilen kompostların 13,3-16,7 arası değişen EC değeri kontrol altına alınabilmesi için Ü.Ç miktarını azaltmanın tuzluluğu düşürmekte etkili olduğu tespit edilmişdir.

SONUÇ

Pakmaya Düzce fabrikasından atık olarak çıkan A.Ç. ve Ü.Ç'larının kompostlaştırılması sonucu elde edilen toprak düzenleyicileri sera domates yetiştiriciliğinde kullanılmış ve domates veriminin %8-20 arası artması sağlanmıştır. Ekonomisinin büyük kısmı tarımcılığa dayalı olan ülkemizin düşük kaliteli ve nadasa bırakılmadan kullanılan tarım arazilerinin içeriğini zenginleştirmek amacıyla toprak düzenleyicilere olan ihtiyacını kompost gübrelerle gidermek maliyeti azaltacaktır.

Bertaraf ve navlun maliyetlerinin yüksek olmasından dolayı ülke ekonomisine girdi sağlamak amacıyla gerçekleştirilen bu çalışmanın önemli kazanımlarından biriside %15-20 kuru maddeye sahip olan 2 çeşit çamurun kompostlaştırma sonucu %55-60 kuru maddeye çıkarılması ile ilgili maliyetleri 2/3 oranında azaltacağının tespit edilmesidir. Bu tür atık çamur miktarlarının sanayinin gelişimine ve nüfus artışı hızına paralel olarak artacağı görülmektedir. Bu duruma hazırlıklı olmak adına kompostlaştırma, paketleme ve satışının sağlanacağı birimlerden oluşan entegre geri dönüşüm tesislerinin desteklenmesi, hatta zorunlu hale getirilmesi faydalı olacaktır.

Her ne kadar çalışmamızda elde edilen kompostlar ağır metal yönünden sınır değerlerin çok altında bulunsa bile sürekli ya da aşırı kullanımın toksik etki yaratarak verimi düşürme riski derinlemesine araştırılmalıdır. Kompost kullanılacak olan arazinin toprak analizi yapıldıktan sonra uygun kompost seçimi yapılarak ağır metal yükünün kontrol altında tutulması tarım sektörünün geleceğini uzun vadede olumlu etkileyecektir.

TEŞEKKÜR

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➢ *ORAL PRESENTATION*

Sportswear for aromatherapy with encapsulated peppermint oil

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Abstract

Herbal products are used in many industrial areas due to their antimicrobial properties, therapeutic effect and pleasant fragrances. Aromatherapy is using the fragrant essential oils obtained from different parts of the plants in the field of natural / herbal therapy. These oils can be encapsulated for preserving their expected features and extending their lifetime by limiting the release rate and applied to the textile materials avoiding oil spots and colour change. In this study, peppermint oil, was obtained from *Mentha piperita* plants by water vapour distillation method, which is a fragrant herb that contains large amounts of menthol and menthone, cultivated in the Aegean region. The peppermint oil has been used in many research because their potential therapeutic property besides its use in the field of food and cosmetics. Composition of the peppermint oil was evaluated by gas chromatography mass spectroscopy (GC-MS) system. Peppermint oil was encapsulated by employing spray-drying method with ethyl cellulose shell. Morphological features, chemical structures, and thermal properties of the microparticles were examined by scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR) spectrometer, and thermogravimetric analysis and differential thermal analysis system (DT / TGA) respectively. The optimum microparticle formulation was transferred to the six different fabrics used in sports and leisure clothing according to the exhaustion method. In order to determine the presence of capsules on fabrics and the effect of sequential washing and rubbing, SEM images of the fabrics was taken. To analyse the change in colour of fabrics due to the finishing applications was measured by a spectrophotometer.

Keywords: Ethyl Cellulose, Microencapsulation, Peppermint Oil, Spray Drying, Textile Finishing

INTRODUCTION

The importance of functional processes that add value in the textile sector, create a competitive environment and difference, increase the market share, and add different features to the product is increasing day by day. As a result of the sports and leisure clothing market researches, it has been observed that the concepts that offer aesthetics and functionality together have recently come to the fore. The prominent ones among these design concepts were identified as athletic luxury and conscious clarity. In the concept of athletic luxury; functional designs and clothings that aim to improve health and fitness come to the fore while in the concept of conscious clarity; products using clean lines that increase minimalism, technically strong functional features with simple aesthetic appearances, and fabrics with healing and well-being additives draw attention. One of the value-creating functions that can be used for these concepts is aromatherapy. Aromatherapy can be defined as the use of fragrant essential oils obtained by various methods from different parts of the plants such as shell, leaf, flower, fruit, seed, stem and root in the field of natural / herbal therapy.

Microcapsules are small droplets that protect functional materials such as fragrances, antioxidant and pharmaceutical from external factors with a thin shell film. Thus, these perishable materials can preserve their effects for a longer period (Ghosh, 2006). Peppermint oil (*Mentha piperita L.)* is one of the most produced and consumed essential oil and it is cultivated world-wide for its pleasant smell, flavor, and medicinal properties. The

oil of this fragrant plant constitutes carvacrol, menthol, carvone, methyl acetate, limonene and menthone active agents. The many medicinal features such as analgesic, anti-inflammatory, antimicrobial/fungicidal, digestive, vasoconstrictor properties of the active ingredients have been investigated in many scientific studies. Since it is highly volatile, its useful life is limited (İşcan, 2002; Herro and Jacob, 2010; Ali et al., 2015; Danila et al., 2019). The volatile oils can be encapsulated for preserving their expected features and extending their lifetime by limiting the release rate and applied to the textile materials avoiding oil spots and colour change (Türkoğlu et al., 2020).

In this study, peppermint oil was obtained from *Mentha piperita* plant which is cultivated from Aegean region. Essential oil was obtained from the plant and characterized by the active agent content (GC-MS). The obtained peppermint oil was encapsulated using different polymer shells (gelatin and ethyl cellulose) according to spray drying technique. The morphological features of the microparticles were examined by scanning electron microscopy (SEM). The chemical structures of microparticles were investigated by Fourier transform infrared (FT-IR) spectrometer, and their thermal properties was discussed using thermogravimetric analysis and differential thermal analysis system (DT / TGA). The selected optimum microparticle formulation was transferred to the fabrics used in sports and leisure clothing according to the exhaustion method. In order to determine the presence of capsules on fabrics and the effect of washing and rubbing, SEM images of fabrics were examined. The effect of capsule application on mechanical properties were determined with a burst strength method. A spectrophotometer is employed to determine the change in colour after microcapsule application.

MATERIALS AND METHODS

Materials

To prepare aromatherapic textiles, microparticles transferred to six different fabrics used in sports and leisure clothing. The knitted fabrics are named after their color and their fiber compositions and important fabric parameters are given Table 1.

Table 1. Properties of the Fabrics

Fabric	Fabric Name	Fiber Composition	Yarn Count (Ne)	Mass (g/m2)
	Ecru	50% PET 50% MO	30/1	140
	Orange	95% CV 5% EL	30/1	250
	Pink	65% PET 35% CV	28/1	140
	Red	95% PET 5% EL	30/1	140
	Green	50% CO 50% PET	30/1	150
	Blue	95% CO 5% EL	30/1	140

Gelatin and ethyl cellulose are used as shell material. Gelatin was donated by Sel Gel firm. Ethyl cellulose were obtained from Sigma-Aldrich. Peppermint oil used as core material was extracted by Doğal Destek A.Ş. (Tabia) via water vapor distillation from *Mentha piperita L* plant. TanaPUR One based on nano polyurethane chosen as binder was donated by Tanatex company.

Extraction of Peppermint and Determination of the Composition

For the peppermint essential oil, plants were harvested from South and Central Aegean. The essential oils chosen for use within the scope of the project are widely used in different fields due to their therapeutic or aromatic effects, they have no reported toxic effects on their topical use and are considered GRAS (Generally Recognized as Safe) (Başer, 2019; Beyaz 2014). For the extraction of the oil water vapour distillation method is employed. Production parameters were optimized by Doğal Destek, and the composition of the essential oil was obtained by AGILLENT brand gas chromatography mass spectroscopy (GC-MS).

Microparticle Production and Characterization with Peppermint Oil

The solutions prepared at the different polymer to oil ratio with addition of 2% Tween 80 surfactant, given in Table 2 were sprayed from the 0.5 mm nozzle into the cabinet with a pump speed of 2.5-5 mL/min. The compressor and air circulation speed were operated at maximum. The microencapsulation was performed in a Lab Plant brand SD-Basic spray drying device with a main cabinet size of 380 mm x 110 mm. Inlet and outlet temperature are set 120˚C and 89-86 ˚C, respectively.

Table 2. Parameters of Microparticle Formulations

	Polymer	Polymer Concentration (%)	Peppermint Oil (%)	Yield (%)
M1	Gelatin	3	5	38,5
M2			7,5	29,9
M3	Ethyl Cellulose	3	3	46,4
M4			6	40,3
M5			9	31,9

The morphological features and the particle sizes of the microparticles were determined using SEM micrographs. Samples were plated with 8 nm thick gold to ensure electrical conductivity. The production yield of the microparticles was calculated according to the following equation.

$$(\%)Yield = \frac{Actual\ capsule\ amount\ (g)}{Theoritical\ capsule\ amount\ (g)} \times 100 \quad (1)$$

Chemical structure of microparticles were examined in the 650-4000 cm^{-1} wavelength range, using FT-IR spectrophotometer. The thermal properties of the materials were studies using a DT-TGA under nitrogen atmosphere. Samples weighing 5-10 mg were compressed into the sample holder to be airtight and the studies were carried out at a scanning speed of 5°C/min, in the range of 0-250°C with 0.001°C sensitivity.

Finishing and Characterization of the Textile Surfaces with Peppermint Oil

The capsule application onto knitted fabrics were realized according to exhaustion method at 25°C for 15min. Laboratory stenter was used for combined drying and fixation process for 7 min at 120°C. To determine the effect of washing on the presence of microparticles, the fabrics were washed in 10 sequential washings for 30 minutes at 40°C according to the A1S program of the TS EN ISO 105-C06. Also, dry and wet rubbing tests were applied to fabrics according to TS EN ISO 105-X12. SEM was employed to evaluate the microparticles on the fabrics after washing and rubbing. The color change of microparticle applied fabrics was measured with a spectrophotometer. The color was measured with spectrophotometer before and after microparticle finishing.

RESULTS and DISCUSSION

When the composition of the essential oil obtained from the *Mentha piperita L* plant was examined (Figure 1a), it was determined that there was 26% methone, 23% menthol, and 18% methyl acetate, and the essential oil also contained compounds such as limonene, eucalyptol, pulegone.

SEM micrographs of gelatin and ethyl cellulose microparticles comprising peppermint oil are given in Figure 2. The effects of polymer type and peppermint oil concentration on the morphological properties of microparticles were investigated. When microparticle formulations (M1 and M2) using gelatin as the shell material were examined, it was determined that they were morphologically non-spherical and the microparticles were agglomerated. It has been determined that the structure is more spherical in formulations prepared using ethyl cellulose (M3, M4 and M5). It was seen that particles with a smooth surface and close to the spherical shape were obtained in M4 studies compared to M3 and M5 formulations. Moreover, the solubility of gelatin in water complicates the process for the textile application.

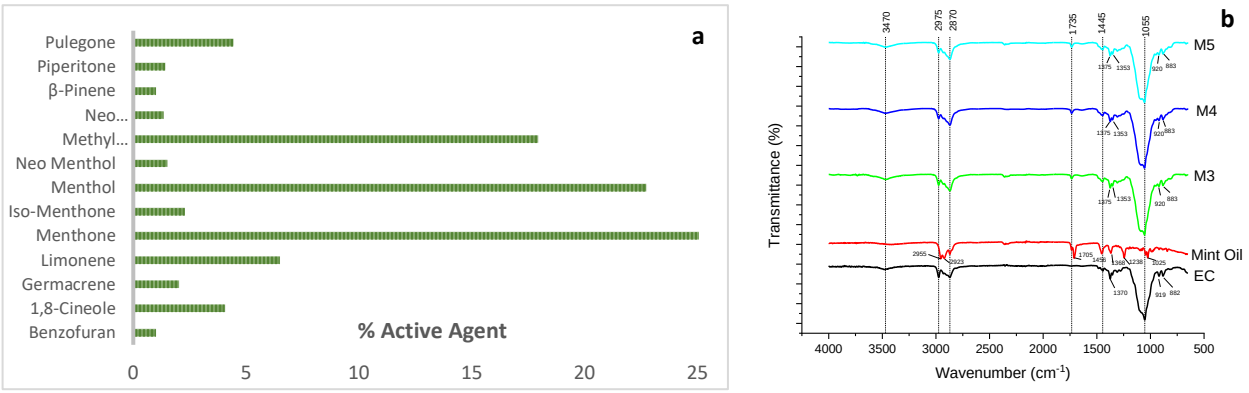

Figure 4. a) Compositions of Active Agents in Peppermint Oil b) FT-IR spectra of Microparticles

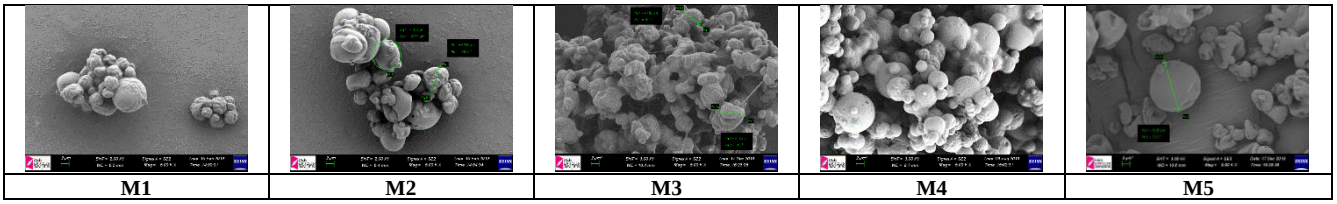

Figure 2. SEM Micrographs of Peppermint Oil Microparticles

FT-IR spectra of the microparticles containing ethyl cellulose, peppermint oil and peppermint oil are shown in Figure 1b. Characteristic –C - O - C– stretch vibration (1053 cm^{-1}) and C - H stretch bands (2865 and 2975 cm^{-1}) were observed in the infrared spectra of ethyl cellulose. C - H bending was seen at 1380 cm^{-1} (Türkoğlu et al., 2017). When the main peaks of peppermint oil are examined, the wide peak of O-H groups is seen in the wavelength range of 3600-3200 cm^{-1}. Peaks belonging to CH_3 groups are double peaks seen at 2955 and 2923 cm^{-1}, peaks seen at 1456 cm^{-1} and 1368 cm^{-1}. The peak belonging to the mentone group was determined at 1705 cm^{-1}. Peaks of both ethyl cellulose and peppermint oil were detected in the FTIR spectra of the microparticles. In the spectra of microparticles, it was determined that the CH_3 peaks of the essential oil shifted to the 2975 and 2870 cm^{-1} band and the menthon peak to the 1735 cm^{-1} band. It was concluded that the presence of peppermint essential oil in the polymeric matrix was ensured for all formulations.

In Figure 3, TGA graph of ethyl cellulose is examined and a weight loss caused by the moisture contained in the material at 100 ° C was observed. Pyrolysis starts around 225 ° C temperature, its speed increases at 300 ° C. It is known that the degradation temperature of ethyl cellulose is around 440 ° C (Türkoğlu, 2013). The TGA thermograph of peppermint oil revealed that degradation took place around 160°C. DSC thermographs of peppermint oil show the phase change that occurs around 50°C. Microparticles containing peppermint oil display thermal behaviors similar to ethyl cellulose, and the mass loss in microparticles does not exceed 5% at 180°C, where the mass loss of peppermint oil reaches up to 99%.

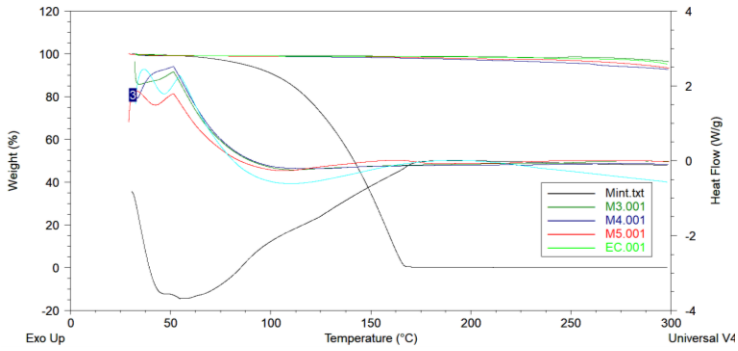

Figure 3. DSC and TGA Thermographs of Peppermint Oil Microparticles

The M4 formulation were selected as the optimum formulation for the textile according to the morphology, chemical and thermal properties of the microparticles. The SEM micrographs of the blue fabrics before and after microparticle finishing according to the exhaustion method are given in Figure 4. The microparticles were detected on the fabric and it was concluded that the microparticles were successfully transferred to the fabric. After 1 and 10 sequential washings, the microparticles existed on the fabric surface and in the spaces between the fibers.

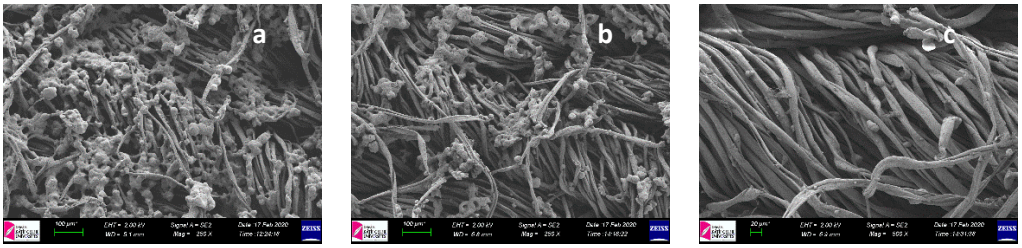

Figure 4. SEM Micrographs of Blue Fabrics Comprising Peppermint Oil Microparticles
a) No Wash b) After 1 Wash c) After 10 Washes

Table 3. Color Measurements of Blue Fabrics Before and After Microparticle Treatment

Fabric	**Color Measurement Results**							
	Chromatic Point	**K/S**	**L***	**a***	**b***	**C***	**H***	**ΔE**
Untreated	600 nm	2.0122	56.147	1.898	-32.104	32.160	273.383	2.012
Treated	600 nm	1.8970	56.646	1.268	-30.260	30.286	272.400	

The color change in blue fabrics after microparticle treatment is shown in Table 3. When these values are examined, no change was observed in the chromatic point of the fabrics. It was determined that the microparticle applied fabric color was slightly darker, greenish and yellowish. It was thought that this change in color might be due to the color of microparticles containing essential oil. Results of the other fabric types will be presented in the conference.

CONCLUSION

In this study, it was aimed to develop fragrant and relaxing products that would be an alternative to the clothes used in sports and leisure wear. For this purpose, peppermint oil, whose pharmaceutical and aromatherapeutic effects have been studied extensively, was chosen. Peppermint plants were harvested, and the oil was obtained by water vapor distillation method and its active agent content was determined with the help of GC-MS. Peppermint oil has been encapsulated by spray drying method, which is applicable in industrial scale, in order to prolong the desired effect and prevent stains that may occur on the fabric during textile finishing processes.

In the production of capsules, gelatin, and ethyl cellulose, which are proven safe to contact with the skin, was used as shell polymers. Ethyl cellulose capsules were chosen for their superior morphological properties and being more suitable for textile application. It is considered that with the use of peppermint oil in sportswear, both aromatherapy effects will be provided and unpleasant odors that may occur during exercise will be controlled. The developed product has a high commercialization capability. It is predicted that a new market can be created with this innovation to be brought to the functional textile market.

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➢ *ORAL PRESENTATION*

Oxalis triangularis'in su ve metanol ekstrelerinin DNA koruyucu, antioksidan ve antibakteriyel aktivitelerinin araştırılması

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Özet
Dünya'da birçok hastalığın tedavisinde bitkilerden faydalanılmaktadır. Ülkemizin farklı iklim ve ekolojik koşullara sahip olması, florasının çok sayıda bitki türü ve çeşitliliği içermesi nedeniyle ülkemizde de alternatif tedavi olarak tıbbi bitkilerden yararlanılmaktadır. Yapılan bu çalışmada *Oxalis triangularis* bitkisinin yaprak özütlerinin total oksidan/antioksidan seviyesi (TOS/TAS), *Stenotrophomonas maltophilia* bakterisine karşı antimikrobiyal etkileri ve DNA koruyucu potansiyeli çalışılmıştır. *Oxalis triangularis*'in yaprakları gölgede kurutularak su ve metanol çözücüleri yardımıyla özütler elde edilmiştir. Elde edilen yaprak özütleri TAS ve TOS Assay Kit (Rel Assay Diagnostic) hazır kitleri kullanılarak total oksidan/antioksidan seviyeleri ölçüldü. Antimikrobiyal aktivite disk difüzyon yöntemi ile çalışılmıştır. DNA koruyucu aktivite pBR322 plazmid DNA'sı ile H_2O_2 ve UV kullanılarak çalışılmıştır. Çalışma sonucunda *Oxalis triangularis* yapraklarında TAS değerinin en yüksek metanol özütlerinde olduğu görülmüştür. TOS değerlerinin ise en yüksek su özütünde olduğu tespit edilmiştir. *Stenotrophomonas maltophilia* bakterisi üzerine antimikrobiyal etkisinin olmadığı gözlemlendi. DNA koruyucu aktivite çalışmasında ise su ve metanol özütünün koruyucu bir etkiye sahip olduğu belirlenmiştir.
Anahtar Kelimeler: *Oxalis triangularis,* total oksidan/antioksidan seviye, *Stenotrophomonas maltophilia,* UV, antimikrobiyal

Investigation of DNA protective, antioxidant and antibacterial activities of water and methanol extracts of *Oxalis triangularis*

Abstract
Plants are used in the treatment of many diseases in the world. Since our country has different climatic and ecological conditions and its flora includes many plant species and diversity, medicinal plants are used as an alternative treatment in our country. In this study, the total oxidant / antioxidant level (TOS / TAS) of leaf extracts of *Oxalis triangularis* plant, antimicrobial effects, and DNA protective potential against *Stenotrophomonas maltophilia* bacteria were studied. The leaves of *Oxalis triangularis* were dried in the shade and extracts were obtained with the help of water and methanol solvents. Total oxidant / antioxidant levels were measured using TAS and TOS assay kit (Rel Assay Diagnostic) ready-made leaf extracts. Antimicrobial activity was studied by the disk diffusion method. DNA protective activity was studied with pBR322 plasmid DNA using H_2O_2 and UV. As a result of the study, it was observed that the TAS values in *Oxalis triangularis* leaves were the highest in methanol extracts, while the TOS values were found to be the highest in the water extract. It was observed that there was no antimicrobial effect on the bacterium *Stenotrophomonas maltophilia*. In the DNA protective activity study, it was determined that water and methanol extract had a protective effect.
Keywords: *Oxalis triangularis*, total oxidant/antioxidant level, *Stenotrophomonas maltophilia*, UV, antimicrobial

GİRİŞ
Tıbbi aromatik bitkiler yüzyıllardır halk arasında tedavi amacıyla kullanılmaktadır. Son yıllarda ise modern tıp alanında ve gıda takviyesi olarak kullanımları oldukça yaygınlaşmıştır. Dünya pazarında tıbbi aromatik bitkilere ilgi her geçen gün artmaktadır. Tıbbi ve aromatik bitkiler; hastalıkların önlenmesi ve var olan hastalıkların

iyileştirilmesi için alternatif tedavi yöntemi olarak kullanıma sahiptirler. Çağımızda tıbbi ve aromatik bitkilerin kullanım alanlarının artmasıyla beraber, bitki ve bitkisel ürünlere olan talep artmıştır. Bu durum hem bitkilerin kullanımını arttırmış hem de bitkilerle ilgili olan çalışmaların artmasına neden olmuştur (Bayram vd., 2010).
Antibiyotiklerin kullanım alanlarının sınırlı olması, zamanla etkilerine karşı mikroorganizmaların direnç oluşturması ve bilinçsiz kullanım sonucu yan etkilerinin fazlalığı, tıbbi tedavide bitki özütlerinin kullanımını arttırmıştır. (Sarker ve Nahar, 2007).
Bitkiler, genelde gıda, kozmetik ve farmasötik endüstrisinde kullanılan antimikrobiyal ve antioksidan etkiye sahip karotenoid, terpenoid, flavonoid, polifenol, alkaloid, tanen, saponin, pigment, enzim ve mineral gibi oldukça fazla biyolojik aktif bölümler içerir (Madhuri ve Pandey, 2009). Bu nedenle, gelişmekte olan ülkelerde, dünya nüfusunun yaklaşık % 60-80' i yaygın ve ciddi hastalıkların % 80 den fazlasının tedavisi için bitkisel ilaçların kullanımı son yıllarda yaygınlaşmıştır (Saab vd., 2012).
Gerçekleştirilen bu çalışmada ülkemiz florasında mevcut *Oxalis triangularis* bitkisinde biyolojik aktivite açısından hiçbir çalışma yapılmadığından bu bitki üzerine bakterilere karşı antimikrobiyal etkilerinin belirlenmesi, antioksidan ve DNA koruyucu aktivitelerinin araştırılması amaçlanmaktadır. Bilimsel çalışmalarla biyolojik aktivitelerinin belirlenmesi doğrultusunda ülkemizde bulunan bu tür çalışmalara bir katkıda bulunmak, daha sonra çalışılacak farmakolojik çalışmalara ışık tutmak ve bitkilerin halk arasında bilinçli bir şekilde kullanılmasını sağlamak bu çalışmadaki diğer amaçları oluşturmaktadır.

MATERYAL VE METOD

Oxalis triangularis bitkisinin özütleme işlemi için materyal olarak metanol, distile su, soxhlet, evaporatör kullanılmıştır. Gaziantep Üniversitesi kampüsü Biyoloji Bölümünde kültür bitkisi olarak bulunan *Oxalis triangularis*'in yaprakları toplanıp kullanılmadan önce distile suyla yıkanmış ve kurutma kağıdı üzerine serilerek gölgede kurutulmuştur. Kurutulan *Oxalis triangularis* yaprakları ekstraksiyon için öğütücü ile parçalanmıştır. Ekstraksiyon için çözücü ile beraber basınç uygulaması yapılmıştır. Ekstraksiyonda su ve metanol çözücü olarak kullanılmıştır. Ekstraksiyon metodunda kurutulmuş olan *Oxalis triangularis* 5 gr olarak tartılmış Soxthern cihazının beherleri içine yerleştirilmiştir. Su için 5.1 bar basınçta, 2 saat boyunca 5 grama 150 ml distile su konularak 130 ºC' de işleme tabi tutulmuştur. Metanol içinde aynı miktarlar kullanılarak su yerine 150 ml metanol eklenerek özütleme uygun sıcaklık ve süre belirlenerek gerçekleştirilmiştir. Elde edilen özütler Rotary Evaporator'de 90^0C'de evaporasyon (buharlaştırma) yöntemiyle metanol ve su uzaklaştırılmıştır. Hazırlanan özütler $+4^0$C'de muhafaza edilmiştir. (Gülaçtı vd., 2007).

Çalışmamızda *Oxalis triangularis* özütünün toplam oksidan (TOS), toplam antioksidan (TAS) kapasitesinin belirlenmesi, potansiyel antioksidan potansiyelinin ortaya konulması planlanmıştır. *Oxalis triangularis* bitkisinin yaprak özütlerinin antioksidan kapasitesini belirlemek için ticari olarak satılan kitler kullanılmıştır. TAS için; TAS assay kit (Rel Assay Diagnostics, Korea), *Oxalis triangularis*'in yaprak özütleri, spektrofotometre ve eliza plate kullanılmıştır. Kit içerisinde Reagent 1: (Buffer), Reagent 2 (Renkli ABTS Radikal Çözeltisi), Standart 1 (0,0mmol Trolox Equiv./L), Standart 2 (1.00mmol Trolox Equiv./L) bulunmaktadır. Reagent 1'den 200 µl alınıp kuyucuğa eklenip üzerine 12 µl % 5'lik su özütünden hazırlamış olduğumuz konsantrasyonlar konulur. Başlangıç absorbansı 660 nm spektrofotometrik olarak ölçülüp üzerine 30 µl Reagent 2 ilave edilip, 5 dakika 37^0C' de inkübasyona bırakılıp İnkübasyon sonrası 660 nm'de ikinci absorbans ölçümü yapılır. Aynı işlem metonol özütü için de yapılıp ölçümler tamamlanır.

$$\text{TAS (mmol/L)}=[\Delta\text{Abs Std1}-\Delta\text{Abs Örnek}]/\ [\Delta\text{Abs Std1}-\Delta\text{Abs Örnek}]x20 \quad (1)$$

Hesaplamada kullanılacak veriler:

ΔAbs Std1:(Std1'in ikinciAbs.- Std1'in birinci Abs.)

ΔAbs Std2:(Std2'in ikinciAbs.- Std2'in birinci Abs.)

ΔÖrnekAbs:(Örneğin ikinci Abs.-Örneğin birinci Abs.)

Toplam oksidan kapasitesini belirlemek için TOS assay kit, *Oxalis triangularis* bitkisinin yaprak özütleri, Spektrofotometre (Vantaa, Finland),Eliza plate kullanılmıştır. Reagent 1 (Assay Buffer) 1x50ml, Reagent 2 (Prokromojen Solusyon) 1x10ml,Standart 1 (Blank Solusyon: deiyonize su) ,Standart 2 solusyon 1x5ml bulunmaktadır. Reagent 1' den 200 µl eklenerek üzerine %5'lik su özütümüzden 30 µl eklenir. 530 nm' de ilk

spekrofotometrik okuma gerçekleştirilir. Daha sonra 10µl Reagent 2 eklenerek oda sıcaklığında 10 dakika bekletilip 530 nm' de ikinci spektrofotometrik okuma yapılır. Aynı işlemi metanol özütü için de yapılıp ölçümler tamamlanır.

TOS (µmol/L) = (Abs. Örnek / AbsStandart2) x 20 (Standart2 Değeri) (2)

Oxalis triangularis yapraklarının DNA Koruyucu Aktivitesinin Belirlenmesi için kullanılan materyaller; pBR322 DNA(vivantis), H_2O_2, % 1,25'lik agoroz jel, distile su (dH_2O), UV translüminatör (DNR-IS), jel dökümantasyon sistemi (DNR-IS, MiniBIS Pro)

Oxalis triangularis özütlerinin DNA'yı UV ve oksidatif stres etkilerinden koruma potansiyellerinin belirlenmesi için pBR322 plazmid DNA'si (vivantis) kullanılmıştır. Plazmid DNA'sı, özütlerin varlığında H2O2 ve UV uygulanarak hasara uğratıldı. Daha sonra % 1.5'lik agaroz jel üzerinde yürütülme işlemi yapılarak jel görüntüleme cihazında görüntüleme yapılmıştır. Farklı çözücüler ile çıkarılan özütler 50mg kuru ağırlıkları ile tartılarak üzerlerine 1000µl distile su eklendi ve özütlerin çözünmesi sağlandı. %1,5 oranında hazırlanan agaroz jele örnek yüklemeleri yapılarak 60dk ve 100 voltta DNA örnekleri yürütülmüştür. Stok olarak hazırlanan pBR322 1:3 oranında distile su ile seyreltilmiştir. Yükleme yapım aşamasında 5µl hazırlanan özüt, 5µl yükleme tamponu eklenerek jelde var olan kuyucuklara yüklenmiştir. Bu deneyde bromfenol mavisi ve sükroz içeren yükleme tamponu kullanılmıştır.

K1: Kontrol: Plazmit DNA (3µl) + dH2O (6µl)

K2: Kontrol: Plazmit DNA (3µl) + dH2O (6µl)+ UV (5dk) + H2O2 (1µl)

S: Plazmit DNA(3µl)+*Oxalis triangularis yaprağı* Su özütü 5µl + UV(5dk)+ H2O2 (1µl)

M: Plazmit DNA(3µl)+*Oxalis triangularis yaprağı* Metanol özütü 5µl + UV(5dk)+ H2O2 (1µl)

Stenotrophomonas maltophilia Bakterisine Karşı Antibakteriyel Etkinlik Testinde kullanılan materyaller ise; Mikropipet, Mueller-Hinton Agar,Bakteri suşu *S.maltophilia*, İnkübatör, Vorteks, McFarland Cihazı, Petri kapları, Eküvyon çubuk, Blank disk (6mm),Otoklav, Hassas Terazi.

Oxalis triangularis özütlerinin, antimikrobiyal aktivitelerinin değerlendirilmesi için *Stenotrophomonas maltophilia* bakterisi kullanılmıştır. Antimikrobiyal belirlenebilmesi için Disk difüzyon yöntemi kullanılmıştır. % 5 *Oxalis triangularis* su ve metanol özütleri 6mm çapındaki steril blank (boş) disklere 30µl olmak üzere emdirilmiştir. Daha sonra önceden besiyerlerinde üreme kontrolleri yapılan *S. maltophilia* bakteri suşundan bir miktar alınarak ayrı ayrı SF içerisinde 0.5 Baryum Sülfat Bulanıklık Standartı baz alınarak Mcfarland cihazında belirlemeler yapılmıştır. Süspanse hale getirilerek vortekslenerek steril eküvyon yardımı ile alınıp örnek Mueller-Hinton agar yüzeyine 100 µl inoküle edilip disk difüzyon için kullanılmak amacıyla ekimi gerçekleştirilmiştir. *Oxalis triangularis* su ve metanol emdirilmiş diskler steril bir pens ile agar yüzeyine yerleştirilmiştir. Besiyerleri 24 saat süreyle 37°C' de inkübe edilmiştir. İnkübasyonun tamamlanmasının ardından meydana gelen zonların çapları cetvel ile ölçülüp kaydedilmiştir.

BULGULAR VE TARTIŞMA

Oxalis triangularis yapraklarının su ve metanol özütlerinin, ticari kit ile Total antioksidan ve Total oksidan seviyelerine bakılmıştır.

TABLO 1. TAS Referans Değerleri

TAS REFERANS DEĞERLERİ (mm Trolox Equiv/L)		
>2.0		Çok İyi
1,45	2,0	Normal
1,2	1,45	Normal Kabul Edilebilir
1	1,1	Düşük Antioksidan Seviyesi
<1,20		Çok Düşük Antioksidan Seviyesi

Tablo 2. TOS Refarans Değerleri

TOS REFERANS DEĞERLERİ (µmol H_2O_2 Equiv/L)		
<5.0		Çok İyi
8	5	Normal
12	8	Yüksek Oksidan Seviyesi
>12		Çok Yüksek Oksidan Seviyesi

TABLO 3. Su ile ekstrakte edilen oxalis örneklerinin TAS değerleri(mmol trolox equiv/L)

Örnekler	**Oxalis Yaprak**
TAS Abs. 2	0,150
TAS Abs. 1	0,147
Toplam Örnek Abs.	0,003
TAS Değeri	4,307

TABLO 4. Metanol ile ekstrakte edilen oxalis örneklerinin TAS değerleri(mmol trolox equiv/L)

Örnekler	**Oxalis Yaprak**
TAS Abs. 2	0,221
TAS Abs. 1	0,232
Toplam Örnek Abs.	-0,011
TAS Değeri	4,359

TABLO 5. Su ile ekstrakte edilen oxalis örneklerinin TOS değerleri(mmol trolox equiv/L)

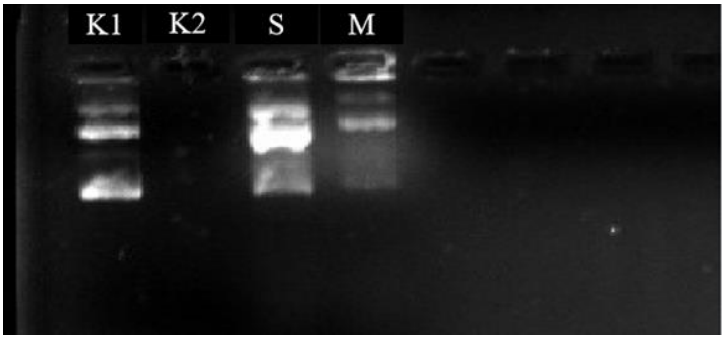
Örnekler	**Oxalis Yaprak**
TOS Abs. 2	2,190
TOS Abs. 1	2,146
Toplam Örnek Abs.	0,044
TOS Değeri	4,362

TABLO 6. Metanol ile ekstrakte edilen oxalis örneklerinin TOS değerleri(mmol trolox equiv/L)

Örnekler	**Oxalis Yaprak**
TOS Abs. 2	2,270
TOS Abs. 1	2,230
Toplam Örnek Abs.	0,040
TOS Değeri	3,966

DNA koruyucu aktivite çalışması ile *Oxalis triangularis* yapraklarının su ve metanol özütünün DNA koruyucu etkisine bakılmıştır. Bu metod ile DNA'da hasara neden olan UV ışınları ve H_2O_2 varlığında oluşturduğumuz ekstraktların DNA hasarını engelleyebilme potansiyelinin olup olmadığının belirlenmesi amaçlanmıştır. Yapılan bu çalışma ile elde edilen jel görüntü kaydı Şekil de verilmiştir.

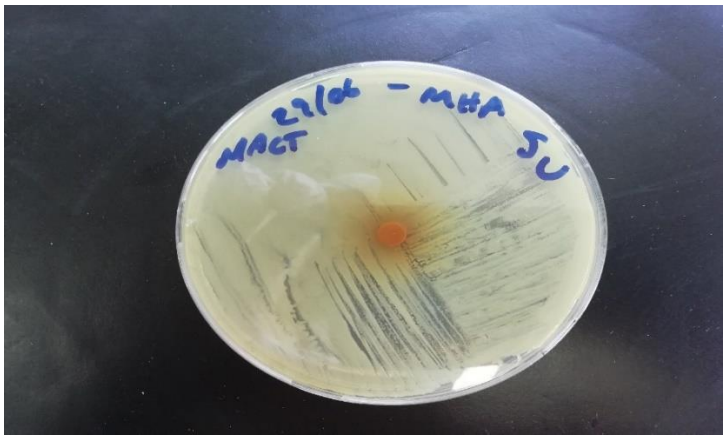
Sonuçlara göre su ve metanol özütünün DNA koruyucu etkisi vardır.



K1: Kontrol: Plazmit DNA (3µl) + dH2O (6µl), **K2:** Kontrol: Plazmit DNA (3µl) + dH2O (6µl)+ UV (5dk) + H2O2 (1µl), **S:** Plazmit DNA(3µl)+*Oxalis triangularis yaprağı* Su özütü 5µl + UV(5dk)+ H2O2 (1µl),
M: Plazmit DNA(3µl)+*Oxalis triangularis yaprağı* Metanol özütü 5µl + UV(5dk)+ H2O2 (1µl)

Şekil 1. *Oxalis triangularis* yaprak ekstraktlarının jel görüntüsü

Oxalis triangularis yapraklarının su ve metanol özütlerinin *Stenotrophomonas maltophilia* bakterisi üzerine antibakteriyel etkisinin olup olmadığı disk difüzyon yöntemi ile çalışılmıştır. Yapılan bu çalışmalar sonucunda *oxalis triangularis yapraklarının* su ve metanol özütlerinin *S. maltophilia* bakterisi üzerine antibakteriyel bir etkisinin olmadığı gözlemlenmiştir.



Şekil 2. *Oxalis triangularis* su özütünün disk difüzyon sonucu

Şekil 3. *Oxalis triangularis* metanol özütünün disk difüzyon sonucu

SONUÇ

Çalışma kapsamında *Oxalis triangularis* yaprak özütlerinin oksidan ve antioksidan kapasiteleri ortaya çıkarılmıştır. Çıkan oksidan ve antioksidan seviyeleri kıyaslanarak çalışma kapsamındaki özütlerin en düşük oksidan ve en yüksek antioksidan seviyelerine sahip olanları kayıt altına alınmıştır. Yapılan çalışmada *Oxalis triangularis* yapraklarının su ve metanol özütlerinin antioksidan yönünden zengin olduğu belirlenmiştir. Oksidan yönünden ise su ve metanolün çok iyi oksidan seviyesinde olduğu belirlenmiştir. Serbest radikallerin süpürülmesinde *Oxalis triangularis* yapraklarından faydalanılabileceği belirlenmiştir. Antioksidanların da oksidatif DNA hasarına ve kansere karşı koruyucu etkiye sahip oldukları yapılan çalışmalarda gösterilmiştir. Çalışmamızdan elde edilen sonuçlar göz önünde bulundurulduğunda *Oxalis triangularis* özütlerinin DNA koruyucu aktivitesinin özellikle su özütünde çok fazla olduğu belirlenmiştir. Kozmetik sektöründe, özellikle güneş koruyucu kremlerde doğal UV koruyucu olarak kullanılabileceği söylenebilir. Çalışmamızda hastane infeksiyonu olan *Stenotrophomonas maltophilia* bakterisine karşı *Oxalis triangularis* yapraklarının antibakteriyel etkileri araştırıldığında bu bakteriye karşı herhangi bir etkinliğinin olmadığı gözlenmiştir.

Tüm bu sonuçlar; biyolojik aktivitelerin belirlenmesi doğrultusunda yapılan çalışmalara katkıda bulunacaktır. Ayrıca farmakolojik çalışmalara yol gösterici olup bitkilerin halk arasında bilinçli bir şekilde kullanılmasına yardımcı olacaktır.

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➢ *ORAL PRESENTATION*

Importance of seminal plasma malate dehydrogenase and lactate dehydrogenase activity on male fertility

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Abstract

Many studies are related to understand the causes of the male infertility, defects in sperm cell structure and biochemistry that underlie the loss in fertilizing potential. Malate dehydrogenase (MDH NAD/NADP) and lactate dehydrogenase (LDH) are important enzymes for energy metabolism of sperm and also sensitive indicators for male reproductive system and fertility potential. In this study, MDH and LDH activities were determined by spectrophotometric method in seminal plasma samples of normozoospermics and other study groups. Glutathione concentration was measured for its importance on enzymatic antioxidant defence mechanism in these samples. Also free testosterone and leptin concentrations were measured in seminal plasma samples to evaluate the possible relation with MDH and LDH enzyme activities. Semen analysis was considered according to the WHO criteria. MDH-NAD activity (the mean±SD, mU/ml) was significantly lower in asthenoteratospermics (42.55±5.85) and in azoospermics (31.13±3.91) than normozoospermics (103.30±6.00). LDH activity (U/L) was significantly lower in oligospermics (599.08±53.96) than normozoospermics (1226.08±109.38) and other groups. Glutathione level (µmol/L) was significantly lower in oligospermics (30.94±2.18) and azoospermics (19.36±1.39) than normozoospermics (44.05±1.59). Free testosterone level (pg/ml) was detected significantly higher in asthenoteratospermics (3.54±0.24) than other groups but it was significantly lower in azoospermics (1.11±0.16). Leptin level (ng/ml) was significantly lower in oligospermics (0.12±0.02) than normozoospermics (0.21±0.01). Negative correlation was detected between MDH-NAD and LDH activity in oligospermics ($p = 0.046$ $r = -0.402$) and also in asthenoteratospermics ($p = 0.006$ $r = -0.591$). Biochemical composition of seminal plasma have important contribution to support sperm functions and fertility potential. MDH (NAD/NADP) and LDH enzymes control and support sperm energy metabolism and activities. These enzymes may work with similar mechanisms that support each other.

Keywords: Malate dehydrogenase, lactate dehydrogenase, glutathione, testosterone, leptin, seminal plasma

INTRODUCTION

Infertility is defined as the failure of conception after at least 12 months of unprotected intercourse. In almost 50 % of cases infertility results from abnormality of the male partners. There is still lack of understanding of the causes of the male infertility and nature of the defects in sperm cell structure and biochemistry that underlie the loss in fertilizing potential. Impairment of normal spermatogenesis and sperm function are the most common causes of male factor infertility. The development of recent approaches to diagnosis and treatment must be related to investigate the cellular pathologies that result in the production of defective sperm (Roldan ERS 2018).

Malate Dehydrogenase (MDH) and Malic Enzymes (ME) are important enzymes of many metabolic pathways, including anaerobic respiration, tricarboxylic acid cycle, gluconeogenesis, maintenance of oxidation/reduction balance, NADPH production and fatty acid biosynthesis. MDH (E.C.1.1.1.37) catalyzes the interconversion of L-malate and oxaloacetate using NAD^+ as a coenzyme (Christopher et al.,1994; Minarik et al.,2002)

L-malate + NAD^+ $\longleftrightarrow$ oxaloacetate + NADH + H^+

MDH1 (cytoplasmic) is localized on chromosome 2 and 2p15 region. Broad expression in heart, fat and other tissues detected. MDH2 (mitochondrial) is localized on chromosome 7 and 7q11.23 region. Broad expression in

heart, duodenum and other tissues detected. ME catalyzes the oxidative decarboxylation of malate to pyruvate together with the reduction of the cofactor NAD^+ or NADP (Yingwu et al., 1999; Yang et al., 2002).

$$NAD(P)^+ + \text{L-malate} \xleftrightarrow{Mg^{+2}} NAD(P)H + \text{pyruvate} + CO_2$$

In previous studies importance of MDH explained in human and animal samples. Prasad et al.,(1976) studied MDH electrophoretically in a total of 99 semen samples obtained from normal, vasectomized, oligospermic, and infertile males and enzymatic patterns were compared with total sperm count and infertile males. MDH-NAD has an effective role on energy metabolism of sperm. Cordoba et al.,(2007) studied with cryopreserved bovine spermatozoa and explained that MDH-NAD(P) and IDH-NAD(P) enzymes play a major role in supplying reduction equivalents and/or energy required for capacitation and acrosome reaction in spermatozoa. Matsuzawa and Sawada (1987) explained that MDH-A and MDH-B originate from the cytoplasm and mitochondria of the sperm respectively.

Spermatozoa are dependent on an efficient generation of ATP to fuel progressive motility, capacitation, hyperactive motility and acrosome reaction crucial for fertilization (Ho et al., 2002). The levels of MDH increased significantly during capacitation and spontaneous acrosome reaction, indicating that participation of the malate-aspartate shuttle may be required to maintain the levels of reduced coenzyme necessary for capacitation and spontaneous acrosome reaction (Choi et al.,2008).

The Lactate deyhdrogenase isoenzyme LDH-C4 (EC 1.1.1.27) in the human testis, spermatozoa and seminal plasma was originally demonstrated by Blanco et al. (1963), Clausen and Ovlisen (1965). The isoenzyme is present only in primary spermatocytes and later stages of the germinal cell line, and its activity in seminal plasma has been regarded as a sign of leakage from the spermatozoa or from their precursor cells. LDH-C4 activity accounted for about 80% of the total LDH activity in spermatozoa. The amount of LDH-C4 was related to testicular maturity and some forms of testicular dysfunction were related with the disapperance of LDH-C4. Since LDH-C4 specifically related to certain types of cells within the seminiferous epithelium it could be a marker for these cells (Goldberg 2010). Semi-quantitative analysis of LDH-C4 in seminal plasma have also been used to differentiate between various types of azoospermia and severe oligozoospermia (Gavella et al.,1982). LDH-C4 is closely associated with the starting of active spermatogenesis and it plays an important role in maintaining glycolysisand ATP production in the flagellum during capacitation, therefore essential for male fertility. Studies have been reported that LDH-C_4 making the major contribution to the total LDH activity in fertile individuals.

Many factors may influence male fertility that oxidative stress has developed interest in recent years. Oxidative stress is defined as an imbalance between pro-oxidant stress and antioxidant defense. Oxidative stress affects sperm in two different ways: first, sperm membrane fluidity is affected. Second, many studies have explained that reactive oxygen species (ROS) mediate an increase of DNA strand breaks in infertile men. This pathology is associated with impaired sperm function, altered sperm motility and sperm-oocyte fusion in vitro. Studies with infertile patients demonstrated that increased ROS production was found to be negatively correlated with fertilization success in human IVF and with the achievement of pregnancy. Sperm and seminal plasma possess antioxidant systems that scavenge ROS and prevent internal cellular damage. Enzymatic antioxidant defence mechanisms in seminal plasma and sperm include the glutathione peroxidase/reductase system, superoxide dismutase, and catalase. Non-enzymatic antioxidants include reduced glutathione (GSH), urate, ascorbic acid, vitamin E, caretonoids, ubiquinones, taurine and hypotaurine (Agarwall et al., 2014).

GSH is the most abundant non-protein thiol in mammalian cells. Cellular GSH plays a key role in many biological processes, including the synthesis of proteins and DNA and the transport of amino acids, but notably, it plays a key role in protecting cells against oxidation: the sulphydryl (SH) group is a strong nucleophile, and confers protection against damage by oxidants,electrophiles and free radicals (Meister and Anderson, 1983).Glutathione peroxidase uses GSH to reduce hydrogen peroxide to H_2O and lipoperoxides to alkyl alcohols. The resulting oxidized glutathione (GSSG) is reduced to GSH by glutathione reductase using NADPH as the co-factor.

GSH has been detected in various concentrations in male reproductive tissues in different species including human being. Substantial quantities of GSH are found in the testis, reproductive tract fluids, and epididymal spermatozoa. Its presence has been shown intracellularly within the sperm as well as extracellularly in the seminal plasma (Chaudhari et al., 2008).

Androgens are essential for the development and function of male reproductive organs, for example maturation secondary sexual characteristics, libido and stimulation of spermatogenesis. Testosterone is the major circulating androgen and it is an essential hormone for the maintenance of male fertility and testicular function. The leydig cells are located in the testicular interstitium. These cells are the main source of testosterone and produce approximately 95% of circulating testosterone in men. The metabolic steps required for the conversion of cholesterol into androgens take place inapproximately 500 million Leydig cells. The absence or reduction of the levels of testosterone leads to failures in spermatogenesis and then infertility (Dohle GR et al.,2003).

Leptin is secreted from the white adipose tissue and acts on the hypothalamus controlling eating behaviour (Wasim et al., 2016). Leptin binds to leptin receptors in the central nervous system and peripheral tissues and regulates energy homeostasis and neuroendocrine function (Ellithy et al.,2013), immunity, sexual development and reproduction. Leptin receptors have been identified in Leyding and germ cells, testicular tissue, seminiferous tubules which provide evidence for role of the leptin in sperm maturation, capacitation or mobility (Cheraghi et al., 2018).

The aim of the present study was to determine the activity of MDH, LDH and to measure the levels of GSH in men with different fertility potential compared with normozoospermic males. Also we determined the concentration of free testosterone and leptin in these samples. We investigated the possible relation between enzyme activities and other parameters to evaluate the fertility potential of sperms in different seminal plasma samples.

MATERIALS and METHODS

Semen samples

Semen samples were provided from patients who attended the Assisted Reproductive Unit of Cukurova University, Medical School Obstetric Department for infertility evaluation. Written informed consents were obtained from all patients, according to the criteria of the Ethical Committee of the Medical Faculty. All participants were aged between 25 and 50 years. Specimens were collected by masturbation after 3-5 days of abstinence. Following liquefaction of the samples at 37˚C, sperm cell concentration, morphology and motility was determined according to the WHO (WHO 2010) criteria. Samples were classified into four groups; normozoospermics (n=40), oligospermics (n=25), asthenoteratospermics (n=20) and azoospermics (n=20). Normozoospermic samples; sperm concentration > $15x10^6$/ml, motility > %40 and morphology > %4 normal. Oligospermics; sperm count < $15x10^6$/ml. Asthenoteratospermics; motility < %32 sperm with progressive motility and morphology < %4 normal. Azoospermics; absence of sperm in the semen. Seminal plasmas were separated from the spermatozoa by centrifugation at 2000 rpm for 10 min at room temperature. Samples were stored at -70˚C until the day of the study.

Biochemical analysis

MDH activity in seminal plasma was measured by the increase in absorbance at 340 nm resulting from the reduction of NAD/NADP with the presence of malate (1993). MDH activity was measured spectrophotometrically using Shimadzu (UV-260) spectrophotometer. The results were expressed as mU/ml for seminal plasma. LDH activity (U/L) in seminal plasma was measured according to Sigma-lactate dehydrogenase kit (228-UV) and spectrophotometrically using Shimadzu (UV-260) spectrophotometer at 340 nm. The results were expressed as U/L for seminal plasma. The GSH concentration in seminal plasma was measured according to the Beutler (1984) spectrophotometrically using Shimadzu (UV-260) spectrophotometer at 412 nm. The results were expressed as µmol/L for seminal plasma. Free Testosterone (FT) concentration (pg/ml) in seminal plasma was measured according to Biosource KIPB19000 kit. Leptin concentration (ng/ml) in seminal plasma was measured according to IRMA DSL-23100 kit.

Statistical Analysis

Since semen parameters were not normally distributed, Spearman's correlation test (SPSS- 20 program) was applied to compare the values between normozoospermic and other groups.

RESULTS

The mean values and SEM of the MDH (NAD/NADP) and LDH activity, GSH, free testosterone and leptin concentrations in the seminal plasma samples of normozoospermic, oligoteratospermic, asthenoteratospermic and azoospermic groups are shown in Table 1.

Table 1. MDH (NAD/NADP) and LDH activity, GSH, FT and leptin concentrations in seminal plasma samples of different study groups

Group	**MDH-NAD** mU/ml	**MDH-NADP** mU/ml	**LDH** U/L	**GSH** µmol/L	**FT** pg/ml	**Leptin** ng/ml
1	103.30±6.00	149.86±10.24	1226.08±109.38	44.05±1.59	2.51±0.20	0.21±0.01
2	119.92±10.28	134.00±10.51	599.08±53.96*	30.94±2.18*	2.18±0.22	0.12±0.02*
3	42.55±5.85*	127.65±16.11	1281.00±128.93	43.32±2.62	3.54±0.24*	0.16±0.01
4	31.13±3.91*	120.52±10.05	982.48±43.57	19.36±1.39*	1.11±0.16*	0.16±0.01

Results are presented as mean±SD * significant at the $p < 0.05$ level

Group 1 –Normozoospermics (n=40), Group 2 -Oligospermics (n=25),

Group 3 – Asthenoteratospermics (n=20), Group 4 -Azoospermics (n=20).

MDH-NAD activity was significantly lower in Group 3 and Group 4 than normozoospermics. There was no difference in MDH-NADP activity in four groups. LDH activity was significantly lower in oligospermics than normozoospermics and other groups. GSH level was significantly lower in oligospermics and azoospermics than normozoospermics and asthenoteratospermics. Free testosterone level was detected significantly higher in asthenoteratospermics than other groups but it was significantly lower in azoospermics. Leptin level was significantly lower in oligospermics than normozoospermics. Negative correlation was detected between MDH-NAD and LDH activity in Group 2 ($p = 0.046$ $r = - 0.402$) and also in Group 3 ($p = 0.006$ $r = - 0.591$).

DISCUSSION

There has been increased investigations in biochemical composition of the seminal plasma because its composition can often indicate male infertility. The markers most commonly used for the secretory capacity of the accessory glands from which the constituents of seminal plasma originate are acid phosphatase, zinc and citric acid for the prostate and fructose for the secretory function of the seminal vesicles (Valesco JAN 1993). However, researches continue to report importance of any biochemical marker of germinal activity for measure in routine analysis.

Sperms produce ATP through glycolysis and aerobic respiration. Sperm mitochondria possess several enzymes or isozymes, LDH-C_4, which contribute significantly to energy production and sperm motility. Sperm motility is an important predictor of male fertility. Sperms obtain their energy from aerobic respiration and anaerobic glycolysis pathway, which take place in the mitochondria located in the middle segment, and from glycolytic enzymes in the main segment of the flagellum and the surface of the fibrotic membrane. Esmaeilpour et al.,(2014) investigated LDH-C_4 expression by western blotting and reported the expression of LDH-C_4 protein was observed in the normal samples but not in the asthenozoospermic ones with any treatment. They explained that absence of LDH-C_4 may be deemed one of the causes of infertility.

In our study, we determined LDH activity in normozoospermic samples as 1226.08±109.38 U/L. In oligospermics the activity (599.08±53.96 U/L) was significantly lower than normozoospermics. It is reported that LDH activity

in seminal plasma has been regarded as a sign of leakage from the spermatozoa or from their precursor cells. In oligospermics, sperm concentration was < $15x10^6$/ml and also LDH activity was lower than normozoospermics. It is concluded that there is a relation between sperm concentration and LDH activity. Also many authors reported similar results in their studies.

In normozoospermic samples MDH-NAD activity was 103.30±6.00 U/L. But in asthenoteratospermic (42.55±5.85 U/L) and azoospermic (31.13±3.91 U/L) groups MDH-NAD activity was determined significantly lower than normozoospermics. MDH activity in seminal plasma of azoospermics regarded as a sign of leakage from the spermatozoa or any testicular canal. In asthenoteratospermics lower MDH-NAD activity may be resulted from some defects in sperm production, spermatogenesis or sperm maturation (Leventerler et al., 2013). Because these sperms have lower motility and morphology. According to this result, MDH-NAD enzyme activity may be an important factor to support energy metabolism of sperm functions and male infertility.

Raymond L. et al., (1970) investigated LDH and MDH activities in the C57BL/6 mouse testis at different ages. Changes coincident with LDH and MDH activity were two physiological events involving the testes and weaning. Onset of spermatocyte maturation and the attainment of sexual maturity appear as evident demarcations, especially in the LDH/MDH ratio. Maturation of spermatocytes is preceded by an increase in MDH activity and in LDH/MDH ratio. If LDH activity is indicative of anaerobic carbohydrate pathway (TCA cycle), then the LDH/MDH ratio may represent the contribution of each of the tissue's carbohydrate metabolism or potential. It is interesting to note how closely the LDH/MDH ratio parallels the definable parameters of spermatocyte maturation, weaning and attainment of sexual maturity. Similar to these explanations we determined a negative correlation between MDH-NAD and LDH activity in oligospermics ($p=0.046$ $r= -0.402$) and in asthenoteratospermics($p = 0.006$ $r = - 0.591$). If one of these enzyme (MDH or LDH) activity decreases the other dehydrogenase activity increases. We believe that MDH should be consider as a second enzyme and parameter (after LDH) to evaluate sperm functions and male infertility. Because MDH and LDH are important dehydrogenases for NADH/NADPH production and also for energy metabolism of sperm.

Hanse et al. (2017) explained that increased glucose consumption is a hallmark of cancer cells. The increased consumption and subsequent metabolism of glucose during proliferation creates the need for a constant supply of NAD, a co-factor in glycolysis. Regeneration of the NAD required to support enhanced glycolysis has been attributed to the terminal glycolytic enzyme LDH. However, loss of glucose carbons to biosynthetic pathways early in glycolysis reduce the carbon supply to LDH. Thus, alternative routes for NAD regeneration must exist to support the increased glycolytic rate while allowing for the diversion of glucose to generate biomass and support proliferation. Hanse et al. demonstrated that cytosolic MDH1 is an alternative to LDH as a supplier of NAD. They suggest that proliferating cells rely on both MDH1 and LDH to replenish cytosolic NAD and therapies designed at targeting glycolysis must consider both dehydrogenases. Also we think that MDH and LDH enzymes show their activity in a similar way in human seminal plasma samples. MDH support LDH activity as another supplier of NAD. Ruskova S et al., (1972) demonstrated biochemical and morphological studies of the semen from males with diseases of the reproductive system. They explained that the activities of LDH, MDH and acid phosphatase were lowered in all pathological groups including the patients with "functional disturbances" of the reproductive system. They concluded these enzymes may be used as sensitive indicators in the early diagnosis of diseases of the male reproductive system.

Imbalance between pro-oxidant stress and antioxidant defense plays an important role in the pathogenesis of male infertility. Among the endogenous antioxidant systems, GSH plays a significant role in the antioxidant defense of the spermatogenic epithelium, the epididiyms and perhaps in the ejaculated spermatozoa. Presence of GSH has been noticed both intracellulary within the sperm as well as extracellulary in the seminal plasma. In our present study, GSH level in seminal plasma was significantly lower inoligospermic and azoospermic groups compared to normozoospermics. These findings were compatible with that observed by Chaudhari AR et., al (2008).The source of seminal GSH remains scattered all through the male reproductive tract starting from the basement membrane of seminiferous tubule, Sertoli cell cytoplasm, epididymis. A considerable contibution comes from the spermatogenic tissues and is found to be deficient in subjects with azoospermia. Thus it indicates that decreased antioxidant level in the seminal fluid has a negative impact on semen quality.

The first reports on the determination of hormones in the seminal plasma appeared as early the late 1970s. Spermatogenesis under hormonal control and testosterone plays an important role in the process of spermatogenesis. Escallon BM et al., (1982) evaluated whether hormonal analysis of seminal plasma could be used in the evaluation of the process of spermatogenesis. Their results confirm that blood levels of testosterone are not different between fertile and infertile men. Therefore, determination of testosterone in serum is not of diagnostic value in those cases in which the characteristics of the sperm are of doubtful quality. In our study free testosterone level was lower in asthenoteratospermics and azoospermics. These results are compatible with MDH-NAD activities. Because this enzyme activity was also lower in asthenoteratospermics and azoospermics.

The first and rate-limiting step of steroidogenesis is catalyzed by the mitochondrial cholesterol side chain cleavage system that is dependent on NADPH. The pathways of NADPH generation in steroidogenic mitochondria include three major routes catalyzed by: 1. NADP-linked malic enzyme, 2. NADP-linked isocitrate dehydrogenase, and 3. nicotinamide nucleotide transhydrogenase. The main route may differ among cell types and across species. The oxidation of NADPH by the mitochondrial P450 systems is not tightly coupled with substrate metabolism, as these systems can reduce oxygen, by a single electron to produce harmful superoxide radical. To minimize such futile NADPH oxidation, NADPH generation may be regulated by two types of mechanisms: 1. Feedback mechanisms that maintain the ratio of NADPH/NADP+ at a steady-state level by enhancing the rate of NADPH production to keep up with its rate of oxidation, e.g., allosteric regulation of enzymes involved in NADPH production. 2. Hormonal signals that enhance the level of NADPH production in coordination with steroidogenesis (Hanukoglu and Rapoport 1995). We think that MDH is an alternative enzyme in NADPH production and also MDH has an effective role on testosterone synthesis and regulation. As testosterone is the principal androgen which supports spermatogenesis therefore, its synthesis is quite important as it influences the development and behaviour patterns in male. Any deviations from normal testosterone synthesis and signalling that has been described may lead to some serious diseases and problems.

Leptin is a polypeptide and found to play a regulatory role in body adipose store, food intake and energy metabolism. Leptin is expressed in the seminiferous tubules, seminal plasma and also directly acts on testis. Also the source of leptin has been shown either seminal vesicle or prostate tissue. But the leptin receptors have been identified in the Leydig cells. Some other studies explained that human ejaculated spermatozoa secreteleptin (Jorsaraei et al., 2010).

In our study, leptin level was significantly lower in oligospermics than normozoospermics. In asthenoteratospermic and azospermic groups we detected a certain level of leptin. According to our results we believe that seminal vesicles are source of leptin and spermatozoa secrete leptin according to its energy needs, independently by systemic leptin expression . It was suggested that testes may contribute leptin secretion and low levels of seminal leptin may be a risk factor for idiopathic male infertility.

CONCLUSION

It is reported that biochemical composition of seminal plasma can often indicate male infertility. We believe that in addition to LDH activity, MDH-NAD enzyme activity may have an important contribution to evaluate sperm functions, sperm energy metabolism and male infertility. Also another interesting point GSH and leptin levels were consistent with LDH activity of oligospermics. Free testosterone levels were consistent with MDH-NAD activity of asthenoteratospermics and azoospermics. GSH, leptin and free testosterone can be considered as supporting markers of LDH and MDH-NAD activities.

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➢ *ORAL PRESENTATION*

Study of cholinesterase activity and lipid profile levels in experimental diabetic rats

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Abstract

Cholinesterases are the enzymes responsible from hydrolysis of cholinesters. Changes in activities of cholinesterases are observed in different diseases of human. Diabetes mellitus is a heterogenous metabolic disorder which is characterised by hyperglycemia, renal failure, neurological and vascular complications. This study was designed to understand the changes in erythrocyte acetylcholinesterase (AChE), plasma butrylcholinesterase (BChE) activities and lipid profile in Streptozotocin (STZ) induced diabetic rats. Male Wistar (180-200 g body weight) rats were used and diabetes was then induced intra-peritoneally with 60 mg STZ (injection dose maximum 0.1 ml) per kg body weight of each rat of the diabetes group. Then body weight, plasma glucose level, BChE activity, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol levels and erythrocyte AChE activities of control and diabetic rats were investigated for three months. Plasma BChE activity increased with the induction of diabetes (0.33±0.07 U/ml) compared to the control group (0.24±0.05 U/ml). Plasma triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol levels were also increased concurrently with the development of diabetes. Erythrocyte AChE activity decreased in diabetic rats (15.69±3.8 U/g Hb) compared to the control group (19.07±2.8 U/g Hb). We believe that diabetes cause changes in lipid profile of the rats and these changes increase BChE activity. Also diabetes damage erythrocyte structure and result a decrease in AChE activity.

Keywords: Acetylcholinesterase, butrylcholinesterase, diabetes mellitus, lipid profile, Streptozotocin

INTRODUCTION

Cholinesterases (ChE) are enzymes that catalyze the hydrolysis of the acetylcholine into choline and acetic acid to allow a cholinergic neuron to its resting state after activation. These are serine hydrolases that belongs to the esterase family within higher eukaryotes. There are two types of ChE in the human body. One type is acetylcholinesterase (AChE) (EC 3.1.1.7) also known as true, specific, genuine, typeI ChE. This enzyme is found in erythrocytes, nevre endings, lungs, spleen and in the all compartments of brain. AChE is one of the crucial enzymes in the central and peripheral nerve system. Its biological role is the termination of impulse transmissions at cholinergic synapses within the nervous system by rapid hydrolysis of the neurotransmitter, acetylcholine. It has been suggested that AChE may participate in development, differentiation, and pathogenic processes such as Alzheimer's disease and tumorigenesis (Zhang XJ., 2002). The other subgroup, butrylcholinesterase (BuChE) or pseudocholinesterase (PChE) (EC 3.1.1.8) also known as false, serum, plasma, butryl, nonspecific, typeII ChE. BChE is also present in blood plasma, liver, smooth muscle, intestines, pancreas, heart and white matter of brain (Mahmood AAJ 2019, Putocka J., 2004). BChE is found to be increased in a significant number of patients with hyperlipoproteinaemia and obesity. It is accepted that BChE has a role in detoxication of poisons that are eaten or inhaled (Lockridge O., 2015).

Diabetes mellitus (DM) is one of the common and severe metabolic disorders in humans. It is characterised by hyperglycemia due to a relative or an absolute lack of insulin on its target tissue or both. DM cause a variety of complications such as renal failure, neurological and vascular complications and cerebrovascular disease. Type 1 diabetes is the consequence of an autoimmune mediated destruction of pancreatic β-cells, leading to insulin deficiency. Patients require insulin treatment for survival. Type 2 diabetes is characterized by insulin resistance and relative, rather than absolute, insulin deficiency. Type 2 diabetes usually occurs in obese individuals and is associated with hypertension and dyslipidaemia (Shaikh H., 2014), (Ahmed RG., 2005).

There are two major types of lipids in the blood, cholesterol and triglycerides (TG). They are carried on four types of lipoproteins: chylomicrons, low-density lipoprotein (LDL), very low density lipoprotein (VLDL) and high-density lipoprotein (HDL). Many studies indicates that the BChE activity is increased in certain metabolic disorders like hypercholesterolemia, hypertension, obesity and type 1 or type 2 diabetes (Mahmood AAJ., 2019).

The aim of the present study was to compare the activity of cholinesterases and lipid profile levels in Streptozotocin induced diabetic rats and also to evaluate the changes on carbohydrate and lipid metabolism.

MATERIALS and METHODS

Laboratory Animals

Male Wistar (180-200 g body weight) rats were obtained from the Laboratory Animal Center of Faculty of Medicine of the University of Cukurova. The study was approved by the ethical committee of the university. The animals were housed in individual cages under 12h:12h light and dark cycle and fed usual laboratory diet.

Induction of Diabetes Mellitus

Before induction of diabetes, animals were weighed and tail blood taken under anesthesia and fasting glucose level of the rats were determined. Streptozotocin (STZ) was obtained from Sigma chemicals Co.,and dissolved in 0.01 M sodium citrate buffer, pH 4.5. Diabetes was then induced intra-peritoneally with 60 mg STZ (injection dose maximum 0.1 ml) per kg body weight of each rat of the diabetes group. But only sodium citrate buffer was injected to the control group rats. On the second day of the diabetes induction 5 ml saline solution injected subcuteneously to the rats of diabetes group. Animals were weighed after 1 week, 1 month, 2 months and 3 months and their tail blood samples were taken in these periods and some biochemical analysis has been applied. Fasting glucose level, erythrocyte AChE and plasma BChE activity, total cholesterol, triglyceride, HDL and LDL levels were determined.

Biochemical Analysis

Plasma glucose levels of diabetic and control groups were measured with glucose oxidase (Enzymatic Trinder) method. AChE and BChE activities were determined according to modified Ellman method. Total cholesterol, LDL and HDL cholesterol levels were detected with cholesterol oxidase-para-aminotripin (CHOD-PAP) method. Triglyceride levels were determined with glycerol-3-phosphate oxidase- PAP (GPO-PAP) method.

Statistical Analysis

SPSS Version 5.0 program was applied to compare the values between control and diabetic groups.

RESULTS

The mean values and SEM of the glucose concentrations, AChE and BChE activities, total cholesterol, triglyceride, HDL and LDL levels of diabetic and control groups are shown in Table 1.

In the first day of experiment mean glucose value of control group was 130±8.4 and diabetics was 129±10.6 mg/dl. After 3 months, mean glucose value of control group was 140±68.4 and diabetics was reached 317±72.2 mg/dl with two fold increase, so diabetes was confirmed by fasting glucose concentration, polydipsia and polyuria. Again in the first day, mean BChE activity of control group was 0.15±0.04 U/ml and diabetics was 0.14±0.3 U/ml. After 3 months, mean BChE activity of control group was 0.24±0.05 U/ml and diabetics was reached to 0.33±0.07 U/ml with two fold increase. In the first day of experiment, mean erythrocyte AChE activity of rats in control group was 13.07±2.6 U/g Hb and in diabetics 13.33±3.3 U/g Hb. After 3 months, mean AChE activity of control group was 19.07±2.8 U/g Hb and diabetics was15.69±3.8 U/g Hb and this value was lower than the control group.

Table 1. Glucose concentrations, AChE and BChE activities, total cholesterol, triglyceride, HDL and LDL-cholesterol levels of diabetic and control groups

	Control	Group	(n=15)			Diabetes	Group	(n=15)		
	1.Day	1.Week	1. Month	2.Months	3.Months	1.Day	1.Week	1.Month	2.Months	3.Months
Weight (g)	190±9.5	195±11.6	230±16.8	296±22.1	320±20.1	195±9.4	201±10.2	220±22.3	243±39.9**	250±32.4***
Glucose (mg/dl)	130±8.4	132±7.5	134±7.3	140±8.3	140±6.4	129±10.6	224±72.5***	260±92.9***	285±86.6***	317±72.2***
BChE (U/ml)	0.15±0.04	0.14±0.03	0.19±0.05	0.22±0.05	0.24±0.05	0.14±0.03	0.20±0.04***	0.24±0.05**	0.27±0.05**	0.33±0.07**
AChE (U/gHb)	13.07±2.6	16.40±2.8	18.26±2.1	18.20±2.9	19.07±2.8	13.33±3.3	15.95±3.1	16.99±2.2**	15.87±3.5**	15.69±3.8***
Triglyceride (mg/dl)	56±7.1	60±7.2	67±7.8	73±7.6	79±8.1	57±6.9	65±9.4	82±24.3**	99±30.9***	114±35.6***
T.cholesterol (mg/dl)	54±6.0	57±5.9	65±5.1	72±5.0	80±5.5	48±5.7**	54±8.0	72±17.8	97±28.9**	116±32.1***
HDL-chol. (mg/dl)	21±3.4	24±3.5	28±4.3	32±4.6	38±4.1	22±4.1	26±4.4	32±4.8	39±5.3**	47±6.3**
LDL-chol. (mg/dl)	29±4.7	32±5.4	36±5.1	39±4.5	42±2.5	33±4.2***	37±3.9***	42±3.9***	47±5.5***	55±4.5***

Results are presented as mean±SD * significant at the $p < 0.05$ level
** significant at the $p < 0.01$ level *** significant at the $p < 0.001$ level
Significance values were determined after comparing control and diabetes group

When we were evaluated the lipid profile; in the first day of the experiment, mean triglyceride value of the control group was 56±7.1 mg/dl and diabetics was reached to 114±35.6 mg/dl in the third month with two fold increase. When we consider mean cholesterol values of control group in the first day of experiment, following results were obtained: total cholesterol 54±6.0 mg/dl, HDL-cholesterol 21±3.4 mg/dl and LDL-cholesterol 29±4.7 mg/dl. After 3 months total cholesterol value was reached to 116±32.1mg/dl with 2.5 foldincrease, HDL-cholesterol was reached to 47±6.3 mg/dl with 2 fold increase and LDL-cholesterol value was reached to 55±4.5 mg/dl with the progression of diabetes mellitus .

DISCUSSION

Diabetes mellitus is a heterogeneous clinical disorder. In diabetes, hyperglycaemia results from defective insulin secretion, resistance to insulin action or both. A variety of complications arises from chronic hyperglycemia such as neuropathy, nephropathy, retinopathy and increased risk of cardiovascular disease. Animal models play a vital role in the understanding of diabetes metabolism and pathogenesis and also genetic and functional characterization of the syndrome.

In our study Streptozotocin induced diabetic rats were used in experiments to evaluate the changes on carbohydrate and lipid metabolism. One week after administration of STZ, diabetes was confirmed by increased blood glucose concentration (224±72.5 mg/dl). Also polydipsia, polyuria and glucose were detected in the urine of diabetic rats. At the end of third month, blood glucose concentration of control group was 140±6.4 mg/dl and diabetics was 317±72.2 mg/dl. Sugimoto et al. (1997) explained blood glucose level as 305±19 mg/dl in experimentally diabetic Sprague-Dawley rats 6 months after administration of STZ.

Our results show that plasma BChE activity started to increase in STZ induced diabetic rats with the development of hyperglycemia after first week of induction until to the end of third month. At the end of third month BChE activity of diabetic (0.33 ±0.07 U/ml) rats were significantly higher than control (0.24±0.05 U/ml) group. Also plasma total cholesterol, triglyceride, HDL and LDL-cholesterol levels were increased concurrently with the development of diabetes.

According to these results increase in BChE activity in diabetic rats is related with development of hyperlipaemia and hypertriglyceridaemia. Insulin deficiency is associated with increased adipose tissue lipolysis which provides the liver with excess non-esterified fatty acids. The excess fatty acids thus available in diabetes can result in either

ketoacidosis and/or increased synthesis of triglycerides and BChE activity. The source of serum BChE is the liver and serum levels may be indication of its rate of synthesis (Annapurna et al., 1991).

Patel et al., (1999) and Ragoobirsingh et al., (1992) explained a relation between increased plasma BChE activity, total cholesterol and triacylglycerol levels in experimental diabetic rats after six months of induction of diabetes. Also there was a remarkable increase in BChE activity of control group rats due to increase in weight of animals. In the first day ofexperiment BChE acyivity of control group rats were 0,15 ±0.04 U/ml but three months after activity was reached to 0,24 ±0.05 U/ml ($p<0.001$).

Annapurna et al., (1991) and Deshaies et al., (1991) reported increased triglyceride levels in experimental diabetic rats. It has been known that insulin deficiency cause a decrease in lipoprotein lipase activity and increase in triglyceride level. Also Ragoobirsingh et al., (1992) explained a relation between increased serum ChE activity and triglyceride level in diabetic patients and this increase may cause cardiovascular complications.

In another part of our study we investigated erythrocyte AChE activity in control and diabetic rats. At the end of third month, following induction of diabetes we determined AChE activity in control group 19.07±2.8 U/g Hb but in diabetic group 15.69±3.8 U/g Hb. There was a significant decrease in AChE activity of diabetic group. We think that diabetes may cause a defect in structure of erythrocyte and also decrease in AChE activity.

Suhail et al., (1990) reported that lower AChE activity in diabetic patients than control group. It is also explained that the changes in erythrocyte membrane, sialic acid and phospholipid content may be related with decrease in erythrocyte membran fluidty of diabetic patients.

CONCLUSION

According to our results we explain that experimental diabet change lipid profile (increase in total cholesterol, triglyceride, HDL and LDL-cholesterol levels) and cause an increase in plasma BChE activity. Also diabetes defect erythrocyte structure and lead to decrease in AChE activity. This study may be supported further investigations that point importance of insulin and related molecules on diabetes mellitus.

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➢ *ORAL PRESENTATION*

Berberis crataegina Meyvesinin Total Oksidan/Antioksidan Seviye ve *Stenotrophomonas maltophilia* Üzerine Etkisinin Araştırılması

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Özet

Bitkiler yıllardan beri hem ülkemizde hem de dünyada değişik amaçlarla kullanılmaktadır. Bu bitkiler çoğunlukla insanlar tarafından tıbbi amaçla kullanılmış ve kullanılmaya devam edilmektedir. Bu bitkilerin çoğu hem antimikrobiyal hem de antioksidan özellikte olup birçok hastalığın tedavisinde kullanılmıştır. Bu sebeple bitkiler kimyasal, mikrobiyolojik ve farmakolojik açılardan çok yönlü olarak araştırılmaktadır. Yapılan bu çalışmada, *Berberis crataegina* meyve özütlerinin total antioksidan/oksidan seviye (TAS/TOS) ve *Stenotrophomonas maltophilia* bakterisi üzerine etkisinin araştırılması amaçlandı. Çalışma materyali olan *Berberis crataegina* meyveleri gölgede kurutularak etanol, metanol, hekzan ve diklorometan çözücüleri yardımıyla meyve özütleri elde edildi. Elde edilen meyve özütleri TAS ve TOS Assay Kit (Rel Assay Diagnostic) hazır kitleri kullanılarak total antioksidan / oksidan seviyeleri ölçüldü. Ayrıca meyve özütlerinin antimikrobiyal aktivitesinin değerlendirilmesinde *Stenotrophomonas maltophilia* bakteri suşları disk difüzyon yöntemi ile çalışıldı. Çalışma sonucunda *Berberis crataegina* meyvelerinde TAS değerlerinin etanol ve metanol özütlerinde yüksek olduğu, hekzan özütünde çok düşük ve diklorometan özütünde ise normal seviyede olduğu tespit edildi. Benzer şekilde TOS değerlerinin etanol, hekzan ve diklorometan özütlerinde çok yüksek olduğu, metanol özütünün ise ilk ölçümde sonuç vermediği buna karşılık ½ oranında sulandırıldığında ise çok yüksek sonuç verdiği tespit edildi. Ayrıca *Berberis crataegina* meyve özütlerinin *Stenotrophomonas maltophilia* bakterisi üzerine antimikrobiyal etkisinin olmadığı gözlemlendi. Bu çalışma, *Berberis crataegina* meyvelerinin antioksidan ve oksidan özellik gösterdiği önceki çalışmaları desteklemekle birlikte *Stenotrophomonas maltophilia* bakterisi ile yapılan ilk çalışma olma özelliği taşımaktadır.

Anahtar Kelimeler: *Berberis crataegina*, *Stenotrophomonas maltophilia*, total oksidan/antioksidan seviye,

Total Oxidant / Antioxidant Level and of *Berberis crataegina* Fruit Investigation of the Effect on *Stenotrophomonas maltophilia*

Abstract

Plants have been used for different purposes both in our country and in the world for years. These plants have mostly been used by humans for medicinal purposes and continue to be used. Most of these herbs have both antimicrobial and antioxidant properties and have been used in the treatment of many diseases. For this reason, plants are studied in a multifaceted way in chemical, microbiological and pharmacological aspects. In this study, it was aimed to investigate the effect of Berberis crataegina fruit extracts on total antioxidant / oxidant level and Stenotrophomonas maltophilia bacteria. Berberis crataegina fruits, the study material, were dried in the shade and extracts were obtained with the help of ethanol, methanol, hexane and dichloromethane solvents. Total oxidant / antioxidant levels were measured using the obtained fruit extracts TAS and TOS Assay Kit (Rel Assay Diagnostic) ready-made kits. In addition, Stenotrophomonas maltophilia bacterial strains were studied by disk diffusion method to evaluate the antimicrobial activity of fruit extracts. As a result of the study, it was determined that TAS values in Berberis crataegina fruits were high in ethanol and methanol extracts, very low in hexane extract and normal in dichloromethane extract. Similarly, it was determined that TOS values were very high in ethanol, hexane and dichloromethane extracts, and methanol extract did not give results in the first measurement, but when diluted at ratio, it was found to be very high. It was also observed that Berberis crataegina fruit extracts did not have an antimicrobial effect on Stenotrophomonas maltophilia bacteria. Although this study supports

previous studies in which Berberis crataegina fruits have antioxidant and oxidant properties, it is the first study conducted with Stenotrophomonas maltophilia bacteria.

Keywords: Berberis crataegina, Stenotrophomonas maltophilia, total oxidant / antioxidant level,

GİRİŞ

Bitkilerin kullanımı çok eski çağlara dayanmakta olup, hem doğal beslenme hem de tıbbi ve aromatik amaçlı tüketilen bitkiler doğada bir denge ürünü olarak kullanılmaktadır (Faydaoğlu ve Sürücüoğlu, 2011). İnsan sağlığı açısından bitkilerin hayatımızda büyük bir öneme sahip olduğu bilinmektedir. Bitkilerden elde edilen hammaddeler insanların sağlığına faydalı olan ve tedavi edici özellik taşıyan bitkisel ilaç haline gelmektedir. Bu ilaçlar genellikle önemli yan etkilerinin olmamasının yanında birçok olumlu biyolojik etkiye sahiptir. Bu sebeple sentetik ilaçlara kıyasla tıbbi bitkiler ile yapılan ilaçlar daha fazla tercih edilmektedir (Diken vd., 2009). Dünya Sağlık Örgütü tarafından yayınlanan raporlara göre çoğu ülkede insanların %80'inin bitkisel kökenli ilaçları geleneksel ilaçlara tercih ettiği görülmektedir.

Karamuk bitkisi (*Berberis crataegina*), *Berberidaceae* familyasının Berberis cinsine ait önemli bir türü olup, Türkiye ve İran bölgesinde geniş yayılım alanına sahiptir. Ülkemizde Antalya, Ankara, Kastamonu Kütahya, Kayseri, Erzincan, Kahramanmaraş, Konya, Karaman, Şanlıurfa, Niğde, Malatya ve Yozgat çevrelerinde kendiliğinden yetişmektedir. Anadolu'nun değişik yörelerinde karamuk bitkisi, kadıntuzluğu, yılkı çalısı, tavşan ekmeği, karamuk dikeni, sarıağaç gibi farklı isimler kullanılır. (Baytop, 1999; Gedikli, 2006). Bazı yörelerde meyvelerinden hoşaf, reçel gibi tatlılar yapıldığı bilinmektedir. Bitkinin C vitaminince zengin olan meyveleri geleneksel kullanımda; sarılık, cilt hastalıkları, tansiyon düşürücü, kan yapıcı, mide ve bağırsak rahatsızlıkları, safra kesesi rahatsızlığı, soğuk algınlığı, göz rahatsızlıkları, romatizma, baş ağrısı, diyabet, dolaşım, solunum, hemoroid, mayasıl, kısırlık ve kadın hastalıklarının tedavisinde kullanılmaktadır (Üçer, 1977; Tuzlacı, 2016; Arslanoğlu ve Ayna 2019).

Organizmada antioksidanlar ve oksidanlar arasında bir denge bulunmaktadır. Şayet mevcut denge oksidanlar yönünde bozulursa oksidatif stres meydana gelmektedir (Sies, 1991; Deveci, 2017). Yapılan birçok araştırmada bazı bitkisel antioksidanların oksidatif stres sebebiyle meydana gelen hücre ölümlerini engellediğini ve antioksidan savunma sistemini güçlendirdiği bildirilmiştir (Schoeter vd., 2000; Youim ve Joseph, 2001; Parihar ve Hemnani, 2003; Deveci vd., 2016).

Stenotrophomonas maltophilia, aerobik, gram-negatif bir bakteri olup ilk kez 1943 senesinde *Bacterium bookeri* olarak izole edilmiştir. Bu bakteri canlılığını devam ettirmek ve üreyebilmek için özellikle nemli ortama ihtiyaç duyan ve ciddi hastane enfeksiyonlarına sebep olan fırsatçı bir patojendir.

Yapılan bu çalışmada , doğal olarak yetişen Karamuk (*Berberis crataegina*) bitkisinin total antioksidan/oksidan seviye ve *Stenotrophomonas maltophilia* üzerine etkisinin araştırılması amaçlanmaktadır.

MATERYAL VE METOD

Çalışma materyali olan *Berberis crataegina* (Karamuk) bitki meyveleri Kayseri-Sarız ilçesinin değişik köylerinden çalışmaya uygun şekilde toplandı. Daha sonra meyve taneleri kurutma kağıdı üstünde gölgede kurutularak mekanik öğütücü ile öğütüldü. Öğütülen meyve tozları 10 gram tartılıp Soxthern cihazının beherleri içine yerleştirildi. Ekstraksiyon işlemi için çözücü ile beraber basınç uygulaması yapıldı. Ekstraksiyonda etanol, metanol, hekzan ve diklorometan çözücü olarak kullanıldı. Tüm çözücüler için ayrı ayrı olmak kaydıyla 5.1 bar basınçta, 2 saat boyunca 10 grama 150 ml çözücü konularak 130 ºC' de işleme tabi tutuldu. Elde edilen özütler Rotary Evaporator'de 90^0C'de evaporasyon (buharlaştırma) yöntemiyle etanol, metanol, hekzan ve diklorometan uzaklaştırıldı. Hazırlanan özütler +4^0C'de muhafaza edildi (Gülaçtı vd., 2007).

Çalışmada *Berberis crataegina* meyve özütünün toplam antioksidan (TAS) ve toplam oksidan (TOS) seviyenin belirlenmesi için ticari kitler kullanıldı.

Total Antioksidan Seviye (TAS) ölçümü için; TAS assay kit (Rel Assay Diagnostics, Korea), meyve özütleri, spektrofotometre ve eliza plate kullanıldı. Eliza plate kuyucuklarına 200 µl Reagent 1 koyulmuştur. Reagent 1 üzerlerine 0.1 mg/1000 µl lik stok çözeltiden 12 µl bitki özütleri eklendi. Başlangıç absorbansları 660 nm'de spektrofotometrik olarak ölçüldü. Ölçüm sonrası üzerlerine 30 µl Reagent 2 eklenerek, 5 dakika 37 °C' de inkübe edildi. İnkübe sonrası 660 nm'de yeniden absorbans ölçümü yapıldı. Bu işlem farklı çözücüler kullanılarak çıkarılan meyve özütleri için teker teker yapılarak ölçüm tamamlandı.

TAS değeri için aşağıdaki formül kullanılarak hesaplamalar yapıldı:

TAS (mmol/L)=[ΔAbs Std1-ΔAbs Örnek]/ [ΔAbs Std1-ΔAbs Örnek]X20

Δ Standard1 absorbansı= (Std1'in ikinci absorbansı - Std1'in birinci absorbansı)
Δ Standard2 absorbansı= (Std2'nin ikinci absorbansı - Std2'nin birinci absorbansı)
Δ Örnek absorbansı = (Örneğin ikinci absorbansı – Örneğin birinci absorbansı).

Total Oksidan Seviye (TOS) ölçümü için; TOS assay kit, meyve özütleri, spektrofotometre, eliza plate kullanıldı. Eliza plate kuyucuklarına Reagent 1' den 200 µl eklenerek üzerine %5'lik stok çözeltiden 30 µl eklenip, 530 nm' de ilk spekrofotometrik okuma gerçekleştirildi. Daha sonra 10µl Reagent 2 eklenerek oda sıcaklığında 10 dakika bekletilip 530 nm' de ikinci spektrofotometrik okuma yapıldı. Aynı işlem farklı çözücüler kullanılarak çıkarılan meyve özütleri için teker teker yapılarak ölçüm tamamlandı.

TOS Değerinin hesaplanmasında kullanılan formül şu şekildedir:

TOS (µmol/L) = (Abs. Örnek / AbsStandart2) × 20 (Standart2 Değeri)

Abs.Örnek =(Örneğin ikinci ABS.- Örneğin birinci ABS.)
AbsStandart2: (Std2'in ikinci Abs.- Std2'in birinci ABS.)
Standart 2 Değeri = 20 µmol H_2O_2 Equiv./L

Berberis crataegina özütlerinin, antimikrobiyal aktivitelerinin değerlendirilmesi için *Stenotrophomonas maltophilia* bakterisi kullanıldı. Antimikrobiyal aktivite tayini amacıyla disk difüzyon testi European Committee on Antimicrobial Susceptibility Testing (EUCAST) kriterleri göz önüne alınarak uygulandı. Daha önceden hazırlanmış olan *B.crateegina*+etanol, *B.crateegina*+metanol, *B.crateegina*+hekzan ve *B.crateegina*+diklorometan örnekleri 6mm çapındaki steril blank (boş) disklere 30µl olmak üzere emdirildi. Daha sonra önceden besiyerlerinde üreme kontrolleri yapılan S. maltophilia bakteri suşundan bir miktar alınarak ayrı ayrı SF içerisinde 0.5 Baryum Sülfat Bulanıklık Standartı baz alınarak Mcfarland cihazında belirlemeler yapıldı. Vortekslenen bu süspansiyonlar daha sonra Mueller-Hinton besiyerlerine 100 µl inoküle edilerek steril eküvyon çubuk ile yayılıp disk difüzyon için kullanılmak amacıyla ekimi yapıldı. On dakika süreyle kurumaya bırakıldı. Bu aşamalardan sonra, önceden hazırlanıp kurutulmuş diskler steril bir penset yardımıyla besiyeri üzerine yerleştirildi. Hazırlanan besiyerleri 24 saat süreyle 37°C'de inkübe edildi. İnkübasyon süresi sonunca disklerin etrafında zon oluşup oluşmadığı incelendi.

BULGULAR

Aşağıda yer alan bulgular laboratuvarda yapılan işlemler sonucunda elde edilmiştir.

Tablo 1. Karamuk TAS değerleri

Etanol	4,30337079
Metanol	5,09612984
Hekzan	0,89138577
Diklorometan	1,23470662

TAS REFERANS DEĞERLERİ (mm Trolox Equiv/L)		
>2.0		Çok iyi
1,45	2,0	Normal
1,2	1,45	Normal Kabul Edilebilir
1	1,1	Düşük Antioksidan Seviyesi
<1,20		Çok Düşük Antioksidan Seviyesi

Şekil 1. TAS referans değerleri

Tablo 1'de laboratuar çalışmaları sonucu verilen TAS değerleri, Şekil 1'de verilen TAS referans değerleriyle karşılaştırıldığında etanol ve metanol özütlerinin çok yüksek antioksidan özellik gösterdiği, hekzan özütünün çok düşük antioksidan özellik gösterdiği ve diklorometan özütünün ise normal seviyede antioksidan özellik gösterdiği görülmektedir.

Tablo 2. Karamuk TOS Değerleri

Etanol	41,5799041
Metanol	----------
Metanol ½	23,2013006
Hekzan	24,2274004
Diklorometan	14,311684

TOS REFERANS DEĞERLERİ (µmol H_2O_2 Equiv/L)		
<5.0		Çok iyi
8	5	Normal
12	8	Yüksek Oksidan Seviyesi
>12		Çok Yüksek Oksidan Seviyesi

Şekil 2. TOS referans değerleri

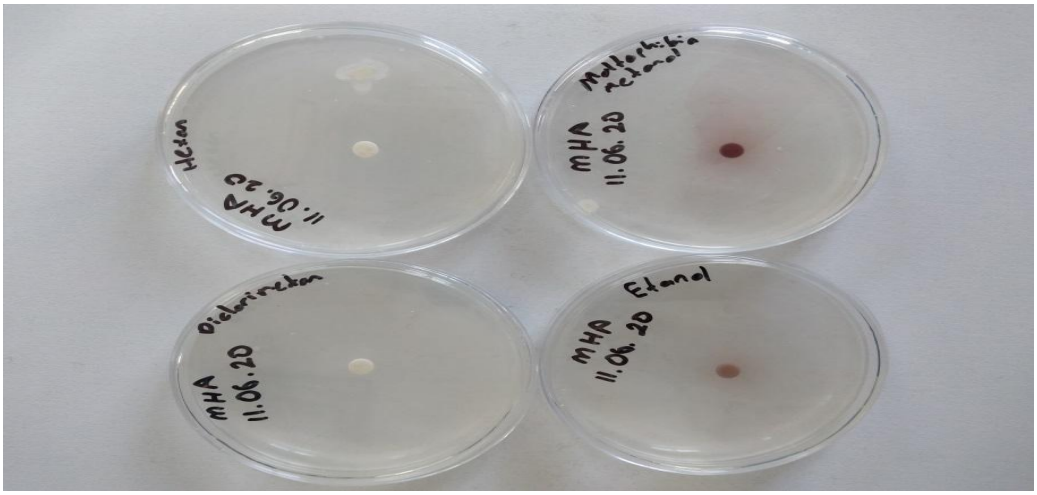
Tablo 2'de laboratuar çalışmaları sonucu verilen TOS değerleri, Şekil 2'de verilen TOS referans değerleriyle karşılaştırıldığında *Berberis crataegina* (Karamuk) meyvesinin etanol, hekzan ve diklorometan özütlerinin çok yüksek seviyede oksidan özellik gösterdiği görülmektedir. Metanol özütünün ise ilk ölçümde sonuç vermediği buna karşılık ½ oranında sulandırıldığında çok yüksek oksidan seviyesinde sonuç verdiği görülmektedir.

Tablo 3. Disk difüzyon yöntemi sonucunda ölçülen zon çapları

Örnek Adı	*Stenotrophomonas maltophilia*
Etanol	X
Metanol	X
Hekzan	X
Diklorometan	X

(“ x“ Zon oluşumu gözlemlenmeyen diskler)

Berberis crataegina meyvesinden elde edilen etanol metanol hekzan ve diklorometan çözücüleriyle ekstre edilen meyve özütleri *Stenotrophomonas maltophilia* bakterisine karşı antibakteriyel etkinliğinin olmadığı gözlenmektedir. Test sonucunda anlamlı denecek bir zon oluşumu kaydedilmemiştir.



Şekil 3. Disk difüzyon test sonucu

TARTIŞMA ve SONUÇ

Çalışma kapsamında *Berberis crataegina* meyve özütlerinin antioksidan ve oksidan seviyeleri ortaya konmaya çalışılmıştır. Böylece çalışmada elde edilen oksidan ve antioksidan seviyeleri kıyaslanarak çalışma kapsamındaki özütlerin en düşük oksidan ve en yüksek antioksidan seviyeleri kayıt altına alınmıştır. TAS sonuçlarına göre; etanol ve metanol özütlerinin çok yüksek antioksidan özellik gösterdiği, hekzan özütünün çok düşük antioksidan özellik gösterdiği, diklorometan özütünün ise normal seviyede antioksidan özellik gösterdiği görülmektedir. TOS sonuçlarına göre; *Berberis crataegina* (Karamuk) meyvesinin etanol, hekzan ve diklorometan özütlerinin çok yüksek seviyede oksidan özellik gösterdiği görülmektedir. Metanol özütünün ilk ölçümde sonuç vermediği buna karşılık ½ oranında sulandırıldığında ise çok yüksek oksidan seviyeye sahip olduğu görülmektedir.
Çalışmada hastane infeksiyonu sebebi olan *Stenotrophomonas maltophilia* bakterisine karşı *Berberis crataegina* meyvesinin antibakteriyel etkileri araştırıldığında bu bakteriye karşı herhangi bir etkinliğinin olmadığı gözlemlenmiştir.

Berberis crataegina (Karamuk) bitkisi ile yapılan değişik çalışmalar ile bizim yaptığımız çalışmanın antioksidan parametre sonuçları paralellik göstermekte ve desteklenmektedir (Yeşilada ve Küpeli, 2002; Khaleghi vd., 2013; Chareszah vd., 2015). Berberis crategina (Karamuk) bitkisinin antimikrobiyal etkisinin araştırıldığı çalışmalara bakıldığında ise oldukça az sayıda çalışma olduğu görülmektedir. Bu çalışmalarda Staphylococcus aureus, Streptococcus pyogenes (Vilinski vd., 2003), Bacillus cereus, Salmonella typhimurium, Yersinia enterocolitica (Eroğlu vd., 2020), E. coli (Shahverdi vd., 2007) gibi bakterilerde Berberis crataegina meyve özütlerinin aktivitesi incelenmiş olup; çalışmalar sonucunda bu bakterilere karşı inhibe edici özellik gösterdiği saptanmıştır.

Bu çalışma ile *Berberis crataegina* meyvesinin antioksidan aktiviteye sahip olduğu, *Stenotrophomonas maltophilia* bakterisine karşı ise antibakteriyel bir etkisinin olmadığı ve bu bitkinin antioksidan ve antibakteriyel etkisinin belirlenmesinin daha sonra yapılacak çalışmalara ışık tutacağı kanısındayız.

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➢ *ORAL PRESENTATION*

Benzimidazol-SiO_2 biyonanokompozitlerin dental uygulamaları

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Özet

Fonksiyonel nanopartiküller. yara bakımı, ilaç dağıtımı, biyomateryal iskeleler, doku mühendisliği ve diş uygulamaları gibi çeşitli biyomedikal uygulamalarda yaygın olarak kullanılmaktadır. Diş uygulamalarının önemli bir kısmını oluşturan dental kompozitler, ağız sağlığının önemli bir parçasıdır. Bu çalışmada, dental kompozit uygulamaları için yeni fonksiyonel nanokompozit tasarımı yapılmıştır. Ortalama boyutu 30-50 nm olan epoksi silika (SiO_2) nanopartikülleri benzimidazol ile fonksiyonel hale getirilmiştir. Fonsiyonel hale getirilen benzimidazol ile titanyum dioksit nanopartikülleri reçineye karıştırılarak foto polimerizasyon yöntemi ile kompozitler hazırlanmıştır. Biyonanokompozitler, benzimidazol-SiO_2 nanopartiküller üzerindeki işlevselleştirmenin bağlanmasını doğrulamak için FT-IR spektroskopisi ile karakterize edildi. X-ışını kırınım çalışmaları, malzemelerin amorf karakterinin benzimidazol-SiO_2 içeriği ile geliştiğini göstermiştir. Termogravimetrik analiz, düşük sıcaklıklarda, biyonanokompozitlerin termal stabilitesinin, içinde polimer bulunmayan numuneye göre daha yüksek olduğunu göstermiştir.Taranan elektron mikroskobik analiz sonuçları, benzimidazol-SiO_2' nin biyonanokompozitlerde homojen dağılımını doğrulamıştır. Azol fonksiyonel benzimidazol-SiO_2' nin antibakteriyel aktivitesi göz önüne alındığında, nanokompozitlerin dental uygulamalarda daha iyi performans sergileyeceği düşünülmektedir.

Anahtar Kelimeler: Dental kompozitler, fonksiyonel nanopartiküller, benzimidazol, titanyum dioksit

Abstract

Functional nanoparticles are widely used in various biomedical applications such as wound care, drug delivery, biomaterial scaffolds, tissue engineering and dental applications. Dental composites, which constitute an important part of dental applications, are an important part of oral health. In this study, a new functional nanocomposite design has been made for dental composite applications. Epoxy silica (SiO_2) nanoparticles with an average size of 30-50 nm were functionalized with benzimidazole. Composites were prepared by photopolymerization method by mixing the benzimidazole and titanium dioxide nanoparticles into the resin. Bionanocomposites were characterized by FT-IR spectroscopy to verify the binding of functionalization on benzimidazole-SiO_2 nanoparticles. X-ray diffraction studies have shown that the amorphous character of the materials is improved with the benzimidazole-SiO_2 content. Thermogravimetric analysis showed that the thermal stability of bionanocomposites at low temperatures was higher than the sample without polymer. Scanned electron microscopic analysis results confirmed the homogeneous distribution of benzimidazole SiO_2 in bionanocomposites. Considering the antibacterial activity of azole functional benzimidazole-SiO_2, it is thought that nanocomposites will perform better in dental applications.

Keywords: Dental composites, functional nanoparticles, benzimidazole, titanium dioxide

GİRİŞ

Polimer bazlı reçineler diş tedavisi teknolojisinde onlarca yıldır kullanılmaktadır. Bu malzemeler gelecek vaat eden adaylar olarak kullanılsa da polimerizasyon sırasında restorasyon başarısızlığı, yetersiz mekanik mukavemet, zayıf aşınma direnci ve yüksek hacimsel büzülme nedeniyle ciddi sorunlarla karşılaşılmaktadır (Wang ve ark., 2018). Hacimsel büzülme, büyük bir sekonder diş çürüğüne, renk değişikliğine ve sızıntı potansiyeline yol açabilmektedir. Bu sorunlar diş kompozitlerinin restorasyon performansını büyük ölçüde etkilemektedir (Li ve ark., 2020; Atalayın ve ark., 2016).

Kompozit diş malzemeleri, klinik diş hekimliğinde uzun süreli performansı sayesinde dezavantajları minimuma indirmek için kullanılan yöntemlerden biridir. Dental kompozitlerin fiziksel özellikleri, partikül boyutu ve dolgu hacminden büyük ölçüde etkilenebilir, bunun nedeni silanizasyon ve yükleme kapasitesidir. Polimer matris içerisindeki dolgu maddesinin hacim fraksiyonu arttıkça polimerizasyon büzülmesinin azalmaktadır (Djustiana ve ark., 2018; Wen ve ark., 2019). Ayrıca, kompozit malzemelerin sertliğini, basınç dayanımı, elastik modülü ve eğilme dayanımı iyileştirmektedir. Dolgu içeriği optimizasyonu, paketleme ve hibrit katkı maddelerinin geliştirilmesini içeren malzemelerin modifikasyonu, polimer kompozit reçinelerin performans iyileştirmesinin alternatif ve etkili bir yolu olarak kabul edilmektedir (Yadav ve Kumar, 2019).

Son zamanlarda, nanopartiküllerin (NP' ler) dolgu maddesi olarak kullanılması, polimerizasyon büzülmesini azaltmakta, mekanik özellikleri iyileştirmekte ve aşınma hızını azaltarak avantaj sağlamaktadır (Rawashdeh ve ark., 2020). NP' lerin büyük yüzey alanı ve yüzeyde fonksiyonel grupları içermesi malzemenin iyileştirilmesini sağlamaktadır. Reçineye NP'lerin eklenmesi bir takım avantajlar göstermiş olsa da, klinik terapide uzun vadeli restorasyon amacına ulaşmak için daha fazla ilerlemeye ihtiyaç vardır (Nguyen ve ark., 2019; Azizi-Lalabadi ve ark., 2019).

Azol iskeleler, etkili antimikrobiyal ajanlar üretmek için en uygun yapılar olarak kabul edilmektedir. Bu çalışmanın amacı, azol bağlantılı nanosilika içeren dental nanokompozit reçinelerin fizikokimyasal ve biyolojik özelliklerini araştırmaktır (Yushau ve ark., 2019). Fonksiyonel SiO_2' ler benzimidazoller ile modifiye edildikten sonra dolgu malzemesi olarak farklı ağırlık yüzdeleri ile reçinelere eklenmiştir. Fonksiyonel gruplar, Fourier dönüşüm kızılötesi spektroskopisi (FTIR) ile karakterize edildi ve hazırlanan nanokompozitlerin yüzey morfolojisi, taramalı elektron mikroskobu (SEM) ile analiz edilmiştir. Nihai ürünlerin termal stabilitesini incelemek için termogravimetrik analiz (TGA) kullanıldı. Malzemelerin antibakteriyel özellikleri ve kimyasal stabilizasyonu analiz edildi.

MATERYAL VE METOD

Kimyasallar

Silika (%99.5), epiklorohidrin, dimetil sülfoksit (DMSO, ≥99.9%), benzimidazol (%98), titanyum dioksit (TiO_2, ≥99.9%), bisfenol A gliserolat dimetakrilat (BisGMA), trietil gliserol dimetakrilat (TEGDMA), kamfokinon (CQ), etil-4-(dimetilamino)benzonat (EDMAB), etil alkol Sigma-Aldrich' den satın alınmıştır.

Fonksiyonel silika nanopartiküllerinin hazırlanması

Fonksiyonel SiO_2 sentezi, için 1 g SiO_2 50 ml DMSO içerisinde ultrasonik banyoda 10 dk disperse edildikten sonra azot atmosfersinde damla damla epiklorohidrin eklenerek 70°C 24 saat karıştırılmaktadır. Etil alkol/distile su ile yıkanarak 80°C' de etüvde kurutulur (Aslan ve ark., 2019).

Fonksiyonel silika nanopartiküllerinin benzimidazol ile modifikasyonu

Yüzeyi epoksi grupları ile kaplanan SiO_2'ye benzimidazol bağlanması için kuruyan örnek 30 ml DMSO içerisinde ultrasonik banyoda 5-10 dk disperse edilmektedir. Disperse solüsyonun içerisine 5 gr benzimidazol eklenerek 100°C de 72 saat karıştırılmaktadır. Sentez sonrası safsızlıkların giderilmesi için SiO_2-benzimidazol(b-SiO_2) biyonanopartiküllerin etil alkol/distile su karışımı ile yıkanır. b-SiO_2 son olarak 72 saat etüvde bekletilerek konjugasyon basamağına geçilmektedir (Han ve ark., 2017; Aslan ve Bozkurt, 2010; Aslan ve ark., 2019).

Dental biyonanokompozitlerin hazırlanması

Dental biyonanokompozit, b-SiO_2, SiO_2 ve TiO_2 farklı ağırlık yüzdelerinde sentezlendi. İlk olarak, monomerler BisGMA ve TEGDMA (ağırlıkça%50:50) karıştırılarak 50°C' de homojenleştirildi. Daha sonra, farklı ağırlık yüzdelerinde b-SiO_2, SiO_2ve TiO_2 elle spatülasyon ile yaklaşık 25 dakika süreyle karıştırıldı. Tablo 1' de biyonanokompozitin bileşim oranları yer almaktadır. Bu işlemi, CQ (ağırlıkça %0.1) ve EDMAB' dan (ağırlıkça %0.4) oluşan bir başlatıcı/koinitatör sisteminin eklenmesi takip etti. Viskoz malzemeler teflon kalıbına yerleştirildi ve karışımı ışınlamak için 60 saniye boyunca dalga boyu: 450-500 nm ve güç yoğunluğu: 1000 mW cm^2 olan LED ışık kaynağı kullanıldı.

Tablo 1. Dental biyonanokompozitlerde yer alan inorganik içerik oranları

Dental biyonanokompozit çeşidi	b-SiO_2 (%w)	SiO_2 (%w)	TiO_2 (%w)
B0	0	50	50
B10	10	45	45
B20	20	40	40
B30	30	35	35

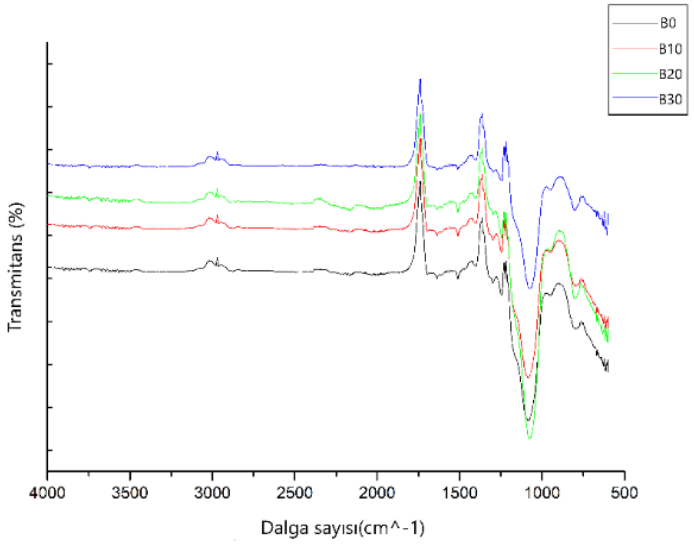
Karakterizasyon

Fonksiyonel yapıların varlığı, FT-IR Pelkin Elmer Spectrum 100 kullanılarak tespit edildi. Fonksiyonelleştirilmiş biyonanokompozitlerin termal stabiliteleri, Termogravimetrik Analiz (TGA), Shimadzu TA-60 W.s kullanılarak gerçekleştirildi. Malzemeler (5 mg), 10°C/dakika hızında oda sıcaklığından 700 ° C'ye tarandı. Sentezlenen nanopartiküllerin morfolojisini gözlemlemek ve boyutu ile ilgili bilgi sahibi olmak için Taramalı Elektron Mikroskobu FEI (PHILIPS) XL30 SFEG SEM kullanıldı. Dental malzemelerin kimyasal stabilitelerinin tayini için fenton test yapıldı. Antibakteriyel çalışmalar için, üniform kuyulara sahip dental kompozitlerin diskleri (3 mm kalınlık ve 5 mm çap) Teflon kontrol ile birlikte kullanıldı.

BULGULAR ve TARTIŞMA

FTIR analizi

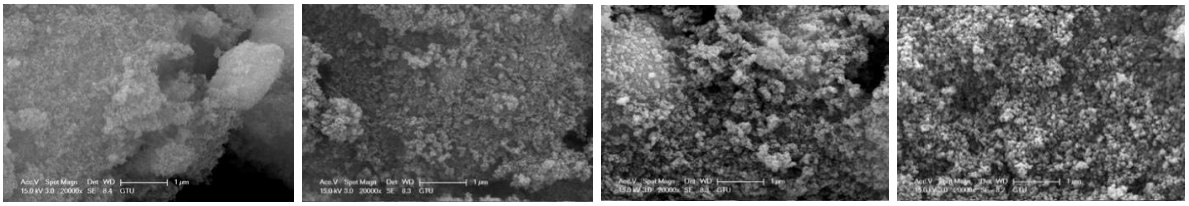
Şekil 1, 1064 cm^{-1} geniş tepeli Si-O-Si oluşumuna bağlanabilir. 1220 ve 1083 cm^{-1}'deki zirveler Si–O uzamasından kaynaklıdır. Yaklaşık 795 cm^{-1} bant, Si–O–Si' nin esneme simetrisine atfedilir. b-SiO_2, azolik halkaya ait 1420, 1520 ve 1670 cm^{-1}' deki absorpsiyon zirvelerine sahiptir (Aslan ve ark., 2019). 2963 cm^{-1} ila 2850 cm^{-1}' deki pikler, polimerin $–CH_3$ ve $–CH_2$ gruplarına (asimetrik ve simetrik esneme, güçlü ve keskin) karşılık gelir. Zirvenin BisGMA' nın C–OH grupları nedeniyle 3479 cm^{-1}' de ortalandığı, 3050–3038 cm^{-1}' deki küçük dorukların =C–H bağlarından (veya aromatik H) kaynaklandığı açıktır. 1170 cm^{-1}' deki zirveler C–O bağlarına atfedilir. 1610 cm^{-1}'de bir absorbans, BisGMA kompozit matrisin aromatik C=C bağlarına aittir (Al-Odayni ve ark., 2019).



Şekil 1. Dental biyonanokompozitlerin FTIR analizi

SEM analizi

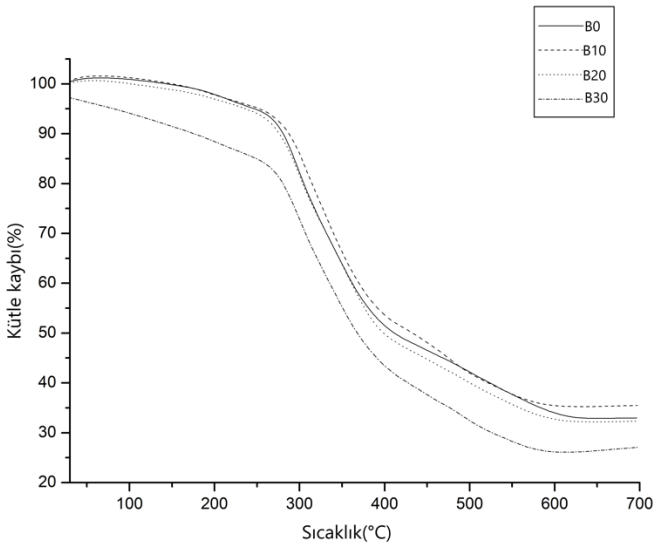
B serisinin yüzey morfolojileri, Şekil 2'de gösterildiği gibi SEM ile incelenmiştir. Nanokompozit B10-B30 serisi resimleri, numunelerin iletken bir tabaka ile kaplanmasından sonra elde edilmiştir. B10' nun hafif pürüzlü bir yüzeye sahip olduğu ve dolgu maddelerinin matris içinde dağıldığı ve sonuçta faz ayrışmasına neden olmadığı açıktır. Ek olarak hem B20 hem de B30 pürüzlü bir yüzeye sahiptir ve nanopartikülllerin dağılımı homojendir. Ayrıca, B30'un yüzey pürüzlülüğü B10'dan daha yüksektir ve biyonanopartiküller oldukça iyi dağılmıştır (Barot ve ark., 2020). Sonuç olarak, nano katkı maddelerinin bu homojen dağılımı, nanokompozitlerin mekanik özelliklerini önemli ölçüde iyileştireceği düşünülmektedir.



Şekil 2. Dental biyonanokompozitlerin SEM görüntüleri

Termal analiz

B serisindeki biyonanokompozitleri temsil eden TGA eğrisi Şekil 3'te gösterilmektedir. Daha önce araştırılan dental reçineler için polimer matrisi, 3 boyutlu çapraz bağlı bir yapı verir. Yapı, inert bir atmosferde termal ayrışmaya karşı oldukça dayanıklıdır. Polimer bağını kırmak için çok fazla enerji gerekir. Organik matris ve azollerin ayrışması, sıcaklık 400ºC' nin altında olduğunda meydana gelir. Bununla birlikte, sıcaklık 400ºC'nin üzerinde olduğunda, kütle kaybı inorganik fazdan kaynaklanmaktadır. Monomerlerin (BisGMA ve TEGDMA) ayrışma davranışları kimyasal yapılarından önemli ölçüde etkilenir (Wu ve ark., 2014).



Şekil 3. Dental biyonanokompozitlerin TGA analizi

Fenton reaksiyon analizi

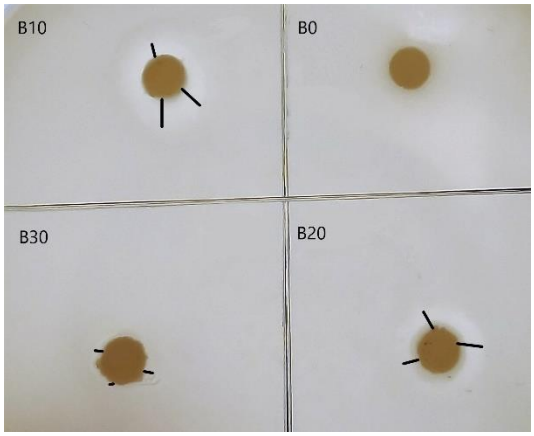
Fenton reaktifi, solüsyonda serbest radikal üretimine yol açmaktadır. Bu nedenle, dental biyonanokompozitlerin kimyasal stabilitesini değerlendirmek için yaygın olarak kullanılmıştır. Fenton solüsyonunda 48 saat bekletilen biyonanokompozit serisi B0-B30'ın kimyasal stabilitesi büyük ölçüde korunduğu Tablo 1' de gösterilmiştir.

Tablo 1. Fenton reaksiyon sonuçları

Örnek	M1(g)	M2 (2 gün bekletilip dH_2= ile yıkanıp 40 derecede kurutuldu)	Malzeme Kaybı (mg/mm^3) %	Malzeme Kaybı(g) %
B0	0.0892	0.0840	%7.63	6.52
B10	0.1354	0.1344	%1.39	0.73
B20	0.0966	0.0941	%1.78	1.55
B30	0.0696	0.0678	%1.92	1.46

Antibakteriyel analiz

Optimize edilmiş koşullar altında hazırlanan biyonanokompozitler, *Escherichia coli* (E.coli) bakterisine karşı antibakteriyel aktiviteleri açısından incelenmiştir. Antibakteriyel aktiviteleri test etmek için agar difüzyon yöntemi kullanıldı. E. coli, lysogen broth (LB) içerisinde gece boyunca 37°C'de büyütüldü. LB agar petrilere E. Coli ekildikten sonra biyonanokompozitler yerleştirilerek 18 saat 37°C'de inkübe edildi (Caneli ve ark., 2020; Jin-Lee ve ark., 2017). Sentezlenmiş biyonanokompozitlerin, E.coli bakterisine karşı oluşturdukları zon bölgeleri Şekil 4' te, çapları Tablo 2' de yer almaktadır. Aynı zamanda, biyonanokompozitlerin E. coli' nin büyümesini yavaşlattığı da görülebilir.



Şekil 4. E. Coli bakterisine karşı dental biyonanokompozitlerin oluşturdukları zon bölgeleri

Tablo 2. Zon çapları

Mikroorganizma	Örnek	n	minimum	maksimum	ortalama	Standart sapma
Escheria coli	B0	3	1	1	1.00	0
	B10	3	5	7	6.34	1,083
	B20	3	4	6	4.67	1,078
	B30	3	1	3	2.00	1.00

SONUÇ

Silika benzimidazol nanopartiküller işlevselleştirilerek, TiO_2 ve SiO_2 ile birlikte BisGMA / TEGDMA' ya dahil edildi. FTIR, materyallerin sentezini doğrulamak için yapıldı. Katkı maddelerinin farklı ağırlık yüzdelerini temsil eden B0-B30 kompozitleri, SEM, TGA, XRD, fenton ve antibakteriyel test ile incelenmiştir. SEM sonuçları, polimer matrisinde iyi bir dağılım işlevi gören nanosilikayı doğruladı. TGA, kompozitlerin ayrışmasının 200ºC civarında başladığını göstermiştir. Ayrıca, kompozitler B serisi üzerinde yapılan sitotoksisite çalışmaları, B10' un diğer örneklere kıyasla antibakteriyel özelliğinin daha iyi olduğu ortaya koyulmuştur. Sonuç olarak, polimer reçinesine yüzeyi modifiye edilmiş silika nanopartiküllerin dahil edilmesi ile oluşturulmuş kompozitlerin klinik uygulamalar için uygun olduğu görülmüştür.

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➢ *ORAL PRESENTATION*

Arı Sütü (Royal Jelly) ve Sağlık İçin Önemi

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Özet

Arı sütü; arının mandibular ve hipofarangal bezlerinden salgılanır. Bu madde Kraliçe arının besin maddesidir. Belirli mevsimlerde ve az miktarlarda üretilebilen değerli bir arı ürünüdür. Eski yüzyıllardan beri bilinen ve kullanılan arı sütünün, biyolojik önemi, insan sağlığına etkileri üzerine ilgi son zamanlarda artmıştır. Hala merak konusu olan arı sütü araştırılmaya devam etmektedir. Bu derlemede arı sütünün yapısı ve içeriği tanıtılacak, insan sağlığı açısından önemine değinilecektir.

Anahtar Kelimeler: Arı Sütü, Royal Jelly, Bal Arısı

Royal Jelly and Its Importance for Health

Abstract

Royal Jellly; It is secreted from the mandibular and hypopharangal glands of the bee. This substance is the food of the queen bee. It is a valuable bee product that can be produced in certain seasons and in small amounts. Interest on the biological significance and effects of royal jelly, known and used since ancient times, on human health has recently increased. Still a matter of curiosity, royal jelly continues to be investigated. In this review, the structure and content of royal jelly will be introduced and its importance for human health will be emphasized.

Keywords: Royal Jelly, Honey bee

GİRİŞ

Arı sütü Royal Jelly olarak anılan sarı beyaz renkli yoğun kıvamlı doğal bir arı ürünüdür. Arı sütü arının Mandibular ve hipofarangal bezlerden salgılanır. Genç larvalar için bir besin maddesidir. Bu ürün kraliçe arı için sadece bir besin maddesi değil, kraliçe arı olması için gereken mekanizmanın etken maddesidir (Khazai vd., 2017). Arı sütünde bulunan royalaktin maddesinin juvenil hormon salgısını arttırdığı ve aynı zamanda kraliçe arıda boy uzunluğu üzerine etkili olduğu yapılan araştırmalarla gösterilmiştir (Kambur vd., 2009). Kraliçe arının gelişmesi için önemli bir besin maddesi olan Arı sütünün insan sağlığı için de çok önemli bir besin olduğu yapılan araştırmalarla keşfedilmeye başlanmıştır. Yapılan çalışmalar arı sütünün antitümör etkisi, antimikrobiyal aktivitesi antialerjik, antioksidan aktivitesi üzerinde durmuş olsa da (Gasic vd., 2007; Vittek 1995; Fontana vd., 2004; Fujiwara vd., 1990; Kloudiny vd., 2005; Okomoto vd., 2003; Kohno vd., 2004; Sver vd., 1996). Bu ürün hakkında henüz yeterince çalışma mevcut değildir ve göstermiş olduğu iyileştirme etkisinin mekanizması henüz tam olarak açıklanmamıştır.

BULGULAR ve TARTIŞMA

Arı sütü ile ilgili kesinleşmiş veriler bulunamasa da Çin'in arı sütü üretiminde önde gelen ülkelerden olduğu bilinmektedir. Dünyada arı sütü üretiminin yaklaşık olarak %90'ını Çin üretmektedir Arı sütü üretiminde Çini Takibeden ülkeler ise Japonya ve Kore'dir. Üretilen arı sütünün en büyük alıcıları, başta Japonya ve ABD olmak üzere kişi başına gelir düzeyi yüksek olan Avrupa ülkeleridir (Piana 1993, Sabatini et al 2009; Ramadan ve Al-Ghamdi, 2012; Clarke ve McDonald, 2017; Cao et al 2016)

Arı Sütünün Yapısı ve Özellikleri

Arı sütü viskoz kıvamında, yoğunluğu 1,1 g/ml ve pH'sı 3,4-4,5 olan bir maddedir. Bej renkli olup depolama süresi uzadıkça rengi koyulaşmaktadır. Kokusu ve tadı karakteristik ve belirleyicidir. Arı sütü güneş ışınlarından, nemden, ısıdan, havadan çok çabuk etkilenir ve özelliğini kaybedebilir. ((Uçar, 2018; Akyol ve Baran, 2015). Bu sebeple arı sütünün koyu renk kaplarda gün ışığından uzak tutarak ve serin bir ortamda saklamak gerekmektedir.

Yapılan çalışmalarda arı sütünün standardizasyonu için en önemli kalite kriterleri, 10-Hidroksi-2-Decenoik Asittir (HDA). Arı sütünün depolanmasıyla 10-HDA içeriği azalır. Arı sütünün kimyasal yapısı üretildiği sezona, bölgeye, arı sütü üretiminde kullanılan kolonilerin ırkına ve besleme durumuna bağlı olarak önemli düzeyde değişim gösterebilmektedir (Antinelli ve ark., 2003, Karacaoğlu ve ark., 2004; Şahinler ve Kaftanoğlu, 2005; Çelik ve ark., 2017).

Karbonhidratlar

Arı sütünün içeriğinin yaklaşık olarak %30'u karbonhidratlardan oluşur (Sabatini ve ark., 2009, Daniele ve Casabianca, 2012) Şekerler bal da olduğu gibi benzer ve sabit oranlarda çoğunlukla fruktoz ve glukozdan oluşmaktadır. Fruktoz daha fazladır. Arı sütünde bunların dışında daha az oranda bulunan diğer şekerler ise maltoz, trihaloz, riboz ve diğer şekerler gibi küçük oligosakkarit izleri de belirlenmiştir (Finke, 2005; Kheyri ve ark., 2012; Çelik ve ark., 2017).

Proteinler ve Peptitler

Arı sütünün içeriğinin %15-50'i proteinler ve peptitlerden oluşmaktadır. Arı sütü proteinlerinin %80'ninden fazlasını çözünür proteinler ve temel arı sütü proteinlerinden (TASP) oluşmaktadır. TASP proteinleri, 49 ila 87 KDa arasında değişen moleküler kütleli 8 protein) tespit edilmiştir (Moriyama ve ark., 2015). TASP'lar pek çok esansiyel aminoasit içerirler. (Furusawa ve ark, 2008; Buttstedt ve ark., 2014). Özelikle TASP-5 adlı protein olmak üzere TASP 1, TASP 2, TASP 4 proteinleri arı büyümesi için çok önemli bir azot kaynağı olduğu bildirilmiştir (Schmitzova ve ark., 1998, Albert et al., 1999; Fratini ve ark., 2016) TASP'ların Kraliçe arı ve işçi arasındaki farklılaşmada önemli rol oynadıkları gösterilmiştir (Buttstedt ve ark. 2013). Diğer proteinler ile yapılan araştırmalar sonucunda, Royalisins'in antibakteriyel özelliklerini (Bilikova ve ark., 2001, Fujiwara ve ark., 1990). Royalactina'nın ise ana arının farklılaşmasında tetikleyici rol üstlendiği bildirilmiştir. (Kamakura,2011; Çelik ve ark., 2017).

Lipidler

Arı sütünün üretildiği koloni bulunduğu coğrafya ve hatta saklama koşullarına göre değişmekle birlikte arı sütünün kuru ağırlığının %3-%19'una sahip olan lipitler, içerik sıralamasında proteinlerden sonra ikinci sıradadır. %80-90'ı serbest yağ asitlerinden oluşur, Pek çok hayvan ve bitki materyallerinin aksine, arı sütünün yağ asitleri 8-10 karbon atomlu ve genellikle ya hidroksi yağ asidi veya dikarboksilik asit şeklinde bulunmaktadır. Bu yağ asitleri arı sütünün bildirilen pek çok biyolojik özelliklerinden sorumludur.

10 hidroksi-2-dekonoik asit en karakteristik asit içeriğidir ve yaklaşık %1,9 oranında görülür, onu doymuş eşdeğeri 10 hidroksidekonoik asit izlemektedir. Serbest yağ asitlerine ek olarak nötral lipitleri, sterolleri (kolesterol dahil) ve balmumunu içerir. 10-HDA da koloni stratejilerinin gelişiminde önemli bir biyolojik role sahip olduğunu göstermiştir (Wu ve ark., 1991; Çelik ve ark., 2017).

Vitaminler ve Mineraller

Mineraller Vitaminler ve diğer elementler arı sütünün kuru maddesinin yaklaşık %4-8'i kadardır. Ana elementler K, P, bunları takip eden oranlarda Ni, Cr, Sn, W, Sb, Ti ve Bi. Bulunmaktadır (Li ve Chen, 2003, Ramadan ve Al-Ghamdi, 2012). Arı sütü vitaminler bakımından oldukça zengindir. İçerdiği vitaminler; riboflavin, tiamin, niasin ve folik asit, piridoksin, biyotin, pantotenik asit ve inositol ve az miktarda vitamin C içerir. (Viuda-Martos ve ark., 2008; Li ve ark., 2012). Arı sütü, A, D, E ve K vitaminleri gibi yağda eriyen vitaminler içermez Genel olarak Arı Sütü, B grubu vitaminler, özellikle B1 vitamini, olmak üzere, B2, B6, B8, B9 ve B12 vitaminlerince çok zengindir (Morita ve ark., 2012; Ramadan ve Al-Ghamdi, 2012).

Minerallerin varlığı ve çeşitliliği çevresel faktörler ile ilişkilidir ve dolayısıyla da değişkenlik gösterebilir (Sabatini ve arkadaşları, 2009). Ayrıca, Arı sütünde heterosiklik maddeler, biopterin ve neopterin gibi çeşitli kimyasal

sınıflar altında sınıflandırılan az sayıda küçük bileşenleri içerdiği belirlenmiştir (Bogdanov, 2012). Bunlara ek olarak arı sütünde düşük miktarlarda serbest nükleotitler (adenosin, guanosin, sitidin ve iridin), fosfatlar, ATP, ADP, AMP, asetilkolin ve glukonik, benzoik, malik, sitrik ve laktik asitler bulunmuştur (Sabatini ve ark., 2009; Bogdanov, 2012). Arı sütünün vitamin içeriği de aynı şekilde işçi arıların topladığı çiçeklerin poleninin varyasyonu olarak mevsimsel değişikliklere maruz kalmaktadır. Çünkü esas olarak vitamin kaynağı polenden gelmektedir (Biondi ve ark., 2003; Sabatini ve ark., 2009; Çelik ve ark., 2017).

Arı Sütünün Sağlık Alanında Kullanımı

Arı sütü biyolojik olarak aktif aminoasitleri içermektedir. Antioksidan etkisinin bu serbest amino asitlere bağlı olduğu düşünülmüştür (Walaa vd., 2014). İçeriğinde çok sayıda biyoaktif molekül içeren arı sütünün, aynı zamanda çok önemli bir antioksidan ve antitümör etkeni olarak sayılan 10-hidroksi-trans-2-dekonoik asit (10HDA) içerdiği de bilimsel çalışmalarla gösterilmiştir (Fujiwara vd., 1990; Khazai vd., 2017). Galay ve arkadaşları (2013), epilepsinin ve ona karşı kullanılan ilaçların oluşturduğu olumsuz etkinin iyileştirilmesi için yaptıkları çalışmalarında arı sütünün genotoksik ve oksidan etkiye karşı savunma özelliği olduğunu göstermişlerdir. Helal ve arkadaşları (2017), arı sütünün biyokimsal abnormaliteye karşı etkisini araştırmışlardır ve kan biyokimyasını iyileştirici etkisini gözlemlemişlerdir. Karaca ve arkadaşları (2013), deneysel diyabet oluşturduktan sonra arı sütünün histolojik olarak etkisini incelemişler ve olumlu ve destekleyici etkisinden bahsetmişlerdir. El-Nekeety ve arkadaşları (2007), arı sütünün antioksidan etkisini inceledikleri çalışmalarında farklı dozların da araştırılmasını önermişlerdir.

Sonuç

Arıcılığın doğru yönlendirilmesiyle sadece bal değil, başta arı sütü olmak üzere diğer arı ürünlerinin üretiminin de yapılması ve yaygınlaştırılması gerekmektedir. Son yıllarda özellikle sağlık alanında yapılan çalışmalarla bu ürünün değeri daha iyi anlaşılmıştır ve bu durum arı sütüne talebi arttırmıştır. Ancak bu talep yeterli değildir. Ürünlerin üretimindeki güçlükler fiyatlarına yansımakta ve insanlar bu ürüne ulaşmakta güçlük çekmektedir. Ayrıca tüketiciler aldıkları ürünlerdeki sahtecilik riskinden korkmaktadır. Hem biyolojik içeriğinin optimizasyonu hem de tüketici hakları açısından bu üründeki denetimler arttırılmalı, biyolojik önemi tanıtılmalı, hakkında daha çok araştırma yapılması teşvik edilmelidir.

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➢ *ORAL PRESENTATION*

Electrocoagulation Technique for Harvesting *Scenedesmus quadricauda sp.*: Assessment of Efficiency

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Abstract
Microalgae is a promising feedstock for various applications and it has gained appreciation in recent years. The high costs related with microalgal biomass harvest hinder its large-scale applications. The application of an efficient, low cost harvesting method is fundamental for achieving a viable process. For this reason, in this study electrocoagulation (EC) technique was used as a harvesting method to obtain microalga biomass from culture media. Electric current, time and pH parameters were investigated in EC experiments. The harvesting efficiency and energy were investigated using *Scenedesmus Quadricauda sp.* Electrocoagulation was tested with Al and Fe electrodes with initial pH values and adjusted values of 4, 7 and 12. The energy consumption and operating cost were calculated for electrocoagulation. The results showed that Al electrodes in EC experiments was more efficient in harvesting than Fe ones. The harvesting efficiency increased by increasing the applied electric current. The obtained results showed that the adjusted pH values with Al electrodes presented higher efficiency values. The power and energy consumption rose with an increase of the applied current thereby increasing the operating costs. The electrocoagulation technique achieved high efficiency values and appear to be a promising harvesting method.
Keywords: Microalgae, Scenedesmus Quadricauda sp., Harvesting, Electrocoagulation.

INTRODUCTION

In the recent years, the world has started to look for renewable energy sources to mitigate the risk of increased GHG (Greenhouse Gases) and researches tend to investigate various renewable energy sources (Cuellar-bermudez et al., 2020). Bioenergy that is obtained from biological sources such as biomass, has many advantages that caught the world's attention. Nowadays, bioenergy is seen as a clean and sustainable alternative to gradually replace fossil dependent energy. Carbon fixation using microalgae has become an option and is being studied extensively as part of a plan to reduce and mitigate greenhouse gases in the atmosphere (Wang et al., 2008). Microalgae can potentially fix carbon dioxide from 10 to 50 times more than terrestrial plants (Usui & Ikenouchi, 1997). With this regard, the use of microalgae is beneficial due to their high productivity, high growth rate and unique properties (Mathimani and Mallick, 2020). Before taking any advantage of microalgae biomass, they must be harvested in an efficient and economical way. Microalgae harvesting means to separate microalgae cells from its aqueous growth medium (Kim, 2015). The methods used for harvesting of microalgae vary such as chemical, biological, electrical and mechanical or combination of different harvesting techniques (Mathimani and Mallick, 2020). However, microalgae harvesting processes still face many challenges, depending on the applied process (Barros et al., 2015; Wan et al., 2015). These difficulties include high costs which require high input energy, expensive equipment, and microalgae contamination in the case of the use of chemical coagulants (Barros et al., 2015). Furthermore, the small cell size and relatively low concentration in the big volume of the growth medium are the main challenges (Mathimani and Mallick, 2020). This paper aims to explain and summarize the electrocoagulation method in the harvesting of microalgae for the *Scenedesmus quadricauda sp.* Microalgae cultivation and purification of culture were explained. The effects of different parameters such as electric current, pH, time and electrode type on harvesting efficiency were investigated.

MATERIALS AND METHODS

Microalgae Species and Cultivation

Experiments were performed with *Scenedesmus quadricauda sp.* and it was investigated by using the light microscope to verify its morphologically. *Scenedesmus quadricauda sp.* is a green fresh water microalgae and the cells attach side by side in small colonies of 2, 4 or 8 cells (Figure 1) (Shawky et al., 2015). The culture was purified in petri dishes and the cultivation of each purified microalgae was followed using one-liter flask as photobioreactor. Cell growth of each microalgae species was determined by daily measuring of absorbance capacity of the microalgae sample using a spectrophotometer at 680 nm via absorbance (UV 1800 UV-VIS spectrophotometer Shimadzu, Japan). A calibration curve was obtained for the convertion between biomass dried mass density and optical density. A calibration curve was needed to modify the optical density (OD680nm) to concentration (mg/L) in dry weight basis. To even increase the cultivation volume, 1 L erlenmeyer flasks with microalgae species were transferred to the 10 L sterilized flasks and filled with autoclaved BG11.

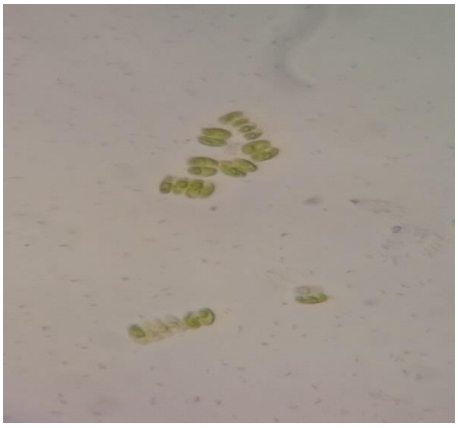


Figure 1. Microscopic view of *Scenedesmus quadricauda sp.* scale bar 100 (oil immersion)

Harvesting Experiments

Electrocoagulation (EC) experiments:

Three different electric currents such as 0.2 A, 0.5 A and 0.8 A were applied. Aluminum and iron electrodes were used in EC experiments and each electrode consisted of 6 parallel plates. Figure 2 shows the EC reactor design with Fe and Al electrodes. The EC cell consisted of a 500 mL beaker equipped with Fe electrodes connected to a DC power supply and put on magnetic stirrer plate. The sample volume was 300 mL and the EC experiment operating time was 30 min. For the experiment, each sample was left to settle for 30 min before reading optical density (OD), also was later left 24 h for more settling time before reading OD. The first run of the EC experiment was performed with Fe electrodes and the electric current was 0.2 A. The mixture was rotated at 200 rpm for 30 min by a magnetic stirrer (Biosan MSH-300, Magnetic Stirrer with hot plate, Latvia). The samples were taken at 5, 10, 20, and 30 min time intervals. The voltages were recorded from the screen of the DC power supply over the experiment at each time interval. The first three runs of the experiments were performed with the initial values of pH medium. The samples were taken to 10 mL tubes and left for 30 min before testing the OD. The pH was measured by the pH meter (edge® Multiparameter pH Meter - HI2020, USA) for the initial microalgae culture and for each sample, likewise the conductivity was measured by conductivity meter (WTW Inolab conductivity meter Level 1, Germany). The second and third runs of the experiments were carried out at electric currents of 0.5 A and 0.8 A, respectively with original pH of the culture. The fourth, fifth, and sixth runs were done with the same processes, but with adjusted pH at 4, 7 and 12 with I: 0.5 A as an average point. The adjustment of pH was done by adding appropriate amounts of 1 M NaOH and 1 M H_2SO_4 to the microalgae solution. For the Al electrodes the same processes were performed with the original pH of the microalgae cultures and at electric currents of 0.2 A, 0.5 A and 0.8 A. Further experiments were performed with the three adjusted pH values at the current of 0.5 A.

Microalgae recovery efficiency was calculated by using Equation 1 (Fayad et al., 2017). The electrode consumption for Al and Fe electrodes was calculated by Faraday 's law (Equation 2) (Gao et al., 2010).

Recovery efficiency % = (OD initial – OD t /OD initial) × 100 (1)

where OD initial is the optical density of the initial culture before the EC treatment and OD t is the optical density at the selected settling time after EC.

Faraday's law: $W = I \times t \times M / n \times F$ (2)

where W is dissolved metal (gram of Al or Fe), I is electric current A, t is the time of electrolysis (seconds), M is molecular weight of Al electrodes or Fe electrodes which (26.98 g mol^{-1}) and (55.8450 g mol^{-1}), respectively. F is Faraday's constant (96.487 C mol^{-1}), n is the number of electrons transferred for Al (3) and Fe is (2).

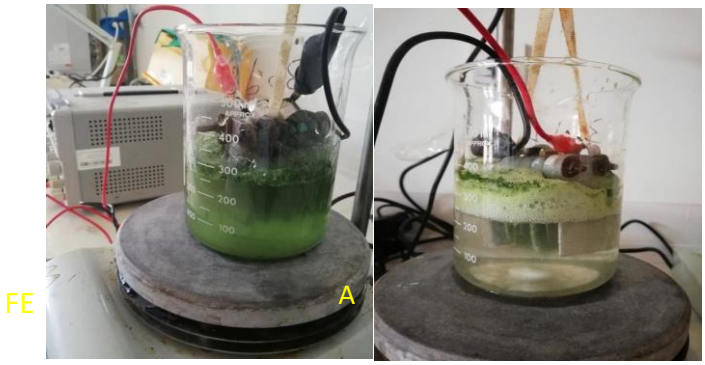


Figure 2. The EC reactor design with Fe and Al electrodes

RESULTS AND DISCUSSION

The effect of the electric current and time

The current of 0.2 A with Fe electrodes showed lower recovery efficiency (RE) after 30 min reaction time and 24 h settling time compared to the higher applied electric currents in the experiments and Golzary et al. (2015) found the same result. The recovery efficiency at 0.8 A electric applied with Fe electrodes was high and reached approximately 97% after 30 min. The increase of the settling time after the EC experiment led to a rise of the recovery efficiency. According to Faraday's Law, the electric current has a direct effect on the rate of dissolution of the anode and has a significant impact on the performance of the EC experiment (Golzary et al., 2015). Therefore, with an increase in the electric current, the amount of coagulant ions increases, and therefore the microalgae isolation rate increases (Kumar et al., 2009). Moreover, the amount and the speed of hydrogen gas depends strongly on the electric current and so that the increase of the electric current results in the increase of the hydrogen produced (Nanseu-Njiki et al., 2009). Then, a higher amount of the isolated microalgae float on the liquid surface by the gas flow (Golzary et al., 2015). According to Faraday's law, the increase of the electric current results in an increase in electrode consumption, thus an increase in operating costs is not avoidable (Martínez-Villafañe et al., 2009). The time of the applied current was an effective parameter in the EC experiment. Based on Faraday's Law, the amount of the dissolved electrodes is proportional to the time of the reaction (Martínez-Villafañe et al., 2009). It has been noticed that the increase of the electric current and reaction time led to an increase of the efficiency and energy consumption that is similar to the results of some studies in the literature (Golzary et al., 2015; Uduman et al., 2011).

The effect of the pH

The pH of the medium plays a significant role in the performance of the EC experiments (Nanseu-Njiki et al., 2009). In this study it was noticed that the effect of the original pH compared to the current and time had a significant effect on the efficiency of harvesting. Additionally, if the original pH of the medium overtakes the original value from 8 to 9, the RE would reduce, and if it decreases from 6 to 5, the harvest efficiency also may decrease (Golzary et al., 2015). In this study the pH was adjusted into values of 4, 7 and 12 and the RE of the

sample with a pH of 4 reached maximum values of 100% in most of the runs. For the pH 7, the RE was not high compared to the pH values 4 and 12. The pH 12 value with Fe electrodes had a significant effect in the EC experiment and *Scenedesmus Quadricauda sp.* had the maximum RE values either after 30 min or 24 h. The original pH values and the conductivity were increased with increasing applied current and the time. It can be concluded that 10 min was optimum for high recovery efficiency and less energy consumption. EC experiments were performed with Al electrodes under the same conditions as for Fe electrodes and it was found that Al electrodes were more efficient since higher RE values were achieved in a shorter time comparing to Fe electrodes that is similar to the results found by Bleeke et al. (2015). The Al electrodes were more efficient than the Fe mostly, because the latter have less current efficiency and they coagulate the suspension less than the Al electrodes (Barros et al., 2015). The RE was high with a pH 4 value and reached 100% after 10 min with 30 min of settling time. For the pH 7, the results were near to that of pH 4 values. The pH 12 value had higher RE values for *Scenedesmus Quadricauda sp.* where the maximum value was 100% achieved after 30 min with 24 h of settling time. Figure 3 (a, b, c and d) shows the recovery efficiency (RE) of *Scenedesmus Quadricauda sp.*with EC experiment using both Fe and Al electrodes, after 30 min of reaction time and 24 h of settling time with original pH and adjusted values 4, 7 and 12.

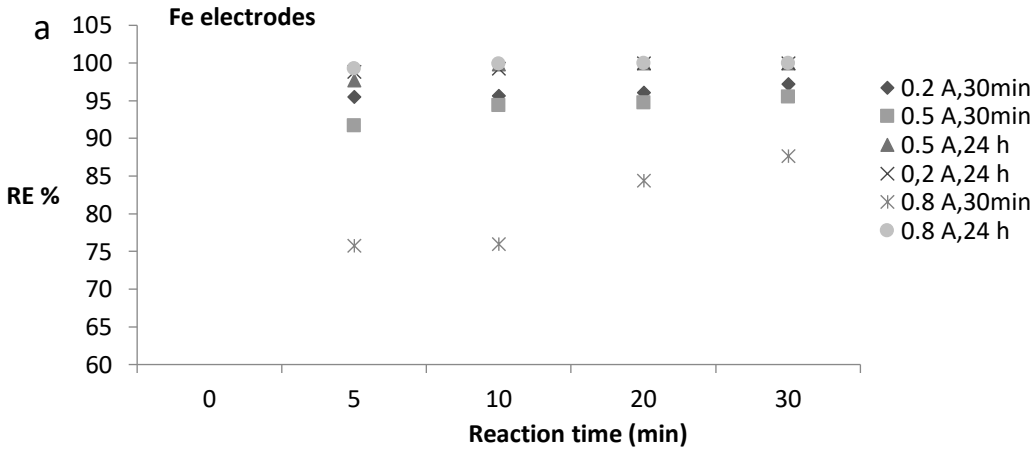
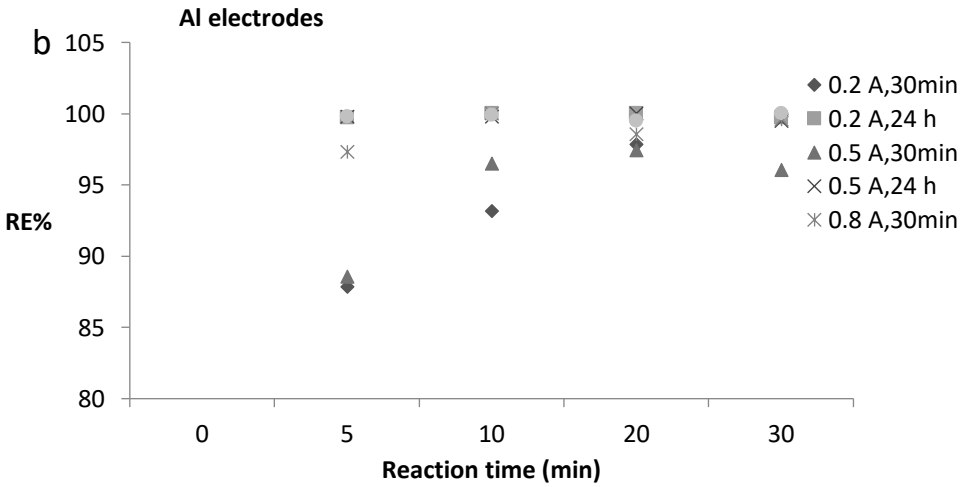


Figure 3. (a, b, c and d) RE of *Scenedesmus quadricauda sp.*with EC experiment using Fe and Al electrodes, after 30 min of reaction time and 24 h of settling time. a and b: original pH; c and d: adjusted value 4, 7 and 12



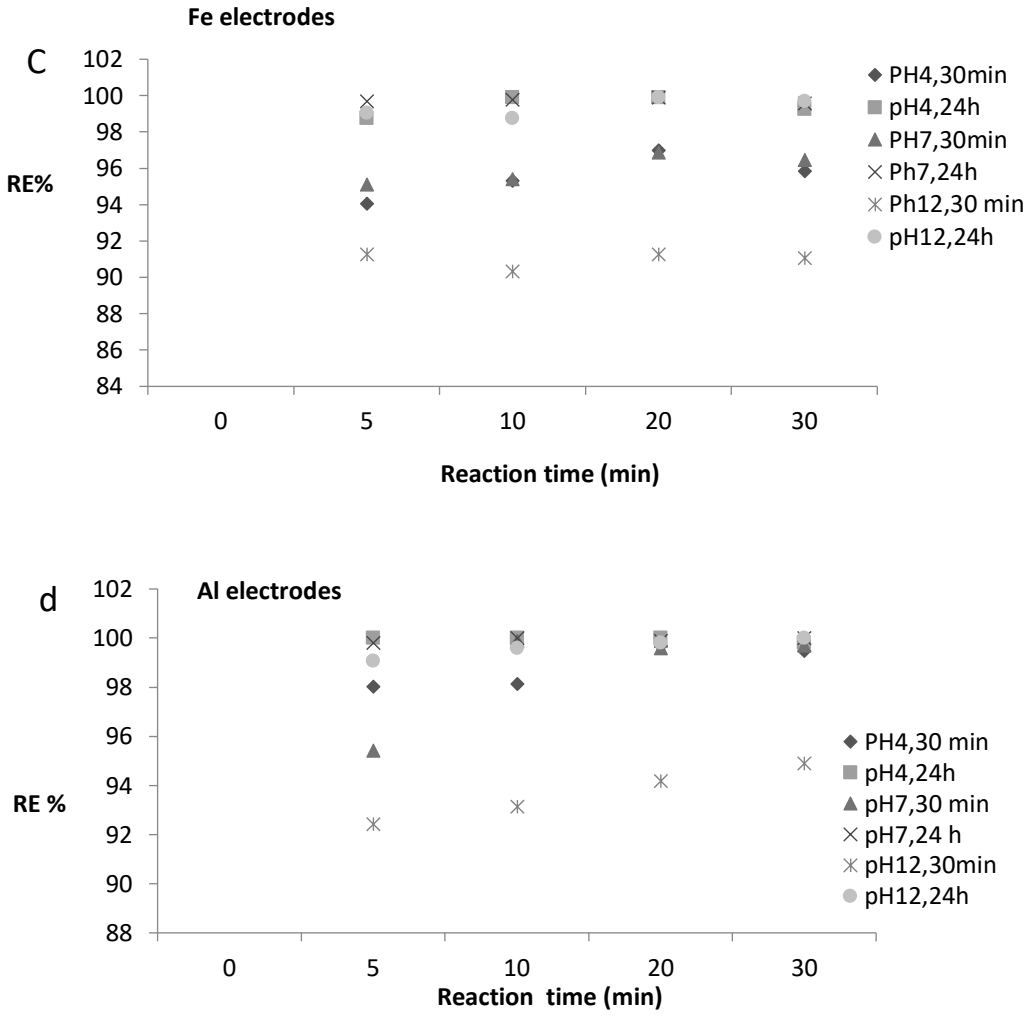


Figure 3 (cont.) (a, b, c and d) RE of *Scenedesmus quadricauda sp.*with EC experiment using Fe and Al electrodes, after 30 min of reaction time and 24 h of settling time. a and b: original pH; c and d: adjusted value 4, 7 and 12

CONCLUSION

Scenedesmus Quadricauda sp. was successfully harvested by electrocoagulation and maximum values of recovery efficiency were reached after 30 min reaction time and 24 h settling time. The achieved recovery efficiency was significantly high with both initial pH values and adjusted values. It can be concluded that pH adjusting on electrocoagulation was a successful method on microalgae harvesting and the recovery efficiency reached almost 100%. In order to increase the efficiency of the microalgae harvesting, the electric current was increased. The time of the applied current was an effective parameter in the EC experiment. Based on Faraday's Law, the amount of the dissolved electrodes is proportional to the time of the reaction. However, the cost of this method was slightly high because of the operating costs such as the energy consumption and the need of replacing the electrodes frequently.

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➢ *ORAL PRESENTATION*

A different approach to nanofiber drug delivery systems: Nanofiber based tablet formulations

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Abstract

Schizophrenia is a serious and often chronic psychiatric disease that affects population. Risperidone (RSP) is a potent antipsychotic agent and used the treatment of schizophrenia. Electrospun nanofibers have large surface area, high porosity and flexibility. In this study, nanofibers based controlled release tablets were developed for oral drug delivery. RSP loaded polycaprolactone (PCL) and poly methyl methacrylate (PMMA) based nanofiber formulations, produced with the electrospinning method. Fiber formulations were coded as F1 (%15 PCL) and F2(%7.5 PCL-%7.5 PMMA), tablet formulations were coded as T1(%15 PCL) and T2 (%7.5 PCL-%7.5 PMMA). Conductivity, viscosity and surface tension of the solutions used in nanofiber production were measured. Nanofibers formulations characterized in terms of mechanical properties and contact angle measurements. Nanofiber formulations compressed, with 6 mm diameter round punch, to produce tablet. Tablet formulations characterized, in terms of weight variation, friability, thickness and, diameter. The possible interactions between RSP and polymers were analyzed with FT-IR and DSC studies. The release profiles of the nanofiber and tablet formulations were compared with in-vitro dissolution studies. The viscosity of the solutions were found 812±14 and 205±1 cPs; conductivity values were found 334.4 and 90.06 mS/cm; surface tension values were found 27.1±0.1 and 27.8±0.1 mN/m for F1 and F2, respectively. Tensile strength of nanofiber formulations was found 8.2±0.1 and 15.2±3.5 MPa for F1 and F2 formulations, respectively. The contact angle of the F1 and F2 formulations was found 59.5±11.9° and 69.1±4.6°, respectively. The thickness of the formulations was found to be 2.69 ± 0.05 and 1.09 ± 0.02 mm and friability values were 0% and % 0,26 for T1 and T2 formulation, respectively. Burst release was observed from nanofibers. In contrast, the burst release prevented in tablet formulations. This study showed that RSP nanofiber tablet formulations were successfully developed for the oral treatment of schizophrenia.

Keywords: Nanofiber, Tablet, Risperidone, Electrospinning

INTRODUCTION

Nanofiber is a dosage form which has advantages such as, high surface area/volume ratio, solubility, stability, flexibility and, possibility of to provide controlled drug release. The control of release rate can be adjusted by polymer selection, porosity, morphology, and nanofiber geometry in the nanofiber formulations (Tuğcu-Demiröz et al., 2020). There are various techniques for producing nanofibers but among all techniques the most commonly used and preferred technique is the electrospinning (Yıldız et al., 2020). Electrospinning allows to produce various dosage forms for rapid or slow release of drugs. Electrospun fibers has feature to be produced quickly and inexpensively (Tort et al., 2019). Various polymers are used in the production of nanofibers. The most commonly used polymers, natural and synthetic, are poly (vinyl alcohol), polyvinylpyrrolidone, PCL, silk fibroin, gelatin, collagen for this purpose.

Nanotechnology provides convenience and, increases patient compliance, in oral administration (Akhgari et al., 2017). These reasons and also the adjustment of drug release in nanofiber dosage form have made the use of oral nanofibers remarkable in the treatment.

Schizophrenia is a chronic mental disorder that negatively affects the quality of life, requires long-term medication and affects brain functions. Since schizophrenia is a disease requiring long-term treatment and release dosage forms are needed to reduce the dosing frequency (Jafarifar et al., 2017). RSP is an atypical and potent antipsychotic agent and effective the treatment of schizophrenia (Khalid et al., 2015). It is widely used in the treatment of

schizophrenia due to its rapid antipsychotic effect, reducing extrapyrimidal effects and being effective against the negative symptoms of schizophrenia (Grant and Fitton, 1994).

In this study, we aimed to develop controlled release nanofiber based tablets for the treatment of schizophrenia. Risperidone loaded PCL-PMMA based electrospun nanofibers are investigated and tablets produced from these nanofibers. Tablet characterization was made and release properties of nanofibers and tablets, were compared.

MATERIALS AND METHODS

1. Materials

Risperidone was donated from Deva Pharmaceuticals. Glacial acetic acid, PCL (80 kDa) and PMMA (120 kDa) were purchased from Sigma Aldrich. All solvents and chemicals were of analytical grade.

2. Methods

2.1 Preparation of polymer solutions

Two different polymer solutions were prepared for electrospinning process. The composition of solutions is given in Table 1. For the preparation of F1 formulation, 15% PCL and 1% RSP were dissolved in glacial acetic acid:formic acid solvent mixture (3:1) and stirred for 24 h at room temperature.

Table 3. Formulation Code

Formulation Code	RSP (%)	PCL (%)	PMMA (%)
F1	1	15	-
F2	1	7,5	7,5

2.2 Characterization of the polymer solutions

Before electrospinning process, polymer mixtures were characterized in terms of; viscosity using multi point viscometer, conductivity using conductivity meter and surface tension using optical tensiometer to determine the electrospinnability of the polymer mixtures. The viscosities of the solutions were measured at 50 rpm using a cone-plate viscometer. Conductivity measurements were made by immersing the conductivity probe in solution at room temperature.

2.3 Electrospinning process

Two nanofiber formulations were prepared using the polymer solutions via electrospinning process. The process parameters are given in Table 2. Process time was kept constant at 3 hours for both formulations.

Table 2. Process parameters of electrospinning

	Feed Rate (ml/h)	Voltage (kV)	Distance (cm)
F1	0.8	16.5	10
F2	0.5	18	10

2.4 Characterization of nanofibers

Mechanical properties (tensile strength and elongation at break) of F1 and F2 formulations were investigated using a texture analyzer (TA.XT. PlusTexture Analyzer, Stable Micro Systems, UK) and wettability of nanofiber determined by contact angle measurements.

2.5 Preparation of tablets

After electrospinning process, nanofibers were directly compressed (Korsh-Erweka GmbH, Germany) with 6 mm diameter punch to prepare nanofiber based tablets. The nanofiber formulations were weighed, filled in the punch physically and compressed without adding any excipient substances (glidants or lubricants). The tablet formulations obtained from F1 and F2 were coded as T1 and T2.

2.6 Characterization of tablets

The tablet formulations were characterized in terms of diameter, thickness, weight deviation, friability and hardness. The weight variation was carried out by weighing each tablets on electronic scales. The friability test was determined by rotating the tablets for 4 minutes in a single drum friabilitor. Diameter and thickness of tablet are determined by measuring with digital caliper.

2.7 Fourier transform infrared spectroscopy and differential scanning calorimetry studies

The possible interaction between risperidone, polymer, and formulations was studied using Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC).

2.9 In vitro dissolution studies

The in-vitro drug release was studied in two different pH media (2 h at pH 1.2 and then 22 h at pH 6.8 buffer solutions) using USP apparatus I. 500 ml volume medium and 50 rpm rotation speed were used at 37 °C (n=3).

RESULTS and DISCUSSION

3.1 Characterization of the polymer solutions

The results obtained from measuring the conductivity of polymer solutions showed that a decrease in conductivity was observed with the addition of PMMA as shown in Table 3. The viscosity of the solutions decreased with the addition of PMMA.

Table 3. Characterization of polymer solution (n=3, ±standart deviation(SD)

	Viscosity (cPs)	Conductivity (mS/cm)	Surface Tension (mN/m)
F1	812±14	334.4	27.1±0.1
F2	205±1	90.1	27.8±0.1

3.2 Characterization of nanofibers

The flexibility of the F2 formulation containing PMMA decreased compared to the F1 formulation, while the tensile strength increased. PCL has good mechanical properties in terms of elasticity. As expected, the contact angle was increased with the addition of PMMA, which is more hydrophobic than PCL.

Table 4. Characterization of nanofiber formulations (n=3, ±SD)

	Tensile strength (MPa)	Elongation at break (%)	Contact Angle (°)
F1	8.2±0.1	80.4±13.9	59.5±11.9
F2	15.2±3.5	75.3±12.7	69.1±4.6

3.3 Characterization of tablets

The weight variation, friability, diameter and thickness of tablet formulations are given in table 5. The diameters of the tablets were found in the range of 5.85±0.068 to 5.73±0.060 mm. The thickness of the formulations was found to be 2.69 ± 0.053 for T1 and 1.09 ± 0.024 mm for T2, depending on the compressing of different weights. The friability values was 0% in the T1 formulation, while it was 1.25% in the T2 formulation. The weight variation for tablet formulations showed a low standard deviation. This shows that the nanofibers can be produced homogeneous tablet compression in the desired mass.

Table 5. Characterization of tablet formulations (n=3, ±SD)

	Weight Variation (%SD)	**Friability (%)**	**Diameter (mm)**	**Thickness (mm)**
T1	0,95	0	5.85±0.07	2.69±0.05
T2	1,64	0,26	5.73±0.06	1.09±0.02

3.4 FT-IR Studies

The FT-IR spectras of RSP, PCL, PMMA, F1 and F2 formulations are illustrated in Fig. 1. Main peaks were observed at 2943 cm^{-1} (asymmetric CH2 stretching), 1723 cm^{-1} (carbonyl stretching), 1239 cm^{-1} (crystalline phase), and 1162 cm^{-1} (amorphous phase) for PCL and 1722 cm^{-1} (C=O stretching), 1386 cm^{-1} (CH_2 deformation) and 2950 cm^{-1} for PMMA (Jang et al., 2020; Abutalib and Rajeh, 2020; Reddy et al., 2016). Pure risperidone showed distinctive absorption bands at 3059 cm^{-1} aromatic C–H, 2941 and 2758 cm^{-1} aliphatic C–H, 1533 cm^{-1} aromatic C=C, 1350 cm^{-1} C–N, 1130 cm^{-1} C–F and 853 cm^{-1} aromatic C–H bending. These bands were found similar to the literature (Ibrahim et al., 2020). FTIR results confirmed the presence of RSP in the all formulations.

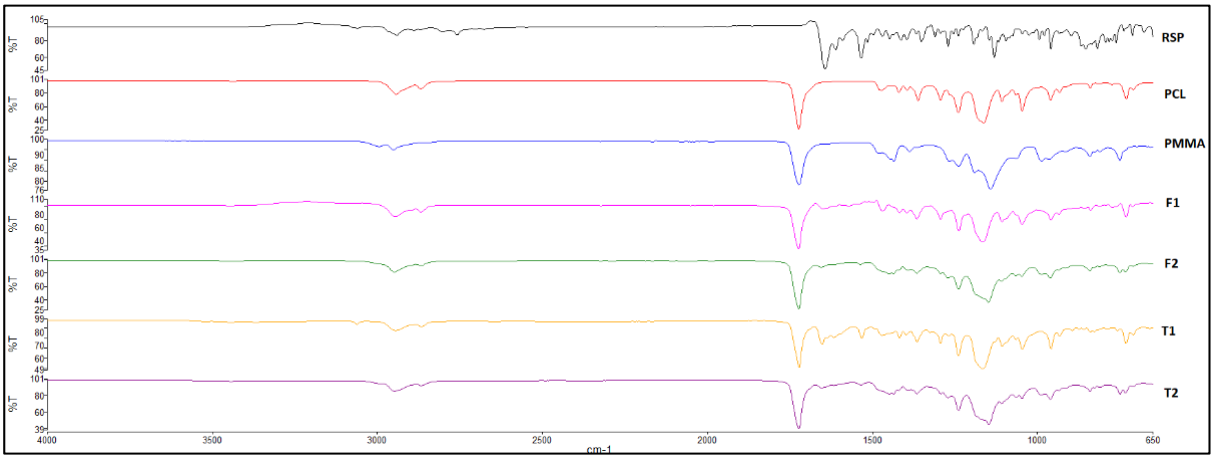

Figure 1. FT-IR spectrum of pure RSP, PCL and PMMA, F1, F2, T1 and T2 formulations

3.5 DSC Studies

Characteristic peaks of PCL was observed exhibiting a melting peak around 60°C and this peak was found with all PCL containing formulations, with broadening in tablet formulations (Fig.2). Pure risperidone showed a sharp endothermic peak at 173°C due to melting. This peak was not observed in nanofiber and tablet formulations. These findings showed that RSP might exist in amorphous form in the formulations.

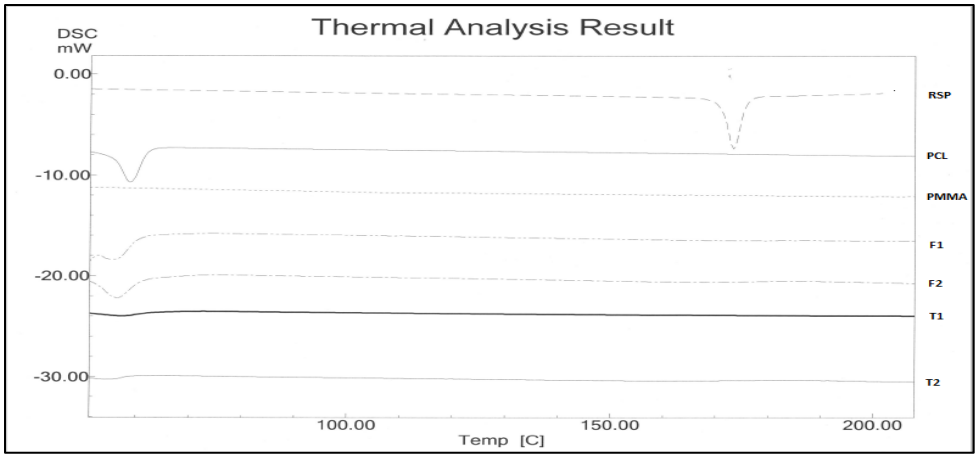

Figure 2. DSC thermograms of pure RSP, PCL and PMMA, F1, F2, T1 and T2 formulations

3.6 *In vitro* dissolution studies

From the data in Figure 3, the initial burst release was observed in the fiber formulations F1 and F2. For F2 fiber formulation, more than 95% of the RSP was released within the first two hours while more than 85% of the RSP was released in F1 fiber formulation. This burst release can be associated with the large surface area and porous structure of the nanofibers. On the other hand, in the tablet formulations, about 17.7% and 5.2 % drug release were observed for T1 and T2 formulations within the first two hours, respectively. The release rate of RSP from PMMA containing formulation slowed compared to the formulation containing only PCL due to the hydrophobic nature of PMMA. The results showed that tableting of the nanofibers provided control of the release of RSP . Controlled release tablets of RSP will increase patient compliance by reducing frequent dosing.

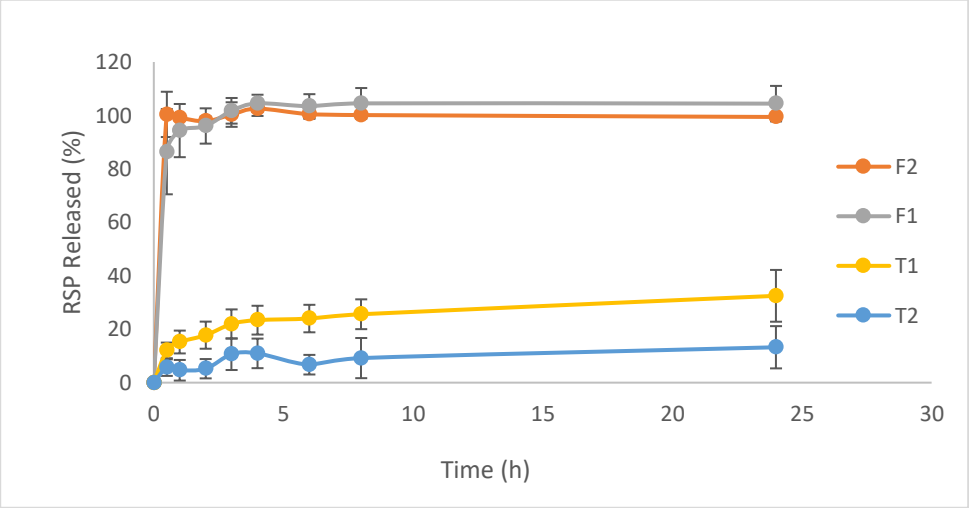

Figure 3. In vitro release studies of formulations (n=3)

CONCLUSION

PCL and PMMA nanofibers containing RSP were successfully produced with electrospinning technique and tablets were prepared from these fibers with the direct compression method. Nanofibers could be easily compressed by the direct compression method without adding an excipient. Compressing nanofibers in tablet form showed sustained drug release of RSP. This study demonstrates the potential of controlled release nanofibers in oral drug delivery systems.

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➢ *ORAL PRESENTATION*

Designing Kaempferol Loaded Microspheres and Evaluation for *In Vitro* Biocompatibility

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Abstract
Fibrotic scar formation is the basis of many skin diseases. This study aimed to synthetize and characterize hyaluronic acid & alginate microspheres and to evaluate kaempferol, a natural substance with an anti-fibrotic effect, either free or loaded on the microspheres for cytotoxic effects on L929 fibroblast cells.

Kaempferol loaded and unloaded microspheres were prepared from hyaluronic acid and alginate (HA&ALG) and characterized by using a thermogravimetric analyzer (TGA) and SEM. Kaempferol release form the microspheres was monitored for 21 days. The L929 fibroblasts were exposed to kaemferol loaded and unloaded microspheres as well as to free kaempferol and mit-c. The MTT cytoticity test indicated that kaempferol, free or loaded on HA & ALG microspheres, exibited a dose-dependent cytotoxicity on L929 fibroblast, higher than mit-c. Furthermore, kaempferol loaded microspheres are promising for prolonged local effect due to controlled release from the microspheres.

Keywords: Kaempferol, microshperes, drug delivery, fibroblasts, cytotoxicity

INTRODUCTION

The flavonoid kaempferol (3,5,7-trihydroxy-2-(4- hydroxyphenyl)-4H-1-benzopyran-4-one), a yellow compound with low molecular weight (that is MW: 286.2 g/mol) is commonly found in plants and has been used in traditional medicine. It is mainly isolated from some plants especially tea and broccoli and other plant sources Kaempferol is a natural flavonoid which has various biological functions, such as powerful antioxidant activity, antidepressant property, anti-inflammatory, increasing metabolism, and cancer fighting properties. (Chen and Chen, 2013). It has also been shown that kaempferol can significantly inhibit hypertrophic scar formation in the mouse model due to mechanical load by attenuating collagen synthesis and suppressing the proliferation and activation of human hypertrophic scar fibroblasts formed in vitro. (Maini et all,2015). Kaempferol can provide protection against ultraviolet ray-induced skin damage through antioxidant activity and direct light absorption (Li et all.,2016).

Materials derived from natural sources (e.g., wood) have a long history of medical use as biomaterials, and have often been used to replace tissues lost to disease, trauma or other reasons. In recent years, however, these materials have began to be replaced by synthetic polymers, mineral enriched materials, ceramics and alloys such as metal due to their better performance and more reproducible properties compared to naturally derived materials(Huebsch and Mooney,2009), (Ratner and Bryant,2004) Some recent development in this field has now led to the definition of a biomaterial as a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body, and boundaries for the use of biomaterials are still expanding.

The design of new biomaterials has focused on mimicking many functions of the extracellular matrices of body tissues because they can regulate host responses in a well-defined way, and naturally derived materials have recently received much attention due to their natural biocompatibility, and new studies in this area are increasing day by day. Alginate is among the most preferred natural polymers in biomaterial production in recent years, and it is a naturally occurring anionic polymer typically derived from brown seaweed and has been extensively

researched in many biomedical applications due to its biocompatibility, hydrophilic nature, low toxicity, relatively low cost, and physical architecture (Lee and Mooney,2012).

Alginate, being hydrophilic, forming matrix structures for cells in temperate conditions without the need for organic solvents, low diffusion restriction due to its large pore size, ability to adjust pore size with various modifications when desired, in pharmaceutical and drug release applications, biomaterials and implantation due to its biocompatible and biodegradable properties It has been widely used in its applications. This applications include; cell encapsulation, wound care industry, drug delivery, stem cell culture, and tissue engineering scaffolds (Lee and Mooney, 2012).

Bioadhesive microspheres are consist of microparticles of 1–1000 μm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively. In general the microspheres (MS), have the potential to be used for targeted and controlled release drug delivery; but using mucoadhesive microparticles provides a potential strategy for improving the retention of drugs, that has additional advantages, e.g. enhanced bioavailability and efficient absorption of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, nasal cavity, or in environments that may have such fluid passage and moisture ,specific targeting of drugs to the absorption site achieved by anchoring plant lectins, bacterial adhesins and antibodies, etc. on the surface of the microspheres(Mathiowitz et all.,2001),(Liao et all.,2005). Hyalurnic acid is a mucoadhesive material and it has been used in many drug release and bioavailability studies and its usage area continues to expans. Huh et al. They stated in their study that microspheres created with HA and producing controlled release can be applied with different formulations in nasal systems and their clinical use may be beneficial(Huh et all., 2010),(Lim et all.,2000).

Drug delivery systems (DDS) that can precisely control the release rates or target drugs or active ingredient to a specific body site have had an enormous impact on the healthcare system. The last years pharmaceutical industry have witnessed an avant-garde interaction among the fields of polymer and material science, resulting in the development of novel drug delivery systems, that being developed gradually (Mathiowitz et all.,1999).

The advancing technology offers a smart approach on drug delivery by combining the drug with a carrier particle such as microspheres, nanoparticles etc., the material can be choosen for specific purpose and absorption properties of the drug to the target organ. Microspheres due to the ease of preparation and application constitute an important part of these particulate drug delivery system by merit of their small size and efficient carrier characteristics. However, there are some disadvantages of these novel one of which is DDS limitation due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the DDS with the absorbing membranes that will eliminate the disadvantages. This may be achieved by coupling bioadhesion characteristics to microspheres and developing novel delivery systems referred to as “bioadhesive microspheres”.

In this study, we aimed to synthetize and characterize hyaluronic acid & alginate microspheres and investigate the cytotoxic effects of a natural substance with an anti-fibrotic effects namely kaempferol, either free or loaded on the microspheres on L929 fibroblasts.

MATERIALS AND METHODS

Materials

Kaemphfero was obtained from Alfa Aesar from Thermo Fisher, India. All other chemicals used were of analytical grade.

Methods

Kaempferol, loaded and unloaded microspheres were prepared from hyaluronic acid and alginate (HA&ALG) and characterized by SEM and TGA (Thermogravimetric Analyzer). Kaempferol release was monitored for 21 days. L929 fibroblasts was exposed to kaemferol loaded and unloaded microspheres as well as free kaempferol and mit-c. Cells were then evaluated for cytotoxicity using MTT.

Cell Culture

Frozen cells were thawed rapidly at 37°C; thawing produce was carried out inside a sterile laminar flow cabin, the cells were transferred to a falcon tube and centrifuged at 2500 rpm for 2 minute A total of 3.5 mL of DMEM (Caprion, CANADA)(10% FBS (Caprion, CANADA), %1 antibiotic (Biological Industries,USA) (for L929cells), mediums were added to the falcon tube and after allowing homogenization, cells were seeded into 25-cm2 flasks. After then this flasks were left to incubate at 37°C and 5% CO2.After 72 hours cells scrapes with 1 mL of Trypsin&EDTA (Biological Industries, USA) for 4 minutes and then the cells were transferred to a 15-mL falcon tube and centrifuged at 2,500 rpm for 2 min. The extracts of the microspheres were adjusted to a concentration of 10 mg / mL in DMEM and kept in an incubator at 37 ° C for 24 hours.

Drug Realase

Release of the drug was monitored for 21 days. The absorbance values were read for 21 days and as a result of the calculations made from the calibration chart.

MTT cytotoxicity tests

MTT assay for cytotoxicity, L929 fibroblast cells ($10x10^3$ cells/well) were seeded into flat-bottomed 96-well plates containing DMEM with L-glutamine and 10% FBS supplemented with 1% PS (Penicilin&Strepyomycin) and incubated overnight. Following this incubation,particles(mit-c and kaempferol) with different concentrations 10 mg/mL to 0,15 mg/mL and Kaempferol+ hyaluronic acid and alginate microspheres (KM) 21 microspheres–1microspheres (each microspheres containing 0.1 mg kaempferol) were diluted with cell culture medium and inoculated into the wells. The plates were kept in to the CO_2 incubator at 37 °C in 5% CO_2 for 24 hour. The cell culture medium was replaced with 50 µl of MTT solution (1 mg/mL, dissolved in without phenol red medium) were added to each well for incubation for 2-2,5 h in a dark condition. After that, 100 µl isopropanol–HCl to dissolve the formed dark blue formazan crsytals was added to each well. The wells were read by the ELISA plate reader using a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, CA) at 570 nm. The number of live and dead cells was counted with a cell counter. Each assay was repeated for three times, and the cytotoxicity test was conducted in triplicate. The cell viability (%) was calculated as follows:

Cell viability(%) = [A]sample/ [A]control x 100,

where the word "[A] sample" means the absorbance of the test sample, and "[A]control" means the absorbance of the control sample.

TGA Analysis

Thermal analysis is a very useful and important method to be used to characterize any materials, including thermoplastic or thermosetting polymer matrix. There are many analysis methods, one of the accepted methods for studying the thermal properties of polymeric materials is the thermogravimetric analysis (TGA). TGA is a thermal analysis technique which has been used to measure changes in the weight loss (mass) of sample that is subjected to a steady increase of temperature so as to quantify reactions involving gaseous emissions (Villain et all.,2007; George et all.,1996). Kaempferol, hyaurnic acid and alginate sphere and also the drug kaempferol loaded hyaurnic acid alginate sphere were analyzed for TGA analysis. For analysis, starting from to 25 °C at 5 °C/min the temperature was increased to 800 degrees.

Scanning Electron Microscope Analysis

Scanning Electron Microscopy is the most preferred device for material characterization and examination of surface morphologies. In this study, we used SEM to determine the morphological states, shape and sizes of the microspheres. The surface morphology of the microspheres has also been studied. Approximately 50 samples were sized and the average values are calculated.

RESULTS

Kaempferol either free or loaded on HA&ALG microspheres causes a dose dependent cytotoxicity to some degree on L929 fibroblast, and kaempferol exibited a higher cytotoxcity compared to mit-C (Table 1). In kaempferol loaded microspheres, kaempferol was released from the microspheres by the 10^{th} day of incubation.

Used as a treatment modality in fibroblastic tissues in routine clinic, mit-c was compared with the microspheres and directly with kaempferol for its cytotoxic effect. It has been found that at the applied concentrations, kaempferol has a lethal effect on fibroblast cells at low concentrations. It is more effective especially when compared with mit-c used. In addition, it has been observed that the microspheres that contain 20(include 2mg kaempferol) and 10(include 1mg kaempferol) microspheres have a lethal effect on fibroblast cells.

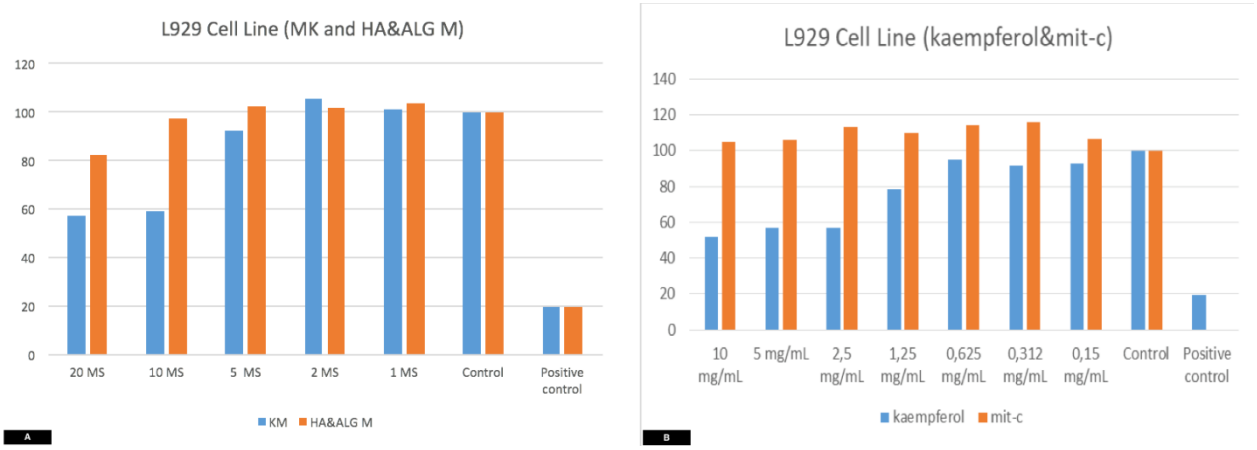

Figure5. Cell Viability of materials 1A. Shows Cell Viability of Kaempferol Loaded Microspheres (MK) and HA&ALG Microspheres 1B. Shows Cell Viability of Kaempferol and Mit-c (%) (compared to negative and positive controls)

In Figure 2a, a mass loss of 60% was determined up to the Tg temperature of 1.4770 g kaempferol material, at 397.66 °C. In Figure 2b, a mass loss of 29.04% was determined for 14.3690 g hyalurnic acid & alginante microsphere up to the Tg temperature at 250 °C. Hyalurnic acid and alginate lose mass at 200-250 °C according to the information in the literatüre (Singh et all.,2014). In figure 2c, Tg temperature for 2.2260 mg kaempferol & hyalurnic acid & alginante microsphere in Figure 2c 4.150% mass loss up to 150 °C, 6.540% mass loss between 150 °C and 210 °C, 210 °C - 380 °C Mass loss of 9.942% at C, 13.70% between 280 °C and 410 °C, 4.378% at 410 °C -580 °C, 3.715% at 580 °C -710 °C. A mass loss of 710 °C - 850 °C has determined a mass loss of 20.62%. Remaining in the final was 36.61% (2,279 mg).

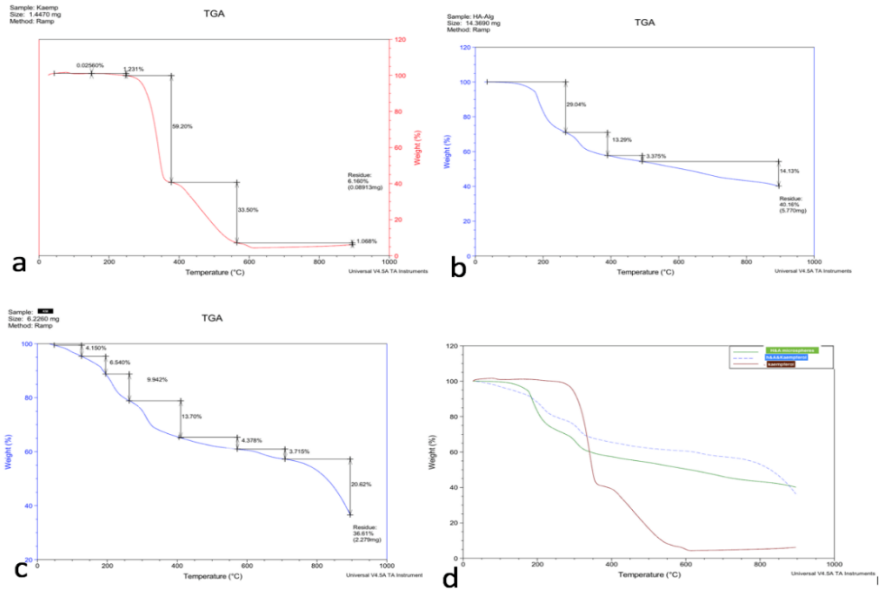

Figure 6. Thermal analysis (TGA) curves of synthesized materials. A) kaempferol, B) hyalurnic acid & alginante sphere, C) kaempferol & hyalurnic acid & alginante microsphere. D) Comparison of all of them.

The surface morphologies were examined by SEM. The pores were observed on the surface. In addition, the average sphere dimensions was 0.374 ± 0.06 micrometers.

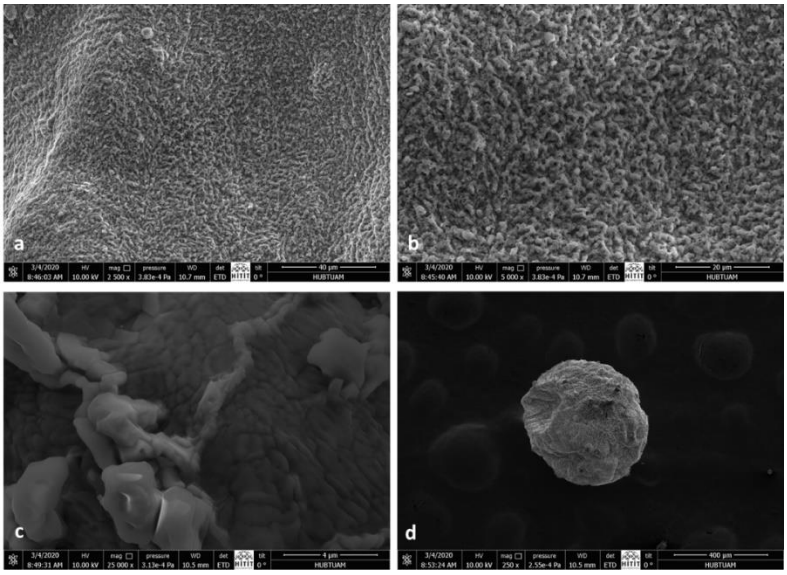

Figure 3. Scanning electromicrographs of a sphere. A, B, and C depicts external morphologies of drug loaded microsphere at the 2500, 5000 and 25000 magnifications. D) A SEM picture of a microsphere.

DISCUSSION

Unlike kaempferol, the viability of the cells increased as the concentration decreased in mit-c, which indicates that the synthesized sample could be successful in clinical practice. The genotoxic effects of materials such as mitomycin-c used in some scar formation in the clinic are known and even used as positive control in standards. In our study, mitomycin-c is not effective on fibroblasts, so it may not be successful in practice, but if the lethal effect of kaempferol on fibroblasts is reflected, it may increase the success in clinical applications. Observation of the surface morphologies which are made in SEM showed that the spheres formed may have adhesion feature in the application areas. In addition, its dimensions have been observed to be applicable according to the area of use to be selected.

CONCLUSION

Kaempferol either free or loaded on HA&ALG microspheres results in a dose dependent cytotoxicity to some degree on L929 fibroblasts. Kaempferol loaded microspheres are promising results for application in scar forming conditions with their ease of application and controlled release since it suppreses fibroblast proliferation.

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➢ *ORAL PRESENTATION*

A preliminary studies of Chironomidae potamofauna on the Eastern Black Sea Region

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Abstract

Turkey is the only country covered almost entirely by three of the world's 36 biodiversity hotspots: the Caucasus, Irano-Anatolian, and Mediterranean. When Turkey is compared to other countries in the temperate zone, it is observed that Turkey has a remarkable amount of fauna biodiversity. In spite of insufficient data, invertebrates have the biggest share within the identified species. There are around 19.000 invertebrate species in Turkey and nearly 4.000 of them are endemic. Nevertheless, the Black Sea region has an insufficient studies regarding the diversity of chironomidae. Therefore the region of the eastern Black Sea's study aims to enrich the diversity of the indicated region.

The eastern area of the Black Sea region has a significant importance regarding freshwater resources in Turkey, some of them are Fırtına Stream (Rize), Büyük Stream (Rize), Tar Stream (Rize), and Çataklıhoca Stream (Rize). In this study the specimens were taken from different places of these streams using a hand net in year 2017.

14 species of Tanypodinae, Prodiamesinae, Orthocladiinae, Chironominae subfamilies were identified that incorporates *Orthocladius thienemanni*, *Polypedilum nubecolusum, Polypedilum pedestre, Cyphomella* sp., *Cardiocladius capucinus, Tanytarsus gregarious, Odontomesa fulva, Parametricneamus stylatus, Prodiamesa olivacea, Cryptochironomus defectus, Rheotanytarsus* sp., *Brilla modesta, Brundiniella* sp.

A numerous number of these species can be located in slow current streams between vegetations, inside mud or in a soft substrates. In these streams *Polypedilum nubecolusum, Polypedilum pedestre, Cyphomella* sp. were recorded the highest diversity among the identified species.

Key words: Black Sea, freshwater invertebrate, Chironomidae.

INTRODUCTION

Turkey is the only country covered almost entirely by three of the world's 36 biodiversity hotspots: the Caucasus, Irano-Anatolian, and Mediterranean. Turkey has remarkable biodiversity due to its geographical and climatic situation and has natural freshwater lakes and important wetlands in the Palearctic region, as evidenced by the recent list of Important Bird Areas (IBAs) in Turkey (Magnin and Yarar, 1997).

The bottom sediment in a freshwater streams is a highly dynamic system where many groups of organisms cohabit. For the majority of these benthic organisms in the bottom of the freshwater streams where they have been studied, insects and specially their larval stages are a substantial item of the benthos community in terms of biomass and transporting energy (Pennak, 1978). The abundance of benthic invertebrates varies basing to many factors such as the distance from the littoral zone, depth, oxygenation and water quality, predation by certain groups, sediment composition, altitude of the stream and the organisms life history (Margalef, 1984; Payne, 1986).

Turkey has a noticeable biodiversity because of its geographical and climatic conditions (Ustaoğlu et al., 2003), and chironomids are one of the most abundant macroinvertebrate group and they often account for the majority of aquatic insects in freshwater environments (Epler, 2001; Freimuth and Bass, 1994). They can be found in many different aquatic environments because of adaptation ability of larvae to extreme environmental conditions of temperature, pH, salinity, depth, flow velocity and productivity (Armitage et al., 1995). Also, chironomid larvae assemblages differs qualitatively and quantitatively among microhabitats, and larvae are highly eclectic in their choice of a locations (Maasri et al., 2008). Larval chironomids are the most useful group which is used as indicators in biological classification of freshwater by their abundance and species compositions (Kırgız, 1988), and are potentially to be as sensitive as (sometimes more sensitive than) other biological indicators such as the EPT

(Ephemeroptera, Plecoptera and Trichoptera), Oligochaeta abundance, etc. (Saether, 1979; Rosenberg, 1993; Barbour et al., 1999; De Bisthoven et al., 2005; Arimoro et al., 2007; Carew et al., 2007). Chironomidae plays an important role in the trophic dynamics of aquatic ecosystem, altering the composition of particulate organic matter and recycling nutrients in the sediment (Silva et al., 2008). Due to their position in food chain which is the lower level chironomid larvae are a source of food for other organisms, especially in winter because of the scarcity of other food sources during this time (Benigno and Sommer, 2008).

The aim of this study is to determine the population density of Chironomidae larvae which were collected with different stations from Fırtına Stream, Çataklıhoca Stream, Tar Stream, Büyük Stream in the year 2017. It was also aimed to fill a gap in the number of the studies made to determine the Chironomidae larvae in these streams.

Material and Method

The study was carried out in Streams Fırtına, Çataklıhoca, Tar and Büyük (Figure 1, Table 1). Fırtına Stream is located in the Eastern Black Sea and is approximately 68 km. The basin, whose length is within the boundaries of Ardeşen and Çamlıhemşin, is one of the largest river basins of the Eastern Black Sea with a surface area of 1177.03 km². Fırtına Stream has been taken under protection as a natural protected area with its ecological structure. 3 stations were sampled in Fırtına Stream (Table 1). Tar Stream, which is about 2 kilometers long on the Çamlıhemşin - Ayder road. Büyük Stream located in Çayeli district of Rize province and flowing into the Black Sea. The source of the northward flowing river is nearly 3000 meters above sea level and the length of the stream is approximately 46 kilometers. Çataklıhoca Stream located in Çayeli district of Rize province and flows into the Black Sea, the length of the Stream is 32.5 km. The river, whose source is Tekfur Hill, flows in a northeast direction and flows into the Black Sea from the west of Çayeli.

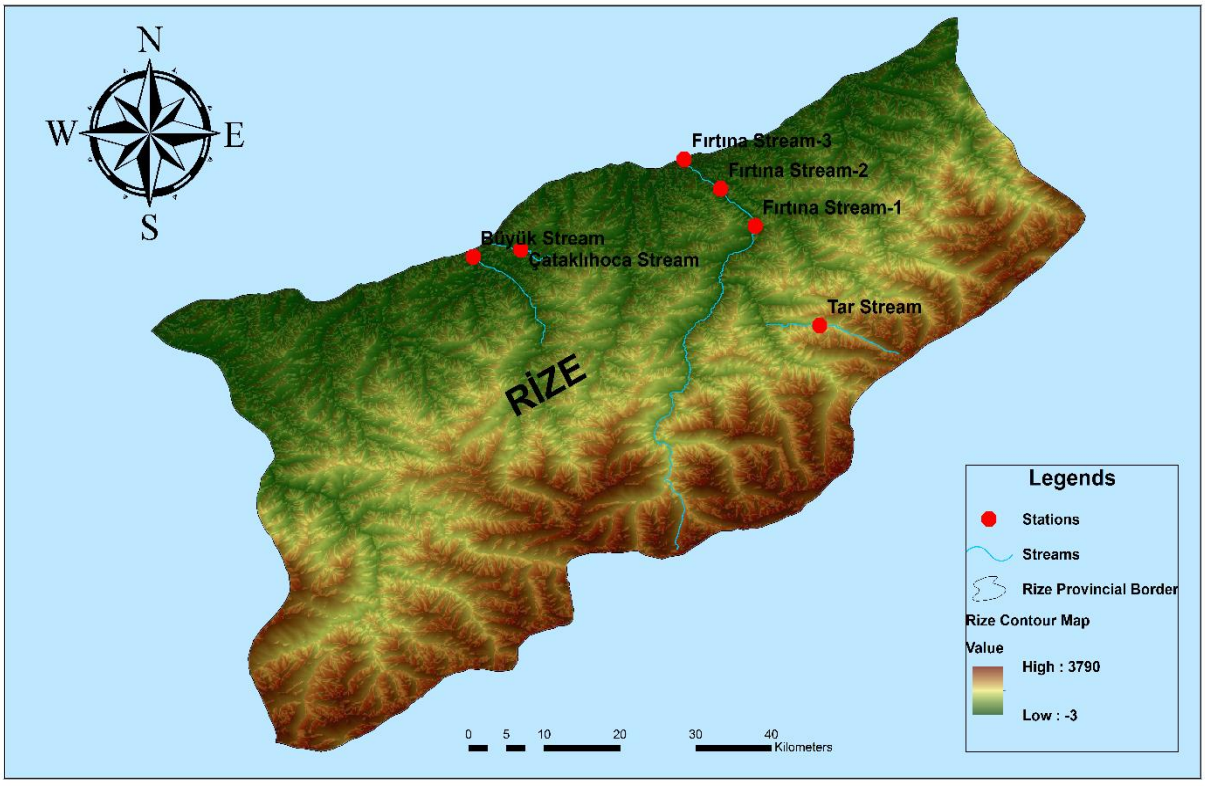

Figure 1. Map of some Rize province's streams

Table 1. Coordinates of sampling stations

Stations	Altitude	Longitude	Latitude
Fırtına Deresi-1	158 m	41.110	41.046
Fırtına Stream-2	63 m	41.153	41.005
Fırtına Stream-3	4 m	41.186	40.962
Büyük Stream	23 m	41.075	40.712
Çataklıhoca Stream	121 m	41.083	40.768
Tar Stream	1475 m	40.998	41.123

Samples were collected from all streams by hand net, then washed in the field on a series of sieves with decreasing mesh sizes. The samples were brought to the laboratory and preserved in 70% ethyl alcohol solution. Samples were sorted, enumerated, decapitated and dipped in a glycerol and identified in the laboratory using both stereo and light microscopes.

All Chironomidae larvae sorted from benthic samples were identified to the lowest possible taxonomic level, usually to genus or species. The chironomid larvae were fixed on slides with glycerol, separating the head capsule from the rest of the body. The taxa were identified using the keys of Epler (1995), Cranston (1982), Pillot (1984) and Şahin (1991). Some Chironomidae species were identified as sp. because of some individuals are in second larval stage that cannot allow determined at level of species.

Results and Discussion

Within the study, benthic macroinvertebrate samples were collected from six different stations in Rize province. According to results, 12 taxa were determined in sampling stations (Table 2). The highest dominance value was determined Oligochaeta taxon in station Fırtına Stream 1 with 63.10%. And also the lowest value was determined Coleoptera, Diptera, Simuliidae, Ceratopogonidae and Plecoptera taxa in Fırtına Stream 1 with 0.74% (Table 2 and Figure 2).

Table 2. The distribution and dominance values of the taxa according to stations

Taxa / Stations	Fırtına Stream 1	Fırtına Stream 2	Fırtına Stream 3	Tar Stream	Büyük Stream	Çataklıhoca Stream
Gastropoda	0.00	0.00	0.00	1.22	0.00	0.00
Oligochaeta	63.10	0.00	23.71	0.00	21.82	3.81
Ephemeroptera	21.03	56.72	21.65	31.71	56.36	39.05
Coleoptera	0.74	0.75	0.00	7.32	0.00	0.00
Diptera	0.74	5.22	0.00	15.85	2.73	0.00
Chironomidae	5.90	8.21	49.48	19.51	4.55	42.86
Simuliidae	0.74	0.00	0.00	0.00	0.00	0.00
Blephariceridae	0.00	6.72	0.00	0.00	10.91	0.95
Ceratopogonidae	0.74	1.49	0.52	0.00	2.73	0.00
Trichoptera	6.27	15.67	2.06	8.54	0.91	4.76
Plecoptera	0.74	3.73	1.55	15.85	0.00	5.71
Hydrachnidia	0.00	1.49	1.03	0.00	0.00	2.86

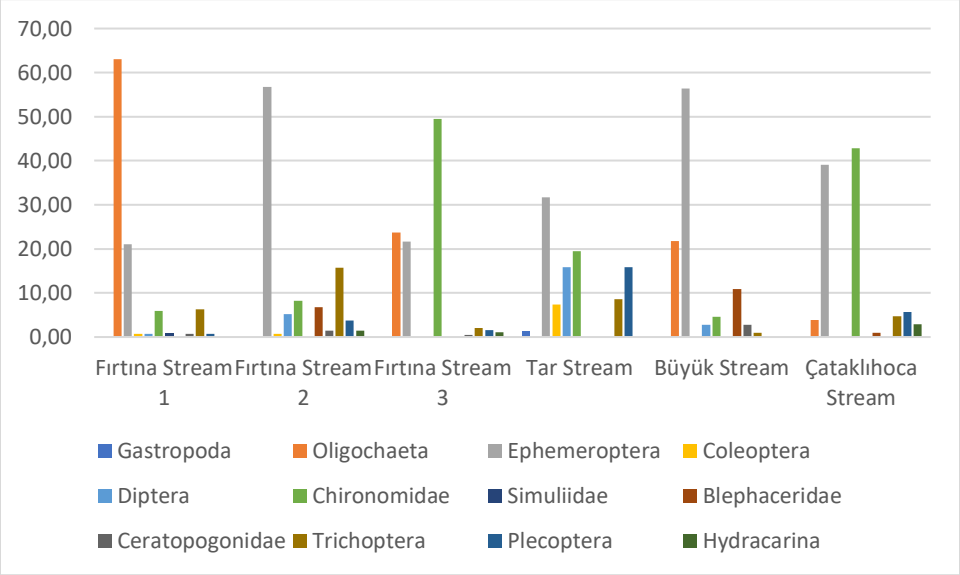

Figure 2. The dominance diagram of determined taxa across the stations

A total number of Chironomidae in sampling stations from Rize province is 118 individuals; 18 taxa, 28 genera, 4 subfamilies (Chironominae, Tanypodinae and Orthocladiinae, Prodiamesinae) of Chironomidae larvae were found (Table 3).

In Fırtına Stream, we have three different stations which are Fırtına Stream 1, Fırtına Stream 2, Fırtına Stream 3, the total number of Chironomidae larvae in this stations combained is 84. In the first station which is Fırtına Stream 1 the total number is 16 individuals; 5 taxa, 4 genera, 2 subfamilies (Chironominae and Orthocladiinae). In the second station, Fırtına Stream 2, the total number of Chironomidae larvae is 7 individuals; 3 taxa, 3 genera, 2 subfamilies (Chironominae and Tanypodinae), while in the third station, Fırtına Stream 3, the total number of Chironomidae larvae is 61 individuals, 10 taxa, 9 genera, 3 subfamilies (Chironominae, Orthocladiinae and Prodiamesinae) (Table 3).

The second stream, Tar Stream, the total number of Chironomidae larvae is 11 individuals, 5 taxa, 4 genera, 3 subfamilies (Tanypodinae, Chironominae and Orthocladiinae). The third stream from this province is Büyük Stream which the total number of Chironomidae larvae is 5 individuals; 5 taxa, 5 genera, 2 subfamilies (Chironominae and Orthocladiinae). Finally the last station is Çataklıhoca Stream, its total number of Chironomidae larvae is 18 individuals; 3 taxa, 3 genera and 2 subfamilies (Chironominae and Orthocladiinae) (Table 3).

The most diverse species in Fırtına Stream are *Cyphomella* sp. (57.14%*)* and *Orthocladius thienemanni* (37.5%). In Tar Stream, *Polypedilum nubeculosum* (45.45%) is the only abundant species, in Çataklıhoca Stream the most abundant species are *Orthocladius theinemmani* (44.44%) and *Polypedilum nubeculosum* (33.33%). While in Büyük Stream there wasn't any abundance recorded.

Fırtına Stream's average flow is 1.460 m^3/sec, Çataklıhoca Stream's average flow 9,460 m^3/sec. (DSİ, 2020), Since *Orthocladius thienemanni* is found under stones in streams where the current is high (Şahin, 1986), *Polypedilum nubeculosum* found in still waters, on stones, sometimes in slow flowing mud and vegetation and *Polypedilum pedestre* is found between vegetation of streams and under stones in the areas of currents (Taşdemir, 2003). Therefore, it can be an indication to the high current of Streams Fırtına and Çataklıhoca. While in Tar Stream *Polypedilum nubeculosum* and *Polypedilum pedestre* was found as dominant species and as was mentioned above both of the species prefere the lentic or the slow flowing stream. In regard to Büyük Stream's average flow is 227 m^3/sec (DSİ, 2020), the Chironomidae larvae that was recorded in this stream was *Chaetocladius dentiforceps* which prefers mud aquatic plant in streams.

Table 3. The dominance value of Chironomidae species in sampling stations from Rize province

Taxa / Station	Fırtına Stream 1	Fırtına Stream 2	Fırtına Stream 3	Tar Stream	Büyük Stream	Çataklıhoca Stream
Tanypodinae						
Brundiniella sp. (Roback, 1978)	0.00	0.00	0.00	9.09	0.00	0.00
Krenopelopia sp. Fittkau, 1962	0.00	14.29	0.00	0.00	0.00	0.00
Prodiamesinae						
Odontomesa fulva (Kieffer, 1919)	0.00	0.00	1.64	0.00	0.00	0.00
Prodiamesa olivacea (Meigen, 1818)	0.00	0.00	4.92	0.00	0.00	0.00
Orthocladiinae						
Brillia modesta (Meigen, 1830)	0.00	0.00	1.64	9.09	0.00	0.00
Cardiocladius capucinus (Zetterstedt, 1850)	6.25	0.00	0.00	0.00	0.00	22.22
Eukiefferiella sp.	0.00	0.00	0.00	0.00	20.00	0.00
Parametriocnemus stylatus (Kieffer, 1924)	0.00	0.00	4.92	0.00	0.00	0.00
Orthocladius thienemanni (Kieffer, 1906)	37.50	0.00	0.00	0.00	0.00	44.44
Chaetocladius dentiforceps (Edwards, 1929)	0.00	0.00	0.00	0.00	20.00	0.00
Chironominae						
Chironomini						
Cryptochironomus defectus (Kieffer,1913)	0.00	0.00	3.28	0.00	0.00	0.00
Cyphomella sp. Sæther, 1977	37.50	57.14	16.39	9.09	0.00	0.00
Polypedilum pedestre (Meigen, 1830)	6.25	0.00	18.03	27.27	0.00	0.00
Polypedilum nubeculosum (Meigen, 1804)						
Tanytarsini						
Micropsectra curvicornis (Tchernovskii,1949)	0.00	0.00	0.00	0.00	20.00	0.00
Rheotanytarsus sp.	0.00	0.00	11.48	0.00	0.00	0.00
Tanytarsus sp. (van der Wulp, 1874)	0.00	0.00	0.00	0.00	20.00	0.00
Tanytarsus gregarius (Kieffer, 1909)	0.00	0.00	24.59	0.00	0.00	0.00

Polypedilum nubeculosum can be found in stagnant and slow flowing water, typically at the bottom, on wood or stones sometimes on plants. This species usually make long tubes, abundant in eutrophic habitats. Its larvae also can be found in flowing water with moderate to heavy organic pollution if the oxygen is not very low. Basing to this information and previously the presented information regarding to the other species, it can be conclude that all the streams that include *Polypedilum nubeculosum* and *Orthocladius thienemanni* are flowing water with moderate to severe organic pollution if the oxygen content is not vey low (Otten, 1986; Peters et al., 1988).

Fırtına Stream 3 has the highest taxa number with 16. And also the same station has the highest value of shannon diversity index with 2.10 (Table 4). Station of Fırtına Stream 3 is close to Black Sea. Structure of station's substrate is muddy and sandy, so Oligochaeta and Chironomidae which prefer to live especially in sand and mud are dominant species in this station.

Table 4. The biotic index values according to macroinvertebrate taxa in sampling stations

Metric / Stations	Fırtına Stream 1	Fırtına Stream 2	Fırtına Stream 3	Tar Stream	Büyük Stream	Çataklıhoca Stream
Abundance	271	130	159	76	109	78
Number of Taxa	12	11	16	11	11	9
Shannon-Wiener-Index	1.21	1.45	2.10	1.87	1.31	1.63
Margalef Index	1.96	2.05	2.96	2.08	1.92	1.84
Evenness	0.49	0.61	0.76	0.81	0.57	0.74

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➢ *ORAL PRESENTATION*

Türkiye'de yetişen *Noccaea* sensu Meyer taksonlarının genetik çeşitliliğin belirlenmesi

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Özet
Sistematik açıdan *Noccaea* Moench cinsi Turpgiller (Brassicaceae) familyası içerisinde en karmaşık cinslerden biridir. Cinsin sistematiği hakkında en detaylı çalışma Meyer tarafından gerçekleştirilse de sınıflandırmada kullandığı karakterler farklı araştırmacılar tarafından yapay olarak değerlendirilmiş ve kabul görmemiştir. Farklı araştırmacılara göre cins Türkiye'de farklı sayıda türle temsil edilmektedir. Meyer'in incelediği herbaryum materyallerinin, doğal popülasyonlarda gözlemlenen, popülasyonun tüm karakteristiklerini yansıtmadığı göz önünde bulundurulduğunda cinse ait üyelerin doğal popülasyonlarının genetik çeşitliliğinin tespit edilmesi sınıflandırmada karşılaşılan problemleri çözmeye yardımcı olacaktır. Bu kapsamda Türkiye'de doğal olarak yetişen ve Meyer tarafından *Noccaea* cinsi içerisinde değerlendirilen 16 taksonun farklı popülasyonlarının genetik özellikleri belirlenmiştir. 50 farklı popülasyondan toplanan *Noccaea* sensu Meyer taksonlarının ITS ve *trn*L-F bölgeleri kullanılarak popülasyon gruplarına ait nükleotid çeşitliliği (π), değişken bölge sayısı (DB), özgün haplotip sayısı (HS) ve haplotip çeşitliliği (HÇ) ayrı ayrı hesaplanmıştır. Nükleotid çeşitliliğinin nötraliteden uzaklaşıp uzaklaşmadığını test etmek için Tajima'nın D ve Fu'nun Fs değerleri kullanılmıştır. Genetik yapılanmanın oluşumunda coğrafik uzaklıkla genetik uzaklık arasında bir ilişkinin [isolation by distance=mesafe yoluyla izolasyon (IBD)] var olup olmadığı, IBD örüntülerinin araştırılmasıyla test edilmiştir. Bu analizlerde populasyonların F_{ST} değerlerine karşı Öklidyen coğrafik uzaklıklarının regresyonuna başvurulmuştur. 650 baz çifti uzunluğundaki ITS veri setine dayalı genetik çeşitlilik çalışmaları neticesinde en yüksek haplotip çeşitliliği (0.916) *N. papillosa* türünde gözlemlenirken bu türü (0.913) *N. microstyla* türü izlemektedir. En düşük haplotit çeşitliliği ise (0.642) *N. violascens* türünde tespit edilmiştir. 811 baz çifti uzunluğunda ki *trn*L-F veri setine dayalı genetik çeşitlilik çalışmaları neticesinde ise en fazla haplotit çeşitliliğinin (0,900) *N. sintenisi* türünde olduğu görülmektedir. İkinci sırada *N. microstyla* (0,721) yer alırken *N. violascens* türünün popülasyon grupları arası *trn*L-F veri setine dayalı haplotit çeşitliliğinin sıfır (0.000) olduğu tespit edilmiştir. Her iki veri setine dayalı nötralite testleri neticesinde Tajima'nın D ve Fu'nun FS değerleri açısından anlamlı bir popülasyon grubuna rastlanmamıştır. Ayrıca Mantel testi sonuçlarına göre coğrafik uzaklık ile genetik yapılanma arasında anlamlı bir ilişki gözlenmemiştir.
Anahtar Kelimeler: *Noccaea*, Brassicaceae, Genetik Çeşitlilik, Popülasyon Genetiği

Determination of genetic diversity of *Noccae* sensu Meyer taxa grow in Turkey

Abstract
Systematically, the genus *Noccaea* Moench is one of the most complex genus among Brassicaceae genera. Although the most detailed study about the systematic of the genus was carried out by Meyer, the characters he used in classification were evaluated as artificial and not accepted by different researchers. According to different researchers total number of species that grows in Turkey are different. Considering that the herbarium materials studied by Meyer do not reflect all the characteristics of the population observed in natural populations, determining the genetic diversity of the natural populations of the members of the genus will help solve the problems encountered in classification. In this context, the genetic characteristics of 16 *Noccaea* taxa that grows in Turkey were determined. ITS and *trn*L-F regions of *Noccaea* sensu Meyer taxa that were collected from 50 different populations, were used to determine the nucleotide diversity (π), variable region number (S), specific haplotype number (H) and haplotype diversity (HD) were calculated separately for the population groups. Tajima's D and Fu's Fs values were used to test whether the nucleotide diversity drifted away from neutrality. Whether there

is a relationship [isolation by distance (IBD)] between geographical distance and genetic distance in the formation of genetic construct was tested by investigating IBD patterns. As a result of genetic diversity studies based on 650 base pairs long ITS data set, the highest haplotype diversity (0.916) was observed in *N. papillosa*, followed by *N. microstyla* (0.913). The lowest haplotite diversity (0.642) was detected in *N. violascens*. As a result of genetic diversity studies based on *trn*L-F dataset of 811 base pairs in length, it is seen that the highest haplotyte diversity (0,900) is in *N. sintenisi*. The haplotite diversity of *N. violascens* was found to be zero (0.000) based on the *trn*L-F dataset between population groups, while *N. microstyla* (0.721) ranked second. As a result of the neutrality tests based on both data sets, no significant population group was found in terms of Tajima's D and Fu' values. In addition, according to Mantel test results, no significant relationship was observed between geographical distance and genetic structuring.
Keywords: *Noccaea*, Brassicaceae, Genetic Diversity, Population Genetics

GİRİŞ

Sistematik açıdan *Noccaea* Moench cinsi Turpgiller (Brassicaceae) familyası içerisinde en karmaşık cinslerden biridir. Cins ilk kez Moench (1802) tarafından Avrupa'da yayılış gösteren *Iberis rotundifolia* L. türünün *Noccaea* cinsine transferi ile ortaya çıkmıştır. İlerleyen dönemlerde Gaudin (1829) tarafından *Thlaspi rotundifolium* Gaudin şeklinde *Thlaspi* L. cinsine aktarılan tür Meyer tarafından (1973) *Noccaea* cinsinin tekrar canlandırılmasıyla *Noccaea* cinsine transfer olmuştur. Ancak Türkiye Florası'nda Brassicaceae familyasının yazarlığını yapan Hedge (1965) gibi birçok araştırmacı (Greuter ve Raus, 1983; Greuter ve ark., 1986) Meyer'in *Thlaspi* cinsinin sınıflandırmasında kullandığı yöntemi kabul etmemişler ve türlerin *Thlaspi* cinsi içerisinde değerlendirilmesi gerektiğini savunmuşlardır.

Meyer'in cinsin sınıflandırmasında kullandığı yöntem Al-Shehbaz (2014) tarafından oldukça radikal bir yöntem olarak değerlendirilmiştir. Meyer (1973; 1979) yapmış olduğu morfolojik çalışmalarda cinsin sınıflandırmasında tohum yüzeyi anatomisini ve tohum testa kalınlığını cins üyelerinin sınıflandırılmasında baskın bir karakter olarak değerlendirmiş ve *Thlaspi* cinsini 12 farklı cinse ayırmıştır. Meyer, *Thlaspi* cinsi üyelerini tohum yüzeyi şekli; tohum testa kalınlığı ve meyve şekli gibi karakterleri kullanarak, bir kısmı kendisi tarafından yeni cins olarak (*Microthlaspi* F.K. Mey.; *Thlaspiceras* F.K. Mey.; *Noccidium* F.K. Mey.; *Kotschyella* F.K. Mey.; *Callothlaspi* F.K. Mey.; *Raparia* F.K. Mey.; *Atropatenia* F.K. Mey.; *Vania* F.K. Mey.; *Masmenia* F.K. Mey.); bir kısmı ise daha önce başka araştırmacılar tarafından bilim dünyasına tanıtılan cinslere (*Neurotropis* (DC.) F.K. Mey.; *Noccaea*) ayırmış ve *Thlaspi* cinsi içerisinde sadece altı türü (*T. alliaceum* L., *T. arvense* L., *T. ceratocarpon* (Pallas) Murray, *T. huetii* Boiss., *T. kochianum* F.K.Mey. ve *T. olivieri* A. Engler) bırakarak bunları *Thlaspi* s.str. olarak değerlendirmiştir.

Meyer'in cinsin sınıflandırmasında kullandığı tohum kabuğu (testa) kalınlığı; tohum yüzeyi epidermal hücrelerinin şekli; moleküler çalışmalarla konvergensi varlığı kanıtlanan meyve şekli ve bu meyvelerin meyve sapına bağlanma şekli gibi morfolojik karakterler bir çok araştırmacı tarafından (Greuter ve Raus, 1983; Greuter ve ark., 1986; Al-Shehbaz, 1986; Artelari, 2002; Appel ve Al-Shehbaz, 2003) tespit edilmesi zaman alıcı veya teşhis yapacak farklı kişiler tarafından farklı yorumlanabilecek karakterler olarak değerlendirilmiş ve Meyer'in çalışmaları bu araştırmacılar tarafından kabul görmemiştir. Ayrıca daha sonraları gerçekleştirilen moleküler çalışmalar Meyer'in cinsin sınıflandırmasında kullandığı yöntemin yapay olduğunu göstermiştir (Mummenhoff ve Zunk, 1991; Mummenhoff ve Koch, 1994; Koch, 1995; Zunk ve ark., 1996; Mummenhoff ve ark., 1997; Koch ve Mummenhoff, 2001; Koch ve Bernhardt, 2004; Koch ve Al-Shehbaz, 2004; Al-Shehbaz, 2014).

Belirtilen çalışmalar neticesinde ülkemizde de doğal olarak yayılış gösteren *Noccaea* türlerinin sınıflandırması daha da karmaşık bir hal almıştır. *Noccaea* cinsi Meyer'e (2006) göre Türkiye florasında 16; Mutlu'ya (2012) göre 18 ve Al-Shehbaz'a (2014) göre 51 taksonla temsil edilmektedir. Farklı araştırmacılar tarafından cinsin Türkiye'deki toplam tür sayısındaki tutarsızlığın yanı sıra, Meyer (2006) ve Al-Shehbaz (2014) tarafından türlerin ayırt edici özellikleri kullanılarak hazırlanan teşhis anahtarlarında da problemlerin olduğu ve yanlış teşhislere neden olduğu gözlemlenmiştir. Meyer'in incelediği herbaryum materyallerinin, doğal popülasyonlarda gözlemlenen, popülasyonun tüm karakteristiklerini yansıtmadığı göz önünde bulundurursak cinse ait üyelerin doğal popülasyonları da incelenerek cinse ait üyelerin doğal popülasyonlarının genetik çeşitliliğinin tespit edilmesi sınıflandırmada karşılaşılan problemleri çözmeye yardımcı olacaktır. Bu kapsamda Türkiye'de doğal olarak

yetişen ve Meyer tarafından *Noccaea* cinsi içerisinde değerlendirilen 16 taksonun farklı popülasyonlarının genetik özellikleri belirlenmiştir.

MATERYAL VE METOD

1. Arazi Çalışması ve Örneklem Bilgisi

Çalışma kapsamında incelenen türler 2015-2017 yılları arasında gerçekleştirilen arazi çalışmaları ile toplanmıştır. Arazi çalışmaları gerçekleştirilmeden önce incelenecek türlerin yayılış alanları ilgili literatürlerden tespit edilmiştir. Literatür çalışmalarının yanı sıra incelenecek örneklerin literatürde bulunmayan farklı dağılımlarının tespiti; ilgili türlerin habitat tercihleri ve fenolojisinin tespit edilebilmesi için Ankara Üniversitesi Fen Fakültesi Herbaryumu (ANK), Gazi Üniversitesi Herbaryumu (GAZI), Ege Üniversitesi Herbaryumu (EGE), Hacettepe Üniversitesi Herbaryumu (HUB), Selçuk Üniversitesi Fen Fakültesi Herbaryumu (KNYA) ve İstanbul Üniversitesi Eczacılık Fakültesi Herbaryumu (ISTE) doğrudan ziyaret edilmiş ya da dijital fotoğrafların temin edilmesiyle örnekler hakkında bilgi edinilmiştir. Ayrıca birçok türe ait tip örneği yurt dışındaki herbaryumlarda bulunduğundan, Cenevre Herbaryumu (G), Kew Herbaryumu (K), Viyana Herbaryumu (W), Viyana Üniversitesi Herbaryumu (WU) ve Haussknecht Herbaryumu (JE)'ndan faydalanılmıştır. 16 taksona ait 50 farklı lokaliteye arazi çalışmaları gerçekleştirilmiştir.

Arazi çalışmaları ile moleküler çalışmalarda kullanılmak üzere taze yaprak örnekleri, her popülasyondan en az beş farklı bireyden toplanmış ve yaprak örneklerinin küflenmesini veya çürümesini önlemek amacıyla bitkinin bünyesindeki suyu hızlı bir şekilde uzaklaştıran silikajel içerisine alınmıştır.

2. DNA Analizi, Çekirdek ve Kloroplast DNA'ları İçerisindeki İlgili Gen Bölgelerinin Polimeraz Zincir Reaksiyonuyla Çoğaltılması

Sıvı azot ile mekanik parçalaması gerçekleştirilen yaprak örneklerinden DNA izolasyonu "QIAGEN DNeasy Plant" izolasyon kiti kullanılarak yapılmıştır. İzolasyon sırasında izolasyon protokolünde herhangi bir değişiklik (modifikasyon) gerçekleştirilmeden ticari olarak satılan izolasyon kitinde ki protokol sırasıyla takip edilmiştir.

Çalışma kapsamında cinsin üyelerine ait farklı popülasyonların ve türlerin genetik çeşitliliğini belirlemek için daha önce gerçekleştirilen moleküler çalışmalar ile Brassicaceae familyası üyelerinde güvenirliği kanıtlanan çekirdek ribozomal DNA'sına ait Internal Transcribed Spacer (ITS 1, ITS 2 ve 5.8 S RNA) bölgeleri ve kloroplast DNA'sının *trn*L-F (*trn*L (UAA) geni ve *trn*L-F spacer (boşluk bölgesi) bölgeleri kullanılmıştır (Warwick ve ark., 2010). PZR reaksiyonu için her gen bölgesi için farklı ve daha önceki çalışmalarla belirlenen (Warwick ve ark., 2004) protokoller tanımlanmıştır.

3. Genetik Çeşitlilik ve Popülasyon Genetiği

Noccaea türleri ve bu türlerin farklı popülasyonlarına ait genetik çeşitlilik hesaplamaları DnaSP (Librado ve Rozas, 2009) programı yardımıyla gerçekleştirilmiştir. Genetik çeşitlilik ve popülasyon genetiği çalışmalarına başlamadan önce aynı türün farklı lokalitelerinden toplanan bireyler coğrafik yakınlıkları göz önünde bulundurularak daha büyük popülasyon gruplarına dönüştürülmüştür. Oluşturulan bu popülasyon grupları Çizelge 2'de belirtilmiştir.

Nükleotid çeşitliliğinin nötraliteden uzaklaşıp uzaklaşmadığını test etmek için Tajima'nın D (1989) ve Fu'nun Fs değerleri (1997) DnaSP (Librado ve Rozas, 2009) programı kullanılarak her iki veri seti için ayrı ayrı hesaplanmıştır. Belirlenen popülasyon grupları arasında genetik farklılaşmanın tespiti için ikili genetik farklılaşma değerlerini belirleyen F_{ST} parametresi DnaSP (Librado ve Rozas, 2009) programı kullanılarak hesaplanmıştır. Her tür için bütün popülasyon grupları dahil edilerek hesaplanan F_{ST} parametresinin istatistiksel anlamlılığı 1000'li permutasyon testi ile sınanmıştır.

Genetik yapılanmanın oluşumunda coğrafik uzaklıkla genetik uzaklık arasında bir ilişkinin [isolation by distance=mesafe yoluyla izolasyon (IBD)] var olup olmadığı, IBD örüntülerinin araştırılmasıyla test edilmiştir. Bu analizlerde populasyonların F_{ST} değerlerine karşı Öklidyen coğrafik uzaklıklarının regresyonuna başvurulmuştur (Hutchison ve Templeton, 1999). Analizler GenAlEx 6.3 (Peakall ve Smouse, 2006) programında yer alan Mantel opsiyonu kullanılarak gerçekleştirilmiştir. Populasyonlar arasındaki coğrafik uzaklıklar yine aynı paket programında yer alan ondalık düzeyde (decimal degree) Lat/Long koordinatlarından coğrafik uzaklık

hesaplama seçeneği kullanılarak oluşturulmuştur. Tüm Mantel testlerinin istatiksel açıdan anlamlılık düzeyleri ise genetik uzaklık matriksinin 1000 rastgele permütasyonlarıyla değerlendirilmiştir.

BULGULAR ve TARTIŞMA

Coğrafik yakınlıkları göz önünde bulundurularak daha büyük popülasyon gruplarına dönüştürülmüş gruplar arasında ki genetik çeşitliliği saptayabilmek için ITS ve *trn*L-F veri setleri ayrı ayrı analiz edilmiştir. Daha büyük popülsayon gruplarına dönüştürülmesine rağmen özellikle lokal endemik olan türlerde yeterli sayıda haplotip (analiz için her popülasyon grubunda en az 4 farklı bireyeden elde edilmiş veri seti gerklidir) içeren gruplar için nükleotid çeşitliliği (π), değişken bölge sayısı (DB), özgün haplotip sayısı (HS), haplotip çeşitliliği (HÇ) ayrı ayrı hesaplanmıştır.

Ayrıca nükleotid dizileri arasındaki farklılaşmada nötral süreçlerden bir uzaklaşma olup olmadığını belirleyebilmek amacıyla Tajima'nın D ve Fu'nun FS değerleri hesaplanmıştır (Tajima, 1989; Fu, 1997).

650 baz çifti uzunluğundaki ITS veri setine dayalı genetik çeşitlilik çalışmaları neticesinde en yüksek haplotip çeşitliliği (0.91667) *N. papillosa* türünde gözlemlenirken bu türü (0.91379) *N. microstyla* türü izlemektedir. En düşük haplotit çeşitliliği ise (0.64286) *N. violascens* türünde tespit edilmiştir (Tablo 1).

Tajima'nın D testi polimorfik pozisyonların frekanslarındaki dağılımları özetlemektedir. Elde edilen sonuçlarda anlamlılık sergileyen negatif değerler popülasyonlarda nadir değişkenleri, pozitif değerler ise orta düzeyde frekansa sahip pozisyonların fazla bulunduğunu belirtmektedir. Bu nedenle negatif D değeri son dönemde gözlenen bir pozitif seçilime ya da hızlı bir büyüme sergileyen populasyona işaret ederken pozitif D değeri populasyon yapılanmasının varlığına ya da dengeleyici seçilime işaret etmektedir (Pybus ve Shapiro, 2009). Çalışma kapsamında popülasyon grupları arasında gerçekleştirilen nötralite testleri neticesinde Tajima'nın D ve Fu'nun FS değerleri açısından anlamlı bir popülasyon grubuna rastlanmamıştır (Tablo 1). Bu durum popülasyon gruplarının nötraliteden uzaklaşmadığını göstermektedir.

Tablo 1. ITS verisi kullanılarak hesaplanan genetik çeşitlilik indeksleri ve nötralite testlerine (Tajima'nın D'si, Fu'nun Fs'si) ait sonuçlar. N=toplam sekans sayısı; SU=sekans uzunluğu; DB= değişken bölge sayısı; HS=Haplotip sayısı; HÇ=Haplotip çeşitliliği; π=nükleotid çeşitliliği.

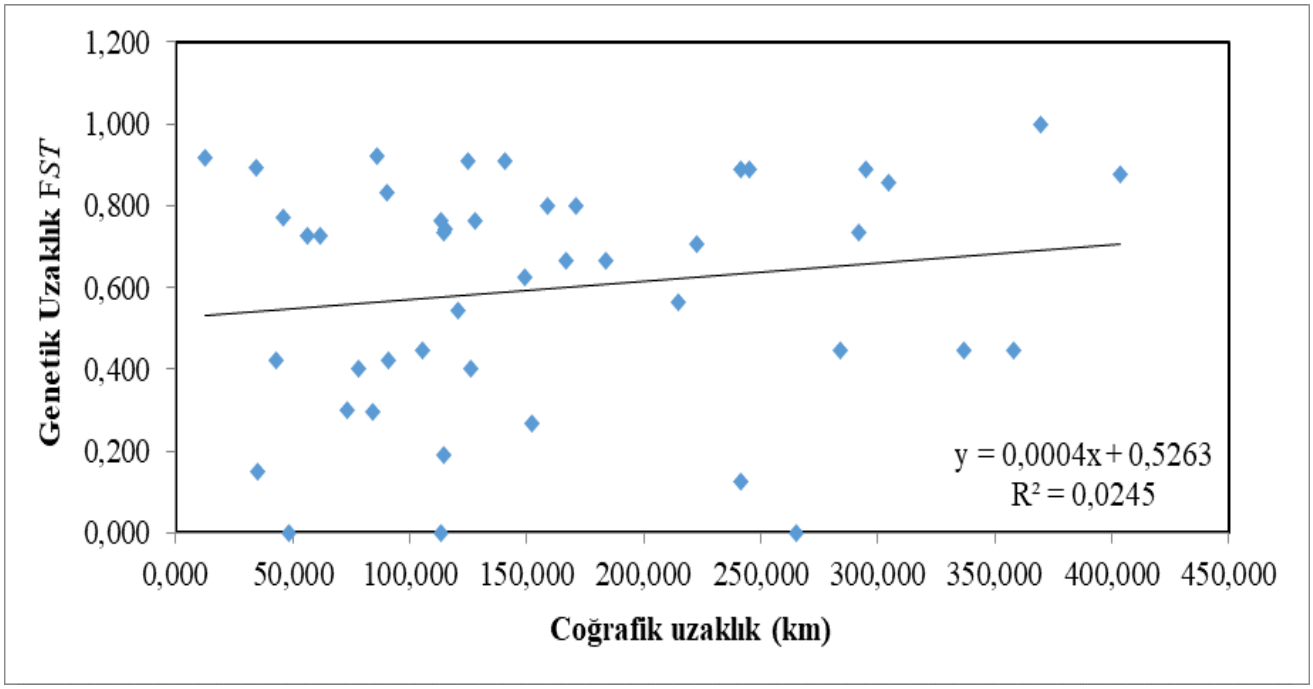
Tür	N	SU	DB	HS	HÇ	π	D	Fs
N. microstyla	29	650	16	12	0.91379	0.0556	-0.55842	0.15805
KAMAD	7	650	5	3	0.76190	0.00382	0,82563	1,40287
SIVAS	4	650	7	3	0.83333	0.00696	1,32331	1,28886
ISPARTA	3	650	2	2	0.66667	0.00213	-	-
AMANOS	15	650	10	4	0,7905	0,0033	-1,2367	-1,3581
N. violascens	8	650	7	4	0.64286	0.00402	-0.41500	-0.07272
Kuzey	4	650	2	2	0.83333	0.00375	0,6501	0,60044
Güney	4	650	1	2	0.50000	0.00080	-0,6124	-0,4787
N. phrygia	7	650	5	4	0.80952	0.01890	0.82563	0.89783
Doğu	3	650	0	1	0.00000	0.00000	-	-
Batı	4	650	4	3	0.83333	0.00373	0,6501	0,60044
N. sintenisii	5	650	6	3	0.8000	0.00489	0.6605	0.69176
Kuzey	2	650	0	1	0.00000	0.00000	-	-
Güney	3	650	3	2	0,6667	0,0032	-	-
N. valerianoides	1	650	-	-	-	-	-	-
N. rubescens	1	650	-	-	-	-	-	-
N. edinensium	3	650	0	1	0.00000	0.00000	-	-
N. papillosa	9	650	9	6	0.91667	0.04480	-0.24847	-0.19091
N. ochroleuca	3	650	1	2	0.66667	0.00109	-	-
N. lutescens	2	650	0	1	0.00000	0.00000	-	-
N. aptera	2	650	0	1	0.00000	0.00000	-	-
N. cataonicum	2	650	0	1	0.00000	0.00000	-	-
N. tatianeae	3	650	6	2	0.66667	0.00657	-	-

811 baz çifti uzunluğunda ki *trn*L-F veri setine dayalı genetik çeşitlilik çalışmaları neticesinde ise en fazla haplotit çeşitliliğinin (0,90000) *N. sintenisi* türünde olduğu görülmektedir. İkinci sırada *N. microstyla* (0,72167) yer alırken *N. violascens* türünün popülasyon grupları arası *trn*L-F veri setine dayalı haplotit çeşitliliğinin sıfır (0.0000) olduğu tespit edilmiştir (Tablo 2). Yine *trn*L-F veri setine dayalı analizler neticesinde Tajima'nın D ve Fu'nun FS değerleri açısından popülasyon gruplarının nötraliteden uzaklaşmadığı görülmektedir (Tablo 2).

Tablo 2. *trn*L-F verisi kullanılarak hesaplanan genetik çeşitlilik indeksleri ve nötralite testlerine (Tajima'nın D'si, Fu'nun Fs'si) ait sonuçlar. N=toplam sekans sayısı; SU=sekans uzunluğu; DB=değişken bölge sayısı; HS=Haplotip sayısı; HÇ=Haplotip çeşitliliği; π=nükleotid çeşitliliği.

Tür	N	SU	DB	HS	HÇ	π	D	Fs
N. microstyla	29	811	5	6	0,7217	0,0021	0,02603	-0,072
KAMAD	7	811	4	2	0,7619	0,7619	0,45159	0,48121
SIVAS	4	811	1	1	0,5	0,0007	0,16766	0,14992
ISPARTA	3	811	0	1	0	0	-	-
AMANOS	15	811	4	2	0,6191	0,0016	-0,4261	0,10493
N. violascens	8	811	0	3	0	0	0,41421	0,9626
Kuzey	4	811	0	1	0	0	-	-
Güney	4	811	2	2	0,6667	0,0019	2,01187	1,79908
N. phrygia	7	811	2	4	0,6667	0,0011	-0,2749	-0,1077
Doğu	3	811	1	2	0,6667	0,0009		
Batı	3	811	1	2	0,6667	0,0009		
N. sintenisii	5	811	8	4	0,9	0,0065	0,98145	0,98145
Kuzey	2	811	0	1	0	0	-	-
Güney	3	811	9	3	1	0,0084	-	-
N. valerianoides	1	811	-	-	-	-	-	-
N. rubescens	1	811	-	-	-	-	-	-
N. edinensium	3	811	0	1	0	0	-	-
N. papillosa	9	811	6	3	0,6667	0,0034	0,03044	0,83189
N. ochroleuca	3	811	0	1	0	0	-	-
N. lutescens	2	811	0	1	0	0	-	-
N. aptera	2	811	0	1	0	0	-	-
N. cataonicum	2	811	0	1	0	0	-	-
N. tatianeae	3	811	1	2	0,6667	0,001	-	-

Genetik çeşitlilik çalışmalarında her iki veri seti ile yapılan analizler her ne kadar popülasyon gruplarının nötraliteden uzaklaşmadığını gösterse de, *N. microstyla* türüne ait popülasyon gruplarının ikili F_{ST} değerleri her iki veri setinde de diğer popülasyon gruplarına oranla daha yüksek çıkmıştır. *N. microstyla* populasyonlarında genetik yapılanmanın oluşumunda gen akışı ve genetik sürüklenmenin önemini belirlemek ve herhangi bir sapma olup olmadığını test edebilmek için populasyonların ITS veri setine dayalı genetik uzaklıklarına karşın coğrafik uzaklıklığın ilişkisi test edilmiştir. Popülasyon gruplarının ITS verilerine dayalı F_{ST} değerleri ile gerçekleştirilen Mantel testi sonuçlarına göre coğrafik uzaklıklık ile genetik yapılanma arasında anlamlı bir ilişki (korelasyon) gözlenmemiştir (r^2=0,0245, p=0,243, Şekil 1).



Şekil 1. *N. microstyla* popülasyon gruplarının ITS verilerine dayalı F_{ST} değerlerine karşın coğrafik uzaklık değerlerinin Mantel Test ile sınanması.

Türkiye'de yetişen *Noccaea* sensu Meyer taksonlarının genetik çeşitliliğini belirlemek için gerçekleştirdiğimiz çalışmada sadece *N. papillosa* türünün batı popülasyonu doğu popülasyonundan ayrılarak coğrafik uzaklıklara dayalı bir örüntü sergilemektedir. Ancak morfolojik çalışmalarda bu popülasyon grupları arasında bir farklılık tespit edilememiştir. Ayrıca ITS veri setine dayalı popülasyon genetiği çalışmaları bu türe ait popülasyon grubunun en yüksek haplotip çeşitliliğine (0.91667) sahip olduğunu göstermektedir. Her ne kadar genetik verilerde farklılıklar olsa da bu farklılıklar, morfolojik verinin de belirttiği gibi, bu türe ait iki farklı popülasyon grubunun ayrı türler olarak nitelendirilmesi için yeterli değildir.

Türler ve popülasyon grupları arası ayrı ayrı gerçekleştirilen genetik çeşitlilik çalışmaları neticesinde, türlerin birbirlerinden farklı olduğu F_{ST} değerleri ile de tespit edilmiştir. Türler arası ikili F_{ST} değeri en düşük (0,3469) olan *N. microstyla* ve *N. violascens* türleridir. Bu iki tür morfolojik olarak da sıklıkla birbirine karıştırılan türlerdir. Türkiye Florası'nın birinci cildinde (Hedge, 1965) *N. densiflora* türü, *N. violascens* türünün yakın akrabası olarak kabul edilmiştir. Gerçekleştirilen çalışmalar ile *N. densiflora* türü *N. microstyla* türünün sinonimi olarak kabul edildiğinden bu iki tür arası F_{ST} değerinin türler arası en düşük değere sahip olması beklenen bir durumdur. Popülasyon grupları arası gerçekleştirilen genetik çeşitlilik çalışmaları neticesinde ise popülasyonlar arası yüksek bir farklılık (türler arası F_{ST} değerlerine oranla) tespit edilmemiştir. Yine her iki veri seti için popülasyon grupları arasında normaliteden herhangi bir uzaklaşmanın olmadığı Tajima'nın D değeri ve Fu'nun Fs değerleri hesaplanarak tespit edilmiştir. Bütün veri setleri ile gerçekleştirilen analizlerde PFs>0,05 ve PD>0,05 olarak tespit edilmiş ve H_0 red edilmiştir.

SONUÇ

Gerçekleştirilen bu çalışma kapsamında gerek morfolojik veriler gerekse moleküler veriler *Noccaea* sensu Meyer cinsinin Meyer (2006) tarafından belirtildiği gibi Türkiye'de 16 takson ile değil 13 tür ile temsil edildiğini göstermektedir. Ayrıca Özüdoğru ve ark. (2019)'nın gerçekleştirdiği filogenetik analizler Meyer'in (1973, 1979) *Thlaspi* cinsini bölerek oluşturduğu (bazı cinsler Meyer öncesinde tanımlanmıştır) cinslerin monofiletik olmadığını göstermiş ve Meyer'in (1973, 1979) *Thlaspi* s.l. cinsinin sınıflandırmasında belirttiği yaklaşımdan daha ziyade Al-Shehbaz (2014) tarafından belirtilen yaklaşımın kabul edilmesinin doğru olduğunu göstermiştir.

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➢ *ORAL PRESENTATION*

H.M.K.Ü Tıp Fakültesi Öğrencilerinin Açlık – Tokluk Durumlarında Biyokimya Parametrelerinin Değerlendirilmesi

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Özet

Amaç: Geleneksel olarak hasta kanları sabah aç karnına laboratuvara teslim edilmektedir. Fakat son dönemlerde hastanelerin 24 saat hizmet vermesinden dolayı günün her saatinde hastalardan kan alınmakta ve açlıktan tam olarak emin olunamamaktadır. Çalışmamızda deneklerin tokluk-açlık biyokimyasal parametrelerini karşılaştırmayı hedefledik. Sonuçlarımızın bu konudaki çalışmalara katkı sağlayacağını öngörüyoruz.

Materyal-Metod: Hatay Mustafa Kemal Üniversitesi Tayfur Ata Sökmen Tıp fakültesi öğrencilerinden (n=723, 297 kadın, 426 erkek) sabah açlık kanları, öğleden sonra tokluk kanları alınarak üniversite hastanesinin merkez laboratuvarındaki biyokimya otoanalizörleri (Siemens Advia-1800) kullanılarak (ALB, AST, ALT, ALP, AMYLAS, BUN, Ca, CHOL, CK, CRE, GGT, GLU, HDL, IP, IRON, TP, TRIG, LIP) ölçüldü ve sonuçlar istatistiksel olarak Paired – t, Wilcoxon Sign Rank testleri kullanılarak SPSS (21) ile değerlendirildi. Gruplar kendi aralarında VKİ, cinsiyet gibi gruba göre ayrılarak sabah açlık ve öğünden sonraki tokluk durumu arasındaki ilişki belirtildi.

Bulgular: Çalışmamızın sonuçlarına göre VKİ için zayıf grupta açlık ve tokluk arasında yalnızca BUN' da farklılık gözlenirken normal grupta Ca, BUN ve TRİG' de gözlemlenmesine rağmen kilolu grupta hiçbir fark gözlenmemiştir. Cinsiyete göre gruplar incelendiğinde ise kadınların açlık-tokluk değerleri arasında TRİG, GLU ve BUN' da istatistiksel olarak anlamlı (p<0, 05) fark gözlenirken erkeklerde Ca, TRİG, GLU ve BUN'da anlamlı (p<0, 05) fark gözlenmiştir.

Sonuç: İncelenen 18 biyokimyasal parametre açlık ve tokluk bulgularımızda yalnızca BUN, GLU, ve TRİG' te anlamlı fark çıkmış olup diğer parametrelerde anlamlı fark gözlenmemiştir. Gözlenen bu farkların ortalama ve standart sapmalarına bakıldığında farklılığın önemli boyutta olmadığı, örnek sayısı arttırılıp klinik önemliliğe bakılırsa kanımızca klinik açıdan önemli olmayacaktır.

Anahtar Kelimeler: Açlık, Tokluk, Biyokimya parametreleri

Abstract

Objectives: Patients fasting blood samples are traditionally delivered to laboratuvary in the morning. However, blood is drawn from patients at all hours of the day and fasting can not be certainly assured. In this study, we aimed to compare the fasting- postprandial biochemical parameters of the subjects.

Materials and Methods: Fasting blood samples in the morning and postprandial blood samples in the afternoon were drawn from students (n = 723, 297 females, 426 males) and this samples were analysed with biochemical autoanalysers.(Siemens Advia-1800) in the central laboratory of the university hospital and results are statisticaly analyzed. Paired-t was evaluated by SPSS (21) using Wilcoxon Sign Ranktests. The groups were seperated; in according to BMI, gender and a general group and the relationship between fasting and postprandial status was indicated.

Results: According to our study when we compared with BMI between fasting and postprandial gruop in underweight there was only differences in the value of BUN, eventhough in the normal weight group there was a differences in the value of Ca, BUN and TG but in the overweight group we stil did not see any differences. When we compared according to gender between male and female, we realized the differences in the value of women in TRIG, GLU and BUN, while in male Ca, TRIG, GLU and BUN were significantly different (p<0, 05).

Conclusions: In our fasting and satiety findings of 18 biochemical parameters examined, a significant difference was found only in BUN, GLU, and TRIG, and no significant difference was observed in other parameters. Considering the mean and standard deviations of these observed differences, the difference is not significant and if the number of samples is increased and the clinical significance is considered, it will not be clinically important in our opinion.
Keywords: Fasting, Postprandial, Parameters of biochemistry

GİRİŞ

Tokluk olarak adlandırılan metabolik durum yemek sonrası 2-4 saatlik süreçtir. Yemek sindirimi sonucunda plazma glikoz, amino asitler ve triaçilgliserol seviyeleri yükselir (J. Koolman 2005). Pankreas tokluğa yanıt olarak insülin sekresyonunu arttırarak glukagon sekresyonunu azaltır ve kan glikoz düzeyini referans aralığı içinde tutmaya çalışır. İnsülin/glukagon seviyelerindeki bu değişme dokularda (özellikle karaciğer kas ve yağ dokularında) anabolik evreyi tetikler. Karaciğer, sağlanan substratlardan glikojen ve lipid oluşturur. Oluşan glikojen karaciğerde depolanırken lipidler çok düşük yoğunluklu lipoproteinlerle (VLDL) kana taşınır. Kas, ayrıca glikojen deposunu doldurur ve sağlanan amino asitlerden proteinleri sentezler. Adipoz doku, serbest yağ asitlerini lipoproteinlerden uzaklaştırır, triaçilgliserolleri sentezler ve bunları çözünmez damlacıklar halinde depolar. Metabolizmanın tokluk sürecinde beyin ve kalp enerji kaynağı olarak temelde glikozu kullanır. Sadece uzun süreli açlıkta glikozdan sonra yalnızca keton cisimlerini kullanabilir (J. Koolman 2005, Ferrier 2011).

Ayrıca adipoz dokuda sentezlenen peptit yapılı olan leptin hormonu, kas ve kahverengi yağ dokusunda glikoz alımını uyarır ve pankreasın α ve β hücrelerinden salgılanan glukagon ve insülin salınımını baskılar. Dahası, leptin, beyaz yağ dokusunda lipolizi arttırır ve karaciğerden glikoz çıkışında genel bir azalmaya neden olur (D'souza et al. 2017).

Tokluktan açlığa geçilirken vücud açlığa cevap olarak pankreasın α hücrelerinden glukagon hormonunu salgılar. Glukagonun temel amacı kan glikoz düzeyini açlık döneminde normoglisemik düzeylerde tutmaya çalışmaktır (Lessan and Ali 2019). İnsülin/glukagon hormonları metabolizmayı düzenleyerek dengeli şekilde lipoliz/lipogenez ve glikojenoliz veya glikojen sentezini düzenler. Yapılan bir çalışmada, insülin seviyelerinin hem açlık hem de toklukta periferal bir peptit olan ghrelin ile ters orantılı olduğunu bu nedenle insülinin, ghrelin salgılanmasını modüle eden güçlü bir faktör olduğunu göstermişlerdir. İnsanlarda akut insülin infüzyonu, ghrelin seviyelerinde hızlı ve geri dönüşlü bir düşüşe neden olur. Ghrelin midede bulunan X/A adlı endokrin hücreleri tarafından salgılanır ve büyüme hormonunun (GH) salgılanmasını sağlar(Öztürk and Arpacı 2018). Dahası ghrelin, pankreasın α ve β hücrelerine doğrudan etki ederek glukagon sekresyonunu stimüle eder ve glikoz kaynaklı insülin artışını inhibe eder bu da kan şekeri artışına neden olur. Dolayısı ile tüm vücud enzim/hormonları tokluk ya da açlığa rağmen metabolizmayı dengede tutma yönünde işlev yaparlar (Pinkney 2014).

Bu çalışmada ülkemizde de pek çok laboratuvar testinin yapılmasında halen tercih edilen açlık kanı ile zorunlu haller dışında çoğunlukla tercih edilmeyen tokluk kanının bazı biyokimyasal parametreler üzerine olan etkisinin karşılaştırılması amaçlandı.

MATERYAL VE METOD

Hatay Mustafa Kemal Üniversitesi Tıp Fakültesi'nde eğitim gören öğrenciler arasından 723 sağlıklı gönüllü çalışmaya dahil edilmiştir. Çalışma için H.M.K.U tıp fakiltesi etik kurulundan onay alındı ve tüm deneklere aydınlatılmış onam formu imzalatıldı. Gönüllülerden sabah ilk açlık ve üniversite yemekhanesinde verilen öğle yemeğini takiben bir saat sonraki tokluk kanları sarı kapaklı jelli biyokimya tüpüne alınmıştır.Pıhtılaşma sonrası 1500xg'de 10 dakika süreyle santrifüj edilmiştir. Klinisyenler tarafından en sık istemi yapılan; Albumin (ALB), Amilaz(AMYLAS), Aspartat Transaminaz (AST), Alanin Transaminaz (ALT), Alkalen Fosfataz (ALP), Demir (IRON), İnorganik Fosfat (IP), gama – Glutamiltransferaz (GGT), Glukoz (GLU), Yüksek Dansiteli Lipoprotein (HDL), Kalsiyum (Ca), Kreatinin (CRE), Kolesterol (CHOL) Kreatin Kinaz (CK), Total Protein (TP), Trigliserid (TRİG) Lipaz (LİP), Kan üre azotu (BUN) gibi 18 parametreleri seçilmiştir. Çalışmaya dahil edilen bireylerimizi cinsiyete göre kadın - erkek ve vücud kitle indeksine göre ise VKI < 20 zayıf, VKI = 20-25 normal ve VKI > 25 hafif kilolu olarak ayırdık. Numuneler Advia Chemistry 1800(Siemens) adlı otoanalizörde ölçülmüştür. İstatistiksel değerlendirmede intra ve interinduvidial varyasyonlar, biyolojik varyasyonlar, Mann Whitney U, Shapiro-Wilks, Kruskal Wallis yöntemleri ile analiz edilip SPSS (21) programı ile değerlendirildi. Böylece açlık ve tokluk durumu arasındaki ilişki ve/veya farklılıklar ortaya konuldu.

BULGULAR

Çalışmaya alınan 723 bireyin 297 kadın ve 426 'sı.erkekti. Bu bireylerde 18 biyokimyasal parameter aç ve tok olarak ölçüldü. Tablo 1 de; 297 kadın ve 426 erkek bireyimizin ortalama ve standart sapmaları ile p değerleri gözlenmektedir. Kadınların açlık-tokluk değerleri arasında TRİG (p=0,001), GLU (p=0,001) ve BUN (p= 0,008)' da istatistiksel olarak anlamlı fark gözlenirken erkeklerde ise Ca (p=0,006), TRİG (p=0,000), GLU (p=0,020) ve BUN (p=0,021)'da anlamlı fark gözlenmiştir. Ayrıca bireylerimizi VKİ' ne göre VKI < 20 zayıf, VKI = 20-25 normal ve VKI > 25 hafif kilolu olarak ayrıldı. Zayıf grubumuzda yalnızca BUN' da (p=0,035) istatistiksel olarak anlamlı fark gözlenirken normal grupta TRİG (p=0,000), GLU (p=0,004), Ca (p=0,012) ve IP (p=0,015) 'de istatistiksel olarak anlamlı fark görülmesine rağmen hafif kilolu grubumuzda 18 parametremizin hiçbirinde istatistiksel olarak anlamlı fark gözlenmedi.

Tablo 1: Cinsiyete göre kadın ve erkek gruplarımızın ortalama ± standart sapma değerleri ile p değerleri

GRUPLAR			**KADIN**			**ERKEK**
PARAMETRELER	Aç Ort ± SS	Tok Ort ± SS	(p)	Aç Ort ± SS	Tok Ort ± SS	(p)
CHOL	152,58 ± 31,36	154,15 ± 33,05	0,633	144,46 ± 37,94	145,62 ± 41,04	0,822
CK	73,65 ± 40,87	85,38 ± 42,3	0,109	122,45 ± 49,49	130,63 ± 52,02	0,246
GLU	74,73 ± 9,55	83,47 ± 15,93	0,001	76,22 ± 13,46	82,32 ± 17,18	0,020
AMILAZ	70,02 ± 23,9	72,02 ± 25,07	0,501	57,84 ± 22,39	59,51 ± 20,89	0,624
IRON	75,34 ± 47,05	66,03 ± 32,85	0,110	83,37 ± 32,75	89,46 ± 35,56	0,321
TRIG	74,19 ± 35,24	97,78 ± 61,27	0,001	97,64 ± 53,18	134,36 ± 70,54	0,000
ALP	55,61 ± 16,55	57,76 ± 18,93	0,164	64,94 ± 18,4	67,6 ± 20,57	0,320
AHDL	51,3 ± 12,96	49,7 ± 11,9	0,236	39,38 ± 9,98	36,68 ± 9,6	0,101
LIP	36,91 ± 9,76	35,58 ± 8,46	0,329	33,81 ± 11,7	32,69 ± 9,12	0,487
AST	16,22 ± 3,22	16,47 ± 3,77	0,602	17,62 ± 4,85	17,55 ± 4,33	0,902
GGT	14,24 ± 4,52	14,17 ± 4,58	0,918	19 ± 6,19	18,26 ± 7,26	0,449
ALT	13,47 ± 5,02	13,64 ± 5,44	0,766	22,55 ± 11,13	21,83 ± 9,27	0,480
BUN	9,84 ± 2,27	10,56 ± 2,67	0,008	11,58 ± 2,76	12,31 ± 3,04	0,021
CA	9,2 ± 0,43	9,28 ± 0,4	0,167	9,36 ± 0,53	9,58 ± 0,44	0,006
TP	7 ± 0,82	6,95 ± 0,84	0,685	6,94 ± 1,02	6,83 ± 1,28	0,598
ALB	4,44 ± 0,38	4,41 ± 0,47	0,628	4,54 ± 0,41	4,61 ± 0,41	0,316
IP	3,7 ± 0,61	3,61 ± 0,54	0,302	3,59 ± 0,88	3,37 ± 0,72	0,074
CRE	0,67 ± 0,11	0,68 ± 0,14	0,374	0,87 ± 0,15	0,85 ± 0,17	0,602

Tablo 2: Vücud kitle indeksine göre grupların ortalama ve standart sapma değerleri değerleri

GRUPLAR	**VKİ< 20**		**VKİ= 20-25**		**VKİ> 25**	
PARAMETRELER	Aç Ort ± SS	Tok Ort ± SS	Aç Ort ± SS	Tok Ort ± SS	Aç Ort ± SS	Tok Ort ± SS
CHOL	138,79 ± 37,89	143,07 ± 30,39	147,6 ± 33,39	148,81 ± 37,3	151,63 ± 39,5	149,83 ± 40,35
CK	72,68 ± 21,71	92,56 ± 38,47	93,41 ± 50,07	103,46 ± 50,98	130,77 ± 56,15	132,25 ± 54,46
GLU	69,79 ± 13,46	81,86 ± 15,64	76,31 ± 11,76	82,44 ± 14,94	77,17 ± 10,15	84,47 ± 20,03
AMILAZ	76,1 ± 31,06	80,9 ± 16,07	65,75 ± 24,62	67,63 ± 25,64	53,81 ± 15,85	53,58 ± 16,32
IRON	90,27 ± 67,44	78,14 ± 38,44	82,22 ± 38,28	83,27 ± 36,92	70,83 ± 25,94	67,43 ± 31,53
TRIG	63,93 ± 26,91	68,79 ± 22,78	80,85 ± 38,31	117,98 ± 67,36	107,53 ± 58,7	130,37 ± 64,03
ALP	55,29 ± 23,84	59,93 ± 23,72	60,38 ± 17,19	62,64 ± 19,94	64,53 ± 17,79	65,73 ± 20,89
AHDL	49,87 ± 16,42	49,19 ± 13,46	46,11 ± 12,31	43,47 ± 12,3	39,31 ± 9,03	37,36 ± 10,24
LIP	33,71 ± 10,79	33,36 ± 5,15	36,56 ± 11,88	34,88 ± 9,61	32,27 ± 7,67	31,03 ± 7,18
AST	16,14 ± 3,88	17,29 ± 3,6	16,59 ± 3,98	16,81 ± 4,21	18,79 ± 4,55	17,93 ± 3,89
GGT	14,46 ± 7,16	15,31 ± 6,07	16,33 ± 4,98	15,78 ± 6,05	19,2 ± 7,5	18,4 ± 7,69
ALT	12,21 ± 4,35	13,5 ± 6,21	17,53 ± 8,84	17,23 ± 8,19	24,4 ± 12,02	23,1 ± 9,52
BUN	10,12 ± 2,13	11,86 ± 3,12	11,02 ± 2,94	11,52 ± 3,2	10,56 ± 2	11,24 ± 2,1
CA	9,06 ± 0,49	9,19 ± 0,44	9,31 ± 0,46	9,46 ± 0,42	9,37 ± 0,53	9,53 ± 0,43
TP	7,1 ± 0,65	6,8 ± 0,99	6,96 ± 1	6,91 ± 1,1	7,03 ± 0,85	6,87 ± 1,19
ALB	4,43 ± 0,36	4,36 ± 0,5	4,54 ± 0,38	4,54 ± 0,46	4,46 ± 0,4	4,53 ± 0,41
IP	3,32 ± 0,92	3,6 ± 0,65	3,71 ± 0,78	3,46 ± 0,66	3,63 ± 0,62	3,45 ± 0,66
CRE	0,67 ± 0,12	0,61 ± 0,11	0,79 ± 0,16	0,79 ± 0,18	0,79 ± 0,16	0,8 ± 0,16

Tablo 3: Vücud kitle indeksine göre grupların p değerleri

GRUPLAR	VKİ< 20	VKİ= 20-25	VKİ> 25
PARAMETRELER			(p)
CHOL	0,687*	00,759	0,782
CK	0,193	0,136	0,859
GLU	0,09	0,004	0,086
AMILAZ	0,241	0,524	0,943
IRON	0,576	0,840	0,583
TRIG	0,526	0,000	0,064
ALP	0,462	0,253	0,718
AHDL	0,867	0,068	0,239
LIP	0,905	0,268	0,425
AST	0,591	0,686	0,241
GGT	0,742	0,642	0,595
ALT	0,597	0,686	0,322
BUN	0,035	0,057	0,072
CA	0,488	0,012	0,09
TP	0,372	0,790	0,568
ALB	0,859	0,995	0,536
IP	0,153	0,015	0,184
CRE	0,169	0,955	0,784

TARTIŞMA

Literatürde 2010' lu yıllara kadar uygun metabolik durumun açlık olduğu kabul edilirken son yıllarda hasta yatış sürelerini azaltmak ve 24 saat laboratuvarın tüm başvuran hastalara hizmet vermesi istendiğinden günün her saatinde hastalardan kan alınmakta ve açlıktan tam olarak emin olunamamaktadır. Sabahın erken saatlerinde kan alma birimlerinin yoğunluğu, sabah 8' de açlık kanı vermeye gelen hastaların öğlen saatlerine kadar beklemelerini ve bu bekleyişin diyabeti olan, hamile, yaşlı ve özellikle çocuk hastalar için zor oluşu veya aç kalamamaları filebotomistler ve hastalar açısından sıkıntıya neden olabilmektedir. Aslında gün içerisinde zorunlu olarak aç kalmak dışında vücut metabolizması günün büyük bir çoğunluğunda tokluk durumundadır. Halen açlık metabolizması testleri ile kişileri değerlendirmek belki de doğru bir durum olmayabilir. İnsanlar üzerine yapılan açlık-tokluk değerleri arasındaki farkın araştırılması genellikle lipidler üzerine iken sınırlı sayıdaki çalışmalar albümin, bilirubin ve ürik asidi de içeren diğer biyokimya parametrelerini kapsamaktadır (Ma, Viczko and Naugler 2017, Plumelle et al. 2014, Pasic et al. 2012).

Tok karnına alınan lipid profili örnekleri ile aç karnına alınan lipid profili örneklerini karşılaştıran Langsted ve ark. yaptığı çalışmada plazma trigliseridlerinde minör artışlar ile toplam LDL kolesterol konsantrasyonunda minör azalışlar olduğunu kaydetmişken, HDL kolesterol değerlerinde herhangi bir değişiklik olmadığını saptamışlardır (Nordestgaard et al. 2016). Yine Nordestgaard BG ve ark. yaptıkları çalışma sonrasında aç karnına örnek kullanımının ilk aşama taramalarda daha az önem taşıdığını, ancak genetik olarak belirlenen dislipidemilerin fenotipik teşhisini belirlemede daha önemli olabileceğini belirtmişlerdir (Nordestgaard 2017).

Çalışmamızda totalde 18 biyokimya parametresi kullanılmış ve VKİ' ye göre 3, cinsiyete göre ise 2 olmak üzere toplamda 5 grubumuz mevcuttur. Bu 5 grup incelendiği zaman 18 parametre arasından yalızca birkaçında istatistiksel olarak ($p<0, 05$) ile anlamlı fark gözlenmesine rağmen farklılık olan parametrelerin Ort ± SS değerleri arasında önemli derecede bir farklılık oluşturmadığı kanısındayız (Tablo 2). Ayrıca VKİ grublarımızın açlık/tokluk p değerlerini incelediğimizde; zayıf grubda yalnızca BUN' da ($p=0,035$) fark bunurken kilolu grubda hiçbir farklılık bulunmadı. Nomal grubda ise TRİG ($p=0,000$), GLU ($p=0,004$), Ca ($p=0,012$) ve IP ($p=0,015$) 'de istatiksel farklılık bulundu. Bu nedenle istatistiksel farkın klinik açıdan ne kadar önemli olabileceği tartışmaya açık bir konudur. Bizler bulgularımıza göre açlık ve tokluk kanı arasında klinik önem arz edecek kadar önemli farklılıklar olduğunu düşünmüyoruz.

SONUÇ

Çeşitli sebepler ile hastanelere başvuran bireylerden rutin biyokimyasal analizlerin değerlendirilmesi için açlık\tokluk durumlarına bakılmaksızın değerlendirilebileceği öngörülebilir. Bu çalışma aynı bireylerin açlık ve

tokluk durumunun karşılaştırılması olarak ülkemizde yapılan ilk çalışma olmakla birlikte bizim de pilot çalışmamızdır. Denek sayısını arttırarak daha detaylı bir araştırma hedeflemekteyiz.

Önerimiz ise, halen klinik laboratuarlarımızda her saat kan alınarak fiilen kullanılan tokluk durumunun kayıtlara alınması ve gerekiyorsa referans aralıkları analizlerimizde küçük değişiklikler yaparak rutinde kullanmaktır. Şüpheye düşülen örneklerde ise aç olarak örnek tekrarı yapılmasıdır. Zaten rutinde kullanılan fiili durumu bilimsel olarak tanımlayabilmek için katkı sunduğumuzu düşünüyoruz. Bu ve benzeri daha kapsamlı çalışmalarla konunun aydınlatılabileceğini umuyoruz.

TEŞEKKÜR

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➢ *ORAL PRESENTATION*

Congo Red Decolourization by Using *Caulerpa prolifera* Based Silver Nanoparticles

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Abstract
Industrial effluent leads to pollution in the stream and affects the micro and macro organisms lived in there. Untreated dyes are still one of the important pollution reasons for aquatic ecosystems. There is a great need for eco-friendly and cheaper treatment methods for polluted waters because of limited water resources all around the world. This paper investigates the decolourization of a model dye, Congo red, by using *Caulerpa prolifera* based silver nanoparticles. Waste *C. prolifera* fronds were used as reducing agents to synthesize silver nanoparticles (CP-AgNPs). Synthesized CP-AgNPs were characterized through UV-VIS and XRD. The results showed that the decolourization of Congo red is strictly dependent on pH, temperature, agitation time and CP-AgNP concentration. It is also found that the synthesized AgNPs was encapped by secondary metabolites from *C. prolifera* which affects the decolourization rate. In conclusion, beach wastes including *C. prolifera* can be used as eco-friendly synthesis of AgNPs and the beach waste can be returned as an economical value.
Keywords: *Caulerpa prolifera*; Congo red; decolourization; Langmuir-Hinshelwood model; silver nanoparticles.

INTRODUCTION

Untreated dyes from industrial effluents are still problems in aquatic environments. Formation of dye layer on the surfaces of aquatic ecosystems such as lakes, rivers and also sea can prevent the penetration of sunlight into water bodies (Kuo, 1992; Kant, 2011). There is still a great need for development of low-cost and eco-friendly solutions for the decolourization of untreated dyes (Forgacs et al., 2004). The members of *Caulerpa* genus are common in the Mediterranean Sea. *Caulerpa prolifera* (Forsskål) J. V. Lamouroux (hereafter *C. prolifera*) is an indigenous species from *Caulerpa* genus in Turkey. The stable meadows of *C. prolifera* have existed in Çeşme, Turkey coastlines for a long time. In addition to other Mediterranean macrophytes, unrooted *C. prolifera* samples are also accumulated in Çeşme coastlines. However, these biomasses contain very important bioactive components and also they are ideal raw materials for the production of many industrial products (Chen et al., 2012; Ellis et al., 2012; Michalak and Chojnacka, 2015; Ariede et al., 2017).

Silver nanoparticles (AgNPs) have been taken interest of the scientists for a long time because of their important and wide application areas such as electronic, medicine, biology, pharmacy and decolourization (Abbasi et al., 2016; Hecel et al., 2017; Modi et al., 2015). Green synthesis methods are of great importance and therefore, it has taken a lot of interest of the scientists. The number of papers related to the synthesis of AgNPs by using green synthesis has been increased from 1 (in 1998) to 1607 (in 2019) (web of science).

In this study, we aimed to investigate the synthesis of AgNPs by using waste *C.prolifera* fronds. After synthesis, decolourization of a model dye, Congo red, was studied. To the best of our knowledge, this is the first scientific study on the synthesis of AgNPs by using waste *C. prolifera*.

MATERIALS AND METHODS

Materials

Silver nitrate was bought from Macron Fine Chemicals. Deionized water used in experiments was obtained from ELGA model OS007XXM1. *C. prolifera* was collected from Çeşme-Turkey coastlines. The geographical coordinates of the beach are 38° 19’ 01’’ N and 26° 17’ 11’’ E. These coordinates were taken from Google Earth

(Version 9.2.77.2). After collection, the impurities and salty water were removed by washing with tap water. The fronds were dried at 70 °C in an incubator (MEMMERT UM500). After the drying process, the samples were ground and the 500 µm sized particles were used in the following experiments.

Extraction of *Caulerpa prolifera*

The methods of Edison et al. (2016), Kathiraven et al. (2015) and the other method which we adapted were studied for the extraction of the *C. prolifera*. The extraction densities were chosen as 0.001 g/ml, 0.01 g/ml and 0.075 g/ml. Briefly, the ground *C. prolifera* samples were waited in deionised water for 30 min 80 °C (Edison et al., 2016) and in the room temperature for 24 hours (Kathiraven et al., 2015). Furthermore, extraction was performed for 60 min at 80 °C. In order to obtain the extract, the solution was filtered by using Whatman A40 paper. The extract was stored at 4 °C until used.

Green synthesis of AgNPs

Optimization of AgNPs synthesis was performed by working on parameters with the concentration of *C. prolifera* extract and $AgNO_3$ solution, temperature, pH, agitation time and agitation rate. 10 mL 0.001 M $AgNO_3$ solution was mixed with 10 mL *C. prolifera* extract and then 30 mL deionised water was added. The pH of the solution is adjusted using NaOH and HCl. Agitation rate were set at different values as 0, 200 rpm, 300 rpm, 400 rpm. The experiments were performed at 25 ˚C, 40 ˚C, 55 ˚C, 70 ˚C and 85 ˚C. Concentration of [$AgNO_3$] were selected as 0.0010 M, 0.0025 M, 0.0050 M, 0.0075 M, 0.0100 M, 0.0250 M, 0.0500 M, 0.0750 M, 0.1000 M and 0.2000 M for optimization. pH values were chosen as 3.0, 4.5, 6.0, 7.5 9.0 and 10.5. Concentration of *Caulerpa prolifera* extract were set as 0.001 M, 0.010 M and 0.075 M.
The formation of silver nanoparticles was followed by the colour change of the solution. The colour of the solution was changed from light yellow to reddish after 20 min agitation. AgNPs were separated via centrifugation at 10,000 rpm for 30 minutes.

Characterization of synthesized AgNPs

In order to characterize the silver nanoparticles synthesized, UV-VIS Spectrum (UV-VIS 1601 Schimadzu), BioTek Microplate Reader, FT-IR (PerkinElmer Spectrum BX) and XRD (Thermo Scientific ARL X'TRA) were used. Since colour changing was seen in the experiment, the spectrum of the solution was recorded between 200-800 nm. FT-IR was carried out for the biosynthesized AgNPs and *C. prolifera* sample. 100 mg of KBr powder and 1 mg of synthesized AgNPs were mixed and pellets were prepared. Thermo Scientific ARL X'TRA was used for X-ray diffraction (XRD) analysis. The analysis was performed between 5-90 degree angles with 2 degrees/minute scan rate and using Ni-filtered CuKα radiation.

Decolourization of Congo red

Decolourization of Congo red was carried out according to the method of Edison et al. (2016). In optimization studies, concentration of Congo red and AgNPs solution, temperature and agitation rate were studied. 0.0005 M, 10 ml of Congo red dye and 0.005 M, 5 ml of $NaBH_4$ solution were mixed and 0.05 ml AgNPs added. Approximately after 10 minutes, the colour of the mixture changed from red to white with regard to the concentration of Congo red dye. For optimization process Congo red concentration were set as $2x10^{-5}$ M-$10x10^{-5}$ M and AgNPs concentration set as $6.20x10^{-4}$ M, $3.10x10^{-4}$ M, $1.60x10^{-4}$ M, $7.75x10^{-5}$ M and $3.39x10^{-5}$ M. Temperature were performed at different values as 25 ˚C, 40 ˚C and 55 ˚C. 200 rpm, 300 rpm and 400 rpm values were chosen as agitation rate.

RESULTS and DISCUSSION

Synthesis Optimization

The extraction optimization is important because reducing agents which are found in *C. prolifera*, should be transferred to the water at high levels. Three optimization methods for extraction were executed and the procedure of Edison et al. (2016) was chosen for extraction because of high absorbance value. According to Figure 1, there are fluctuations in the diagram and 30min/80˚C had a high absorbance value as 0.687 at the 240 minutes.

Three values of extraction concentrations were chosen for study of extraction optimization. A linear increase was

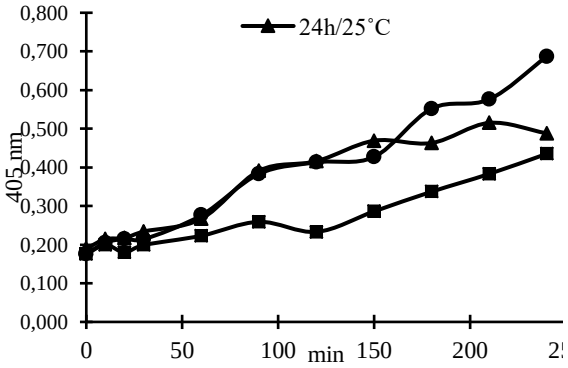


Figure 1. The effect of different extraction protocols for C. prolifera on AgNPs synthesis (pH=6.50, Temperature=60˚ C, C. prolifera concentration=0.02 g/mL, [$AgNO_3$] =0.001 M, Agitation rate=0 rpm).

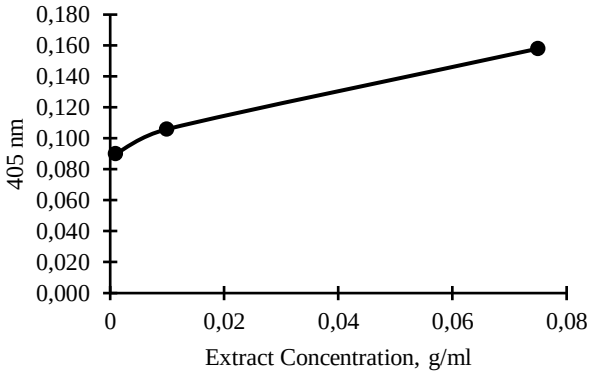


Figure 2. The effect of C. prolifera biomass concentration on the AgNPs synthesis (pH=6.5, Temperature=25˚ C, [$AgNO_3$] =0.001 M, Agitation rate=0 rpm).

observed and high absorbance value was 0.158 at 0.075 g/ml that was seen in Figure 2. This concentration value was chosen for further experiments.

In order to determine the effect of time on the AgNPs synthesis, experiments performed for 390 min. According to Figure 3, the highest absorbance value is 0.147 at 210 min, because of this, experiment time was selected as 210 min.

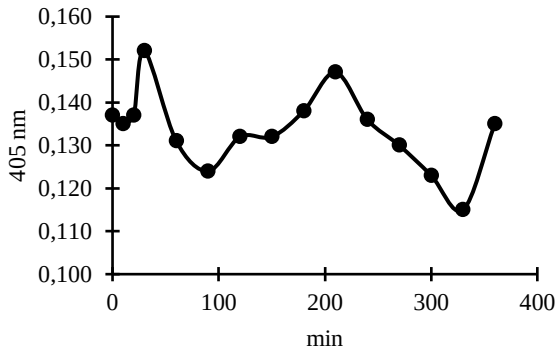


Figure 3. The effect of time on the AgNPs synthesis ([$AgNO_3$] =0.001 M, C. prolifera concentration=0.01g/mL, pH=6.00, Temperature=25˚C, Agitation rate=0 rpm).

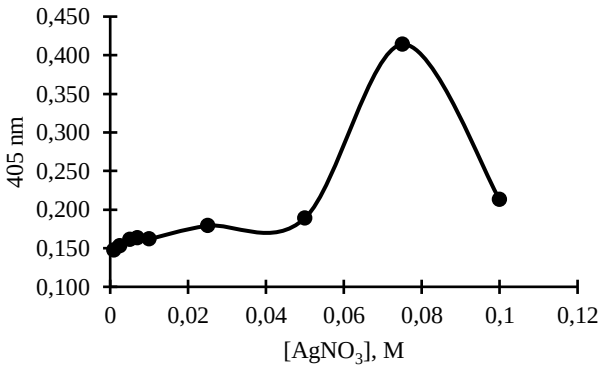


Figure 4. The effect of different $AgNO_3$ concentrations on the AgNPs synthesis (C. prolifera concentration=0.01g/mL, pH=6.00, Temperature=25˚C, Agitation rate=0 rpm, Agitation time=210 min).

The concentration of $AgNO_3$ is one of the important parameters for the green synthesis of AgNPs because it affects the yield of the reaction. According to Figure 4, there is a peak at 0.075 M and this is the highest absorbance value.

The temperature varied between 25 ˚C and 85 ˚C to evaluate the effect of temperature on the synthesis of AgNPs. The highest absorbance value was found as 2.88 at 85˚C. 70˚C was chosen as an optimum value for the synthesis of AgNPs.

The effect of different pH values was studied in the range of 3-10.5 for optimization of AgNPs synthesis. Colour changing was observed in different pH values from light yellow to dark brownish in our experiments. The highest absorbance value was found as 2.854 at pH 6. For further experiments as a pH of reaction solution pH 7 was chosen.

According to Figure 5, there is a decreasing trend based on the increasing agitation rates. The highest absorbance value was found as 1.1 when agitation was not used. Further experiments were performed at 200 rpm.

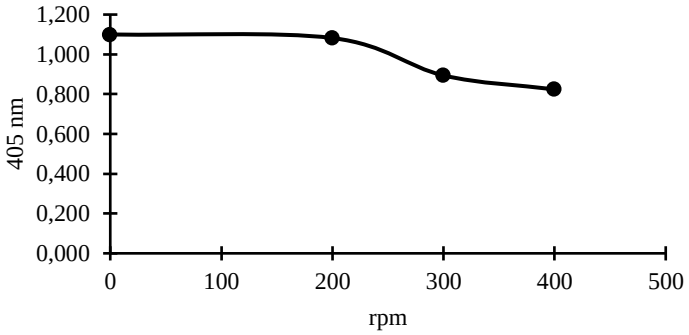


Figure 5. The effect of different agitation rate values on the AgNPs synthesis ([$AgNO_3$] =0.075 M, *C. prolifera* concentration=0.01g/mL, pH=7.5, Temperature=70˚C, Agitation time=210 min).

Characterization of synthesized AgNPs

The UV-Vis spectrum shows the optical properties of the synthesized AgNPs. There is a regular rise for absorbance value in terms of time (Figure 6). The highest absorbance peaks are found in the range of 448-450 nm wavelengths for different times and the highest absorbance value was observed at 451 nm for 24 hours.

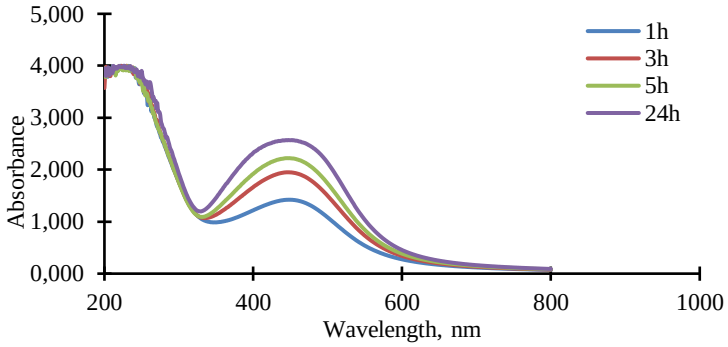
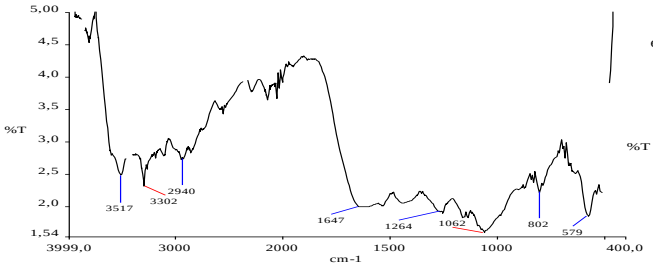


Figure 6. The UV-Vis spectrum of *C. prolifera* based AgNPs.

FT-IR spectrums of *Caulerpa prolifera* extract (Figure 7) have peaks at 3517 cm^{-1}, 3302 cm^{-1} and 2940 cm^{-1} which are attributed to stretching vibrations of OH and CH. Moreover, peaks at 1647 cm^{-1}, 1264 cm^{-1} and 1062 cm^{-1} originate from the (NH=O), C-O and C-N vibration. According to Figure 8, there are peaks at 3421 cm^{-1} and 2361 cm^{-1} which arise from stretching vibration of OH and NH. The sharp peak at 1381 cm^{-1} is characteristic for NO_3^- ion found in $AgNO_3$ solution.



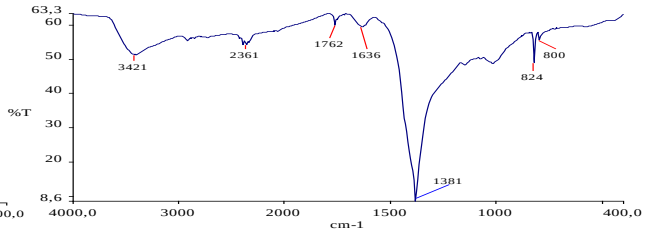


Figure 7. The FT-IR spectrum of *Caulerpa prolifera* biomass. **Figure 8.** The FT-IR spectrum of *Caulerpa prolifera* based AgNPs.

The difference between peaks found in Figure 9 and Figure 10 originates from the calcination step. Calcination process was done for removing the high noise and augmenting intense peaks at 400° for 2 hours. The noise in the spectrum pretty decreased after this step. According to Figure 10, the peaks are found at 38.04°, 44.2°, 64.36° and 77.3°. The size of AgNPs was calculated as 27.45 nm using full width at half maximum belonging to 46.06° with the Scherrer equation.

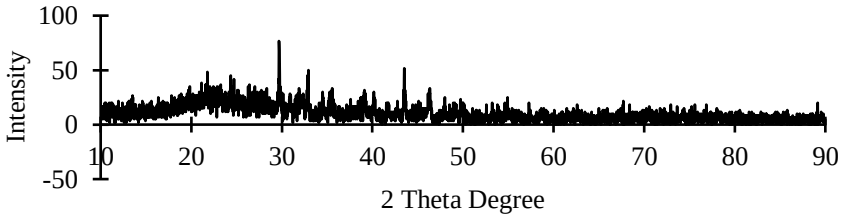


Figure 9. The XRD spectrum of *C. prolifera* based AgNPs without calcination.

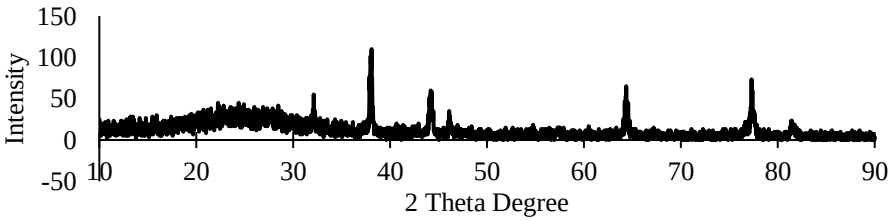


Figure 10. The XRD spectrum of *C. prolifera* based AgNPs with calcination.

If the crystal size of the powder samples is small enough, the max diffraction peak expands inversely proportional to the crystal size and using this additional expansion, the crystal size can be measured Langford et al., 1978). The particle sizes of the powder samples can be determined by the Scherrer equation using XRD peaks. Scherrer equation;

$$L = \frac{K.\lambda}{\beta.\cos\theta} \qquad (1)$$

where K and λ are Scherrer shape factors, θ is Bragg angle and β is full width at half maximum. K varies from 0.62 to 2.08. In our calculation, K and λ were taken as 0.94, 1.45178 Å respectively.

As seen in Table 3, there are different elements as C, N, O, Mg, Al, Si, P, S, and Cl which originate from extract and of Ag shows synthesized AgNPs. Elemental composition analysis of AgNPs according to the reduction agent source can be seen in Figure 11.

Table 4. Results of composition analysis for *C. prolifera* based AgNPs.

	Percentages of Elements										
	C	N	O	Na	Mg	Al	Si	P	S	Cl	Ag
C.prolifera-AgNPs	5.55	5.88	13.48	0.28	0.13	0.20	0.19	0.15	0.26	2.52	71.35

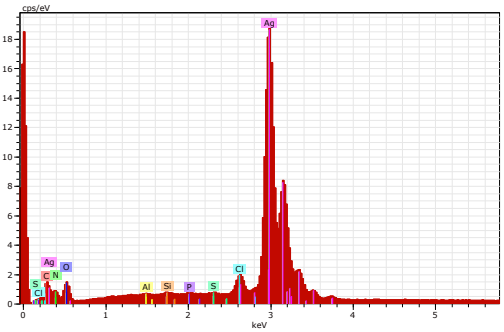


Figure 11. The elemental composition analysis of *C. prolifera* based AgNPs.

Catalytic Activity Assessment

Industrial effluent leads to pollution in the stream and affects the micro and macro organisms lived in there. Congo red as a model dye for investigating decolourization kinetics were chosen and worked. As explained in the Materials and Methods section, the decolourization of Congo red as a model dye was performed.

The pseudo-first order kinetic model is rationalized with the Langmuir-Hinshelwood model, which is arranged for reactions ensuing in a solid-liquid interface (Turchi and Ollis, 1990; Poulios et al., 2003).

$$r_0 = -\frac{dC}{dt} = \frac{k_r K C_{eq}}{1+KC_{eq}} \qquad (2)$$

Where r_0 is the initial fading rate of the organic substrate, C_{eq} is the equilibrium bulk-solute concentration and K symbolizes the equilibrium constant for adsorption. k_r is the limiting constant of the reaction in the case of maximum coating. The equation can be applied when data provides linearity as follows:

$$\frac{C_{eq}}{r_0} = \frac{1}{k_r K} + \frac{C_{eq}}{k_r} \qquad (3)$$

The effect of the change in equilibrium concentration of Congo red at constant AgNPs concentration on the initial rate of degradation is seen in Figure 12. Using the initial concentration of Congo red dye in the range of $2x10^{-5}$-10 x 10^{-5} g/ml, the r_0 values were obtained from C_{eq}-t diagram. At constant AgNPs concentration, the relationship between C_{eq}/r_0 and C_e is shown in Figure 13. The k_r ve K values were calculated according to equation 3 as using *C. prolifera* based AgNPs k_r=0.4192 mg.$l^{-1}$$min^{-1}$, K=1.9368 $mg^{-1}l$.

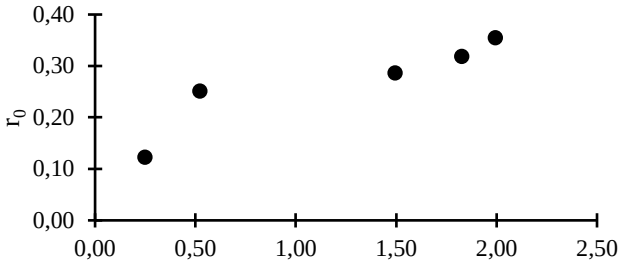


Figure 12. The plot of r_0 vs. C_{eq} for Congo red at different initial concentrations for the constant concentration of C. prolifera based AgNPs.

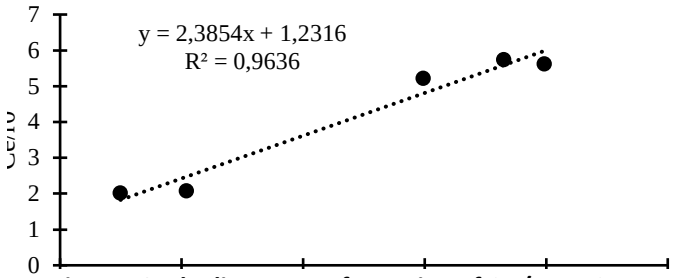


Figure 73. The linear transformation of C_{eq}/r_0 vs. C_{eq} according to Equation 3 (Data from Figure 12).

Decolourization of Congo red occurs by electron exchange between $NaBH_4$ and Congo red. In this reaction, $NaBH_4$ electron is donor and Congo red is acceptor. AgNPs act as catalysts by accelerating electron transfer and reaction occurs on the surface of AgNPs. It is seen from the linear correlation of t versus $\ln A_t / A_0$ graph that the reaction is compatible with pseudo first order kinetic law. Pseudo first order kinetic formulated with the following equation (Lagergren, 1898; Ys and McKay, 1999, Yuh-Shan et al., 2004):

$$\ln \ln \frac{C_t}{C_0}) = -k.t \qquad (4)$$

C_t= the concentration of Congo red in t reaction time.
C_0= the initial concentration of Congo red.
k= Rate constant

According to Figure 14, the rate constants were calculated from the slope of the line and found as 0.0791 min^{-1} for *C. prolifera* based AgNPs.

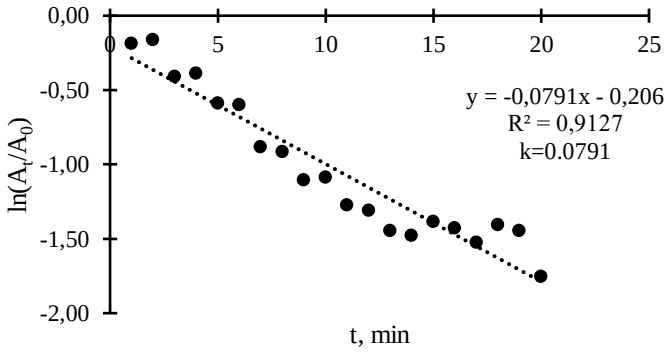


Figure 14. The first order kinetics plot of $\ln(A_t/A_0)$ versus time for decolourization of Congo red using *C. prolifera* based AgNPs.

CONCLUSION

In this work, AgNPs were synthesized by using *Caulerpa prolifera.* Since the accumulated fronds on the beaches are considered as "beach waste", the local municipalities collect the biomass of *Caulerpa prolifera* and then burn it to clean the beaches. On the other hand, the biomass of *Caulerpa prolifera* includes very important bioactive agents and they can be used for eco-friendly and cost effective industrial purposes. From this point of view, this paper can be considered as a model paper for industrial evaluation of the beach wastes. The present study reports that AgNPs can be synthesized by using *Caulerpa prolifera* and the synthesized AgNPs can be used in the decolourization of textile based dyes.

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➢ *ORAL PRESENTATION*

Evaluation of DNA extraction protocols from cottonseed oil

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Abstract

Extraction of pure and intact DNA is the most important step in several techniques for molecular analysis. Depending on matrix different extraction protocols have been studied so far in literature. Oil, as complex matrix, has certain difficulties for getting pure and intact DNA. In this study, DNA isolation study was performed with four different methods from commercially available cotton oil samples used in food industry. The concentrations and purities of DNA samples isolated by cetyl trimethylammonium bromide (CTAB)-hexane-chloroform, CTAB-mercaptoethanol, CTAB and commercial kit were obtained by spectrophotometry. The yields and qualities of DNA extracted by these four methods were evaluated. The maximum amounts of DNA obtained from cottonseed oil samples were 58 ng/µL, 48 ng/µL and 143 ng/µL by CTAB-hexane-chloroform- based extraction method, CTAB-mercaptoethanol based extraction method and CTAB-based method, respectively. Commercial DNA isolation kit yield lower amount of DNA from cottonseed oil samples as compared to manual CTAB based methods. Maximum and minimum amounts of DNA were found as 12,3 ng/µL and 0,6 ng/µL. The ethanol purification step increased the purities of all DNA samples with slight decrease in their concentration.

Keywords: Cottonseed oil, DNA extraction, CTAB

INTRODUCTION

DNA-based technologies with DNA extraction methods are preferred nowadays in order to determine the origin or the adulteration in oil samples extracted from different plant sources. The first and the most important step in DNA-based molecular studies is to obtain intact DNA with high purity. The basic principle in the DNA extraction procedures was the breakdown of cell walls of vegetative cells if it is a plant, cell membrane disruption using detergents such as cetyl trimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS), thereby releasing DNA molecules in the cell. The success criteria in DNA isolation are the amount and the quality of the DNA and, they are mainly affected by the extraction methods and matrix itself. There are various studies in the literature in order to develop DNA isolation methods in oil samples and to provide pure, high quality and appropriate amounts of DNA for further studies. Since determining the source of the raw material and producing extra virgin olive oil are the most important factors for the quality control in foods, the DNA extraction studies use mostly olive oil samples as matrix. However, most of these studies resulted in the fragmentation of the DNA, obtaining lower DNA amount and the presence of impurities which prevent further processes (Testolin and Lain, 2005; Agrimonti et al., 2011; Raieta et al., 2015). Although DNA extraction methods for cotton seed samples were studied frequently, there is only one reporting DNA extraction from cottonseed oil samples so far. Huang et al. (2010) compared CTAB based method with FPLC based method for the purity and applicability of DNA for PCR.

Within the scope of this study, DNA isolation from cottonseed oil samples was performed using three CTAB-based DNA isolation methods previously determined for olive oil in the literature and a general DNA isolation kit developed for DNA isolation from foods. The concentration and purity parameters of the DNA obtained were checked with a spectrophotometer.

MATERIALS AND METHODS

Materials, reagents and samples

In this study, eight cottonseed oil samples from Adana region were obtained from supplier. All chemicals used for DNA extraction were purchased from Sigma-Aldrich (St Louis, MO). DNA extraction kit innuPrep Food DNA Kit was supplied from Analytikjena Company (Germany).

DNA extraction protocols

CTAB-hexane-chloroform- based extraction method

DNA was extracted by a method previously published by Raieta et al. (2015). In this method, oil samples were mixed with 2% CTAB buffer and hexane (1:1:1,v:v:v) and centrifuged at 20°C for 1 hour. Aqueous phase was incubated at 65°C for 30 min and centrifuged at 20°C for 15 min. Pellet was suspended in 0.5 % CTAB buffer and phenol and centrifuged again. Aqueous phase was mixed with chloroform: isoamylalcohol (24:1, v:v) and centrifuged. Aqueous phase was mixed with cold ethanol, amonium asetate solution and glycogen (10µg/mL) and incubated at -20°C over night. Upon incubation mixture was centrifuged and pellet was washed twice with 70% ethanol solution. Dried pellet was suspended in dH_2O and stored at -20°C.

CTAB-mercaptoethanol based extraction method

DNA was extracted by a method previously published by Busconi et al. (2003). Basically, 50 mL of oil sample was centrifuged at 4°C for 30 min and pellet was freezed in liquid nitrogen. Then, pellet was heated at 65°C and freezed again. This freeze-thaw step was repeated twice. The pellet was suspended in prewarm CTAB extraction buffer and incubated at 65°C for 90 min. After incubation solution was mixed with chloroform: isoamlyalcohol (24:1, v:v) and incubated at room temperature for 5 min. After centrifugation supernatant was mixed with chloroform: octanol (24:1, v:v) and incubated at room temperature for 5 min. Supernatant was mixed with 10% CTAB precipitation buffer and after centrifugation pellet was dried. It was suspended in NaCl solution and incubated at 37°C for 1 hour. It was washed with chloroform: octanol (24:1, v:v), cold ethanol and 70% ethanol solution separately, and after drying suspended in dH_2O. All extracted DNA samples were stored at -20°C.

CTAB-based extraction method

Oil samples were mixed with distilled water (1:1, v:v) then, 500 µL CTAB buffer was added to this mixture. After centrifugation at 16000x g for 10 min, supernatant was mixed with chloroform and centrifuged again. Upper phase was mixed with CTAP-precipitation buffer and incubated at room temperature for 1 hour. Mixture was centrifuged and pellet was suspended in NaCl solution. Then, suspension was mixed with chloroform and centrifuged to separate phases. Upper phase was then mixed with isopropanol and after centrifugation; pellet was washed with 70% ethanol solution. Pellet was dried and suspended in dH_2O. All extracted DNA samples were stored at -20°C.

DNA extraction by a commercial kit

For DNA extraction from cottonseed oil, innuPrep Food DNA Kit (Analytikjena, Germany) was used with InnuPure® C16 touch automated DNA extraction instrument. According to manual provided by manufacturer; 200 mg oil sample was mixed with lyses solution and protease K and incubated at 65°C for 1 hour. After centrifugation at 11.000xg for 10 min, supernatant was added to reagent plate and inserted into instrument for extraction steps. All extracted DNA samples were stored at -20°C.

DNA quantification

The concentration and purity of DNA samples extracted from cottonseed oils by four different methods was determined by measuring absorbance values of samples at 260nm and 280 nm. The concentrations of samples (µg/mL) were calculated by multiplying absorbance values at 260 nm with dilution factors and 50 for double stranded DNA. The purity values of samples were calculated by dividing the absorbance values at 260 nm to that at 280 nm. For each cottonseed oil samples five biological replicates were used in each extraction method and triple measurements were read for each replicates. All results were given as mean ± standard deviation and coefficient of variation (CV) was calculated by dividing standard deviation to the mean concentration and multiplied by 100 to give percent CV.

RESULTS and DISCUSSION

In order to identify the biological origin and detect adulteration in edible plant oils recent techniques depend on DNA based methods. The first step in all DNA-based methods is the extraction of DNA from different matrix such as seeds, leaves, oils and foods produced from plants. There have been certain studies in the literature for the development of DNA isolation methods from such matrix to obtain high quality and appropriate amounts of DNA. Since identification of original plant source for raw materials and production of extra pure oil sample are the two main factors effecting quality control parameters of olive oil, many of these studies have focused on olive oil samples for DNA extraction. As an example, Busconi et al. (2003) extracted DNA from four olive oil samples produced in Italy at 1999 by a modified CTAB extraction method and the yields of DNA were in the range of 5-10 ng/µL. Testolin and Lain (2005) used a commercial kit and CTAB method to extract DNA from extra virgin olive oil samples and obtained 8.7 ng/µL and 5.4 ng/µL DNA, respectively. However, obtaining lower amount of DNA, fragmentation of DNA during extraction and presence of impurities are the most common problems in extraction methods. In addition to these problems, olive oil production process and storage conditions also affect the DNA yield and quality. For example, while Cresti et al (1996) showed that higher amount of DNA was obtained from olive oil samples produced by cold press, Parfundo et al. (2010) found that olive oils stored in small bottles at dark gave higher amount of DNA when it was fresh or not older than one month.

Cotton DNA has been obtained mainly from seed samples and there is only one study in literature so far that used cottonseed oil samples for DNA extraction. Huang et al. (2010) presented a study showing FPLC based isolation procedure gave higher amount of DNA faster as compared to CTAB and commercial kit.

Within the scope of this study, DNA from cottonseed oil samples was isolated using three CTAB-based DNA isolation methods previously determined for olive oil in the literature and a DNA isolation kit developed for different food matrix and the concentration and purity parameters of obtained DNA were evaluated. With the use of first CTAB-based DNA isolation method the maximum amounts of DNA extracted from cottonseed oil were found as 58 ng/µL and 63 ng/µL for 300 and 500 µL initial sample volumes, respectively. CTAB-Mercaptoethanol based extraction method yielded DNA amounts within the range of 3 ng/µL and 48 ng/µL from cottonseed oil samples. In the last manual method, maximum DNA concentrations were obtained as 143 ng/µL and 42 ng/µL for 300 and 500 µL initial sample volumes, respectively. Commercial DNA isolation kit yield lower amount of DNA from cottonseed oil samples as compared to manual CTAB based methods. Maximum and minimum amounts of DNA were found as 12,3 ng/µL and 0,6 ng/µL. Testolin and Lain (2005) compared Promega Wizard Magnetic DNA purification kit, Qiagen QIAamp stool kit, Qiagen plant DNA extraction kit and LB Link-Biotech ExtMan DNA kit for their DNA extraction performance on olive oil samples and they reported a concentration range from 2,8 ng/µL to 8,7 ng/µL. Our results were our results were compatible with these results.

Figure 1 showed mean concentration of DNA extracted from eight cottonseed oils by four different methods used in this study. Commercial kit gave better results for repeatability with lowest error bars on graphs. Although the mean concentrations of DNA extracted by three manual methods were higher, standard deviations of measurements were also higher for all eight oil samples. Table 1 also presented the means, standard deviations and variation coefficients of DNA samples extracted from eight oil samples by four methods in this study. Among eight samples closer concentration values for DNA were obtained again by commercial kit with highest CV value of 2.1%. While the concentrations of DNA obtained by CTAB-hexane-chloroform-based method showed variance between 8.6% and 66%, the results of DNA isolated by the CTAB-mercaptoethanol based method showed variance between 24% and 89%.

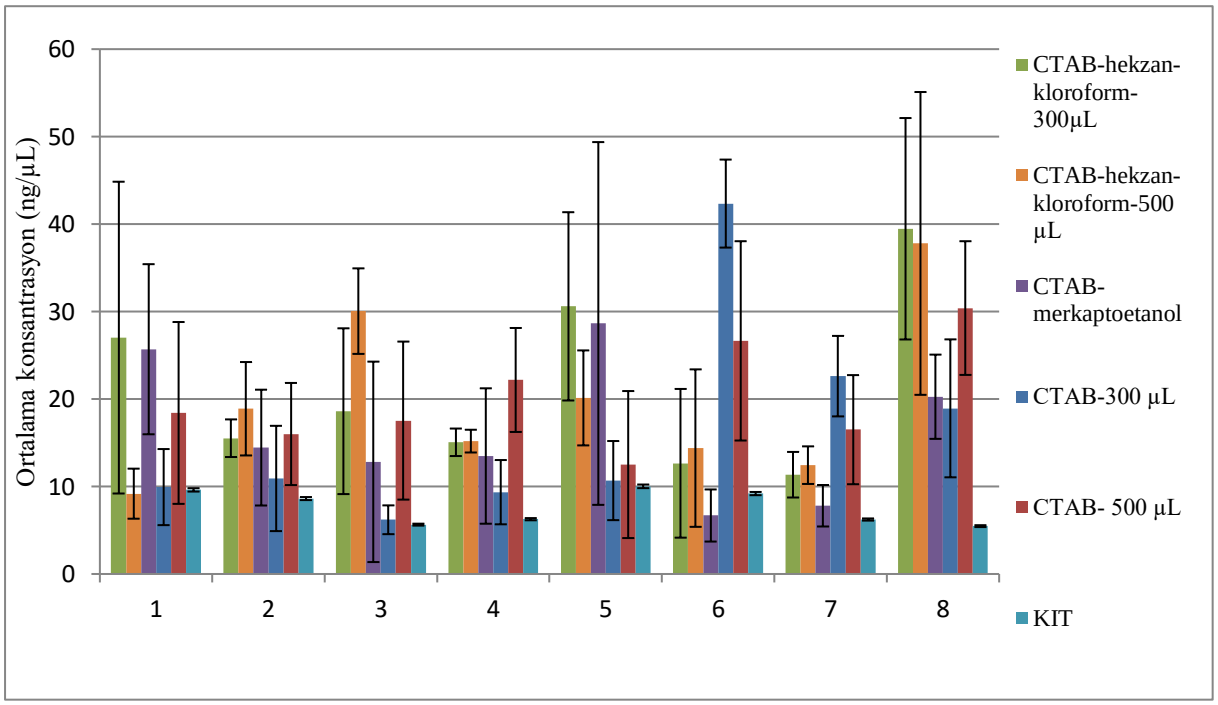

Figure 8. The mean concentrations of DNA extracted by four different methods from five replicates of eight oil samples (For CTAB-hexane-chloroform- based extraction method and CTAB-based extraction method two different initial sample volumes were used and 300 and 500 µL were representing these initial volumes of samples in the graph).

Table 1. The means, standard deviations and variation coefficients of DNA samples extracted from eight oil samples by four methods in this study

	CTAB-hekzan-kloroform-300 µL			**CTAB-hekzan-kloroform-500 µL**			**CTAB-merkaptoetanol**			**CTAB-300 µL**			**CTAB- 500 µL**			**KIT**		
DNA	**$\bar{x}$ (ng/ µL)**	**s (ng/µL)**	**CV (%)**	**$\bar{x}$ (ng/ µL)**	**s (ng/µL)**	**CV (%)**	**$\bar{x}$ (ng/µL)**	**s (ng/µL)**	**CV (%)**	**$\bar{x}$ (ng/µL)**	**s (ng/µL)**	**CV (%)**	**$\bar{x}$ (ng/ µL)**	**s (ng/ µL)**	**CV (%)**	**$\bar{x}$ (ng/ µL)**	**s (ng/µL)**	**CV (%)**
11	27,02	17,82	65,96	9,18	2,86	1,10	25,70	9,72	7,84	9,93	4,35	3,79	18,41	10,40	56,48	9,62	0,19	2,00
22	15,52	2,15	13,85	18,89	5,34	8,29	4,45	6,62	5,83	10,92	6,02	5,12	16,00	5,84	36,49	8,62	0,17	2,02
33	18,61	9,48	50,93	30,05	4,89	6,27	2,82	11,46	9,37	6,20	1,65	6,58	17,53	9,03	51,52	5,64	0,11	1,99
44	15,06	1,57	10,44	15,19	1,30	8,57	3,49	7,74	7,41	9,35	3,68	9,30	22,18	5,94	26,80	6,26	0,13	2,02
55	30,60	10,76	35,18	20,13	5,43	6,96	28,64	20,74	2,41	10,68	4,52	2,36	12,51	8,40	67,18	10,02	0,21	2,07
66	12,65	8,50	67,19	14,39	9,00	2,51	6,69	2,98	4,57	42,35	5,03	1,88	26,65	11,39	42,75	9,19	0,18	2,00
77	11,35	2,61	22,95	12,44	2,15	7,24	7,80	2,37	0,41	22,62	4,60	0,34	16,50	6,24	37,81	6,23	0,12	1,99
88	39,47	12,66	32,07	37,79	17,31	5,80	20,26	4,82	3,76	18,94	7,89	1,64	30,40	7,64	25,14	5,47	0,11	2,00

In order to have higher DNA yield and to remove undesired molecules (such as polysaccharides, proteins and phenols) DNA samples were purified by ethanol. Table 2 showed DNA concentration and purities of extracted and purified DNA samples in this study. Although there was a slight decrease in the amount of DNA samples due to washing steps with ethanol, the purities of DNA extracted from cottonseed oil samples by four methods was increased as observed by higher ratio of absorbance values at 260 nm to 280 nm. These purity values should be higher to perform further processes like PCR; however, the impurities were removed successfully from extracted DNA samples via ethanol precipitation.

Table 2. The concentration and purity values of DNA obtained after ethanol precipitation of DNA isolated from cottonseed oil samples by four different ways

	CTAB-hekzan-kloroform*		**CTAB-merkaptoetanol***		**CTAB***		**KIT***	
DNA	**ng/µL**	**A260/ A280**	**ng/µL**	**A260/ A280**	**ng/µL**	**A260/ A280**	**ng/µL**	**A260/ A280**
1	40,21	1,36	29,60	1,11	115,20	1,71	55,26	1,22
2	42,16	1,22	35,26	1,12	99,45	1,88	50,20	1,61
3	36,40	1,64	37,21	1,26	121,44	1,79	49,60	1,44
4	35,45	1,43	43,20	1,36	116,37	1,92	48,33	1,29
5	30,55	1,51	34,54	1,44	71,36	1,96	49,47	1,45
6	31,09	1,68	36,25	1,29	59,36	1,79	50,10	1,64
7	33,92	1,34	40,05	1,28	64,20	1,84	47,24	1,38
8	34,28	1,65	34,67	1,33	69,75	2,01	46,31	1,41

*The values are the means of three replicates.

CONCLUSION

The most important step in DNA-based molecular studies such as PCR, real-time PCR analysis, sequence analysis, single point mutation analysis and genetically modified organism analysis is to obtain intact DNA with high purity. In this study, DNA isolations were carried out with four different methods from commercially available cottonseed oil samples used in the food industry. The concentration and purity values of DNA obtained by CTAB-hexane-chloroform, CTAB-mercaptoethanol, CTAB and kit method were determined by spectrophotometry. The CTAB-based manual methods yielded higher amount of DNA from cottonseed oil samples as compared to those extracted by commercial kit; however, the repeatability of DNA extraction method was higher for commercial kit. Also, it was concluded that polysaccharide and other impurities in extracted samples upon isolation by CTAB-based manual methods prevented amplification. Although the concentrations of DNA were decreased slightly for all samples extracted by four methods, DNA became more pure after ethanol precipitation.

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➢ *ORAL PRESENTATION*

Simmental ırkı sığırlarda *K*-Kazein (CSN3) geni polimorfizmi ve bazı performans özellikleri arasındaki ilişkileri

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Özet

Erzurum'da özel bir işletmede yetiştirilen 70 baş Simmental sığırlarda *κ*-Kazein (*CSN3*) gen lokusu bakımından genotipik yapılarının incelenmesi, ilgili genler bakımından sığırlara ait genotip ve allel frekanslarının dağılımının belirlenmesi ve belirlenen genotiplerin bazı performans özellikleriyle ilişkilendirmesi amaçlanmıştır. Çalışmada kullanılan Simental sığırlardan alınan kan örneklerinden izole edilmiş DNA'larda, *PCR-RFLP* yöntemi kullanılarak *CSN3/HinfI* gen polimorfizmleri tanımlanmıştır.

Popülasyondaki *CSN3* genine ait AA, AB ve BB genotip frekansları sırasıyla %57,14, %3,86 ve %4,89, A allelinin frekansı 0,74 ve B allelinin frekansı 0,26 olarak tespit edilmiştir. AA, AB ve BB genotiplerinde ortalama değerler sırasıyla gerçek süt veriminde 5151±308,6, 5805±370.3 ve 5772±547,3 kg; 305 günlük süt veriminde 5313±233.9, 5784±280.7 ve 6458±414,8 kg; günlük süt veriminde 17,9±0.75, 18,6±0.89 ve 19,6±1,32 kg; laktasyon süresinde 294±13,7, 316±16.5 ve 294±24,4 gün olarak saptanmıştır.

İncelenen sığır ırkı popülasyonda *CSN3* gen lokusu dağılımının Hardy-Weinberg prensibine göre genetik dengede olduğu, *CSN3* gen polimorfizmi bakımından belirlenen genotip ve allel frekansları ırkın genotip çeşitliliğini ortaya koymada yeterli sayılabildiği, *CSN3* genotipleri ile performans özellikleri arasında ki ilişkilerin belirlenmesi için yapılan istatistik analiz sonuçlarına göre *CSN3* genotiplerin sadece 305-günlük süt verimiyle ilişkisinin önemli (P<0,05) olduğu, *CSN3* BB genotipli hayvanların ekonomik olarak sürüde avantaj oluşturduğu ve *CSN3*'ün bu bakımdan markör yardımlı seleksiyon(MAS) amacıyla kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: *κ*-Kazein, polimorfizm, Simmental, performans özellikleri, *PCR-RFLP*

The relationships between *K*-Casein (CSN3) gene polymorphism and some performance traits in Simmental cattle

Abstract

This study aimed to investigate the genotypic structure of *κ-Casein* (*CSN3*) gene locus, determination of the distribution of cattle genotype and allele frequencies in terms of related genes and to associate with performance traits of the genotypes in 70 Simmental cattle raised in a private enterprise in Erzurum. *CSN3/HinfI* genes polymorphism were defined by using the *PCR-RFLP* method in the DNAs isolated from blood samples taken from Simmental cattle used in this study.

The AA, AB, and BB genotype frequencies of the *CSN3* gene found in the population were 40 (%57,14), 23 (%32,86), and 7 (%4,89), and the frequency of the A allele and the B allele was found to be 0,74 and 0,26, respectively. The averages of AA, AB, and BB genotypes of *CSN3* were determined as 5151±308,6, 5805±370,3, and 6458±414,8 kg for the actual milk yield, as 5313±233,9, 5784±280,7, and 6458±414,.8 kg for 305-day milk yield, as 17,9±0,75, 18,6±0,89, and 19,6±1,32 kg for daily milk yield, and as 294±13.7, 316±16,5, and 294±24,4 days for lactation periods, respectively.

In the study was concluded that in the point of *CSN3* gene locus was in Hardy-Weinberg genetic equilibrium, the genotype and allele frequencies detected in terms of *CSN3* gene polymorphism were sufficient to reveal the genotype diversity of the breed, according to the statistical analysis results of the relationships between *CSN3* genotypes and performance traits, only the effect of genotype on 305-day milk yield was significantly(P<0,05), the cattle with *CSN3* BB genotype are economically advantageous in the herd, and therefore *CSN3* can be used for marker-assisted selection(MAS).

Keywords: *CSN3*, polimorfizm, Simmental, performance traits, *PCR-RFLP*

Giriş

Dünya nüfusu 1950 yılında 2,5 milyar iken 2000'li yıllarda 6 milyara kadar ulaşmıştır (Anonim, 2018). Dünya popülasyonundaki artış nedeniyle insanlığın beslenmesinde daha fazla besin madde üretimine gidilmesi gerekmektedir(Akman, 2016). Nüfusun artması, beraberinde beslenme ihtiyacının özellikle hayvansal protein ihtiyacının karşılanmasını gerektirmektedir. Buda hayvancılık sektörünün her yönüyle gelişmesiyle mümkündür. Bu ihtiyacın giderilmesinde hayvancılık sektöründe önemli bir yer tutan sığır varlığının ve verim potansiyelinin özellikle büyük bir önemi vardır. Hayvancılık sektörü içerisinde süt sığırcılığı ayrı ve önemli bir yere sahiptir ve dünyada süt üretiminin yaklaşık %93'ü sığırlardan elde edilmektedir (Hodoğlugil, 1996; Kopuzlu, 2003).

Türkiye'de, tarım sektörü içerisinde en önemli alanlarından biri olan hayvansal üretime ve hayvansal üretim içerisinde de sığır yetiştiriciliğine büyük önem verilmektedir. Nitekim Türkiye'de 2019 yılı istatistiklerine göre yetiştirilen toplam büyükbaş ve küçükbaş hayvan varlığı 66.169.618 baş olup bunun 17.688.139 başı sığırlardan oluşmaktadır. Toplam hayvan varlığı içerisinde sığır varlığı yaklaşık %21,1'ini oluşturur (TUİK, 2020). Türkiye genelinde ise 2019 yılı itibariyle e-ıslah veri tabanına kayıtlı sığır varlığının yaklaşık % 38,93'ünü Siyah Alaca, % 36,11'ini Simmental, % 16,91'ini Esmer, % 4,84'ünü Yerli sığır ırkı ve % 3,21'ini diğer sığır ırkları oluşturmaktadır (Harmandar, 2019).

Simmental ırkı verim potansiyeli değerlendirildiğinde yüksek süt ve döl verimine sahip olduğu aynı zamanda da besi performansı ve hastalıklara dayanıklılığının iyi olması gibi özellikleri nedeniyle üreticiler tarafından tercih edilmektedir. Türkiye'ye, hayvancılığın gelişmesi amacıyla ülke dışından 1925 yılından itibaren Simmental ırkı sığırlarda dahil kültür ırkı hayvanlar getirilmeye başlanmıştır.(Koç, 2016; Akman ve ark., 1990). Simmental ırkı hayvanlarının en yoğun yetiştirildiği bölgeler Orta Karadeniz (Amasya ve Çorum'unda içinde bulunduğu), İç Anadolu (özellikle Afyon), Ege ve Doğu Anadolu bölgeleridir. Yetiştiriciliğin en az olduğu bölge ise Akdeniz Bölgesidir (Yıldırım, 2015).

Süt, memeli hayvanlarda doğum ile salgılanmaya başlayan, yavruların beslenmesi ve bağışıklık sisteminin gelişmesi yönünden büyük öneme sahip doğal halde bulunan sıvı bir besin kaynağıdır (Adıgüzel, 2019). Türkiye istatistik Kurumu 2019 yılı verilerine göre ülkemizde toplam 22.386.594 ton süt üretilmektedir. Üretilen sütün 20.782.374 tonu (%92,83) sığırlardan, geri kalan miktarı mandalardan, koyunlardan ve keçilerden elde edilmektedir. Süt su, protein, yağ, karbonhidrat, mineraller ve vitaminlerden oluşur. Sütün büyük bir bölümünü (%88) su oluşturmaktadır. Geriye kalanı ise kuru maddedir. Kuru madde ise yağ, laktoz, kazein, serum proteinleri ve tuzlardan meydana gelmektedir. Ülkemiz sığır yetiştiriciliğinde verim artışını sağlamak için başta bakım, besleme, alt yapı gibi pek çok çevre faktörlerinin iyileştirilmesinin yanı sıra, hayvan ithali ve melezleme çalışmaları sonucunda sığır popülasyonunun genetik yapısının da büyük önemi vardır (Hodoğlugil, 1996; Erdem, 1997).

Hayvanlardan elde edilen verim fenotip, çevre ve genotipin ortak etkisi ile ortaya çıkmaktadır. Bundan dolayı hem çevreyi hem de genotipi iyileştirmek verim artışına sebep olmaktadır. Günümüzde genotipi iyileştirme çalışmaları, laboratuvar metot ve tekniklerinin gelişmesi, teknolojik şartların ilerlemesiyle melezleme ve seleksiyon gibi klasik ıslah metotlarından daha ileri boyutlara çekilmiştir. Çiftlik hayvanlarından elde edilen ve ekonomik önemi olan çeşitli verimlere (süt, yapağı, yumurta ve et gibi) ait karakterler fazla sayıdaki genin kontrolü altında ve çeşitli çevre faktörlerince büyük ölçüde etkilendiğinden söz konusu kantitatif karakterlerde fenotipik değer çoğu zaman genotipik değeri yansıtmamaktadır. Bu nedenlerden dolayı ele alınan bu tür karakterlerin genotipik değerinin tahmin edilmesi büyük önem taşır (Özdemir, 2001).Ekonomik karakterlerle polimorfik sistemler arasında yeterli derecede bir ilişkinin varlığını ortaya koyabilirse polimorfik karakterleri belirleyen gen, belirleyici gen (markör gen) olarak kabul edilmek suretiyle seleksiyonda bunlardan faydalanmak mümkün olacaktır. Bu sayede süt verme yeteneğinde olmayan erkek damızlıklar ve ilk laktasyonuna girmiş ineklerde daha erken yaşta seleksiyon yapma imkânını mümkün kılacaktır. 1987; Özbeyaz, 1991).

Süt protein sistemlerinin genetik temellerinin belirlenmesine yönelik çalışmalarda, son yıllarda geliştirilen çeşitli moleküler genetik yöntemlerden yaygın bir şekilde yararlanılmaktadır (Formaggioni et al,. 1999). Bir kısım çalışmalarda süt kalitesi, bileşimi ve teknolojik özellikleri ile doğrudan ilişkileri nedeniyle süt proteinlerinden kazeinlerin genetik polimorfizmleri üzerinde durulmuştur (G. Ceriotti et al., 2004; Doğru ve Özdemir, 2002). Hayvanların verimlerinde varyasyona neden olan polimorfik unsurların önemli kısmına hayvan sütlerinin protein yapılarında görülmüştür (Özdemir, 2001). Sütün % 80'lik bir bölümünü oluşturan kazein proteinlerinde çalışmalar yoğunlaşmış ve özellikle son yıllardaki çalışmalarda *CSN3* gibi süt protein lokusları bakımından tespit edilen biyokimyasal genetik varyasyon ile süt verimi ve süt bileşenleri arasında çeşitli ilişkilerin varlığı belirlenmiştir (Martin et al,. 2002).

Bu çalışmada amaç, özel bir işletmede yetiştirilen Simmental ırkı sığırların PCR-RFLP yöntemi kullanılarak süt proteinlerinden *CSN3* gen lokusu bakımından göstermiş oldukları polimorfizmi araştırmak, ilgili gen yeri bakımından hayvanlara ait genotip ve allel frekansların dağılımını ortaya koymaktır. Ayrıca genotipler ile bazı performans özellikleri arasındaki ilişkiler incelenerek farklılığın önemli olup olmadığını araştırmaktır.

Materyal Metod

Araştırmanın materyalini Erzurum'da özel bir işletmede entansif olarak yetiştirilen 6 farklı laktasyonda 70 Baş Simmental ırkı sığırlardan alınan kan örnekleri oluşturmuş ve κ-kazein (*CSN3*) geni polimorfizminin süt verimi ile ilişkilendirmesi için bu hayvanlara ait çeşitli süt verim kayıtları kullanılmıştır. PCR-RFLP yöntemi kullanılarak Simmental ırkı sığırlarda κ-kazein gen lokusunun genotipik yapısı belirlenmiştir.

Genomik DNA izolasyonu Qıagen DNA izolasyon kiti kullanılarak metodta belirlenmiş yörüngeler uygulanarak (Purgene DNA kiti, Gentra Systems, Minnesota, USA) elde edilmiştir. Elde edilen DNA'ların kalitatif ve kantitatif kontrolleri Nanodrop (Drop Plate, Cat. No: 12391) spektrofotometri cihazı kullanılarak tespit edilmiştir.

PCR aşamasında, F: 5'- ATTTATGGCCATTCCACCAA-3' ve R: 5'- ATTAGCCCATTTCGCCTTCT-3' (Doğru ve ark. 2008) primerleri kullanılarak 351-bp'lik DNA bölgesi çoğaltılmıştır. CSN3-*Hinf1* PCR amflifikasyonu için toplam hacim 25 µl'ye tamamlanacak şekilde her bir primerden ve dNTPmix'ten (D7595: Sigma, St. Louis, MO, USA) 2 µl, 0.5 ünite Taq DNA polimeraz (D1806: Sigma), 100-200 ng kalıp DNA, 3 µl 10x PCR Buffer (Cat. No: P2192), 1 µl 25 mM MgCl2 ve ddH2O kullanılmış PCR amflifikasyon koşulları; 95°C'de 5 dk, 95 °C 1 dk. ve 57°C 1 dk. 30 döngü, son uzama sıcaklığı 72°C'de 5 dk ve 1 döngü olacak şekilde ayarlanmıştır.

Amplifikasyonu gerçekleşen her bir örnekten 9 μl alınıp üzerine 2-5 U ilgili bölge için *Hinf1* RE

enzimi, kullanılarak 37 ℃'de 12 saat süreyle inkübasyon işlemi gerçekleştirilmiştir.

HinfI RE'ler ile DNA'ların kesim işlemleri tamamlandıktan sonra, %2,8'lik agaroz jelde örnekler yürütülerek 35 Watt 'da 180 dak süreyle elektroforez işlemi uygulanmıştır. Elektroforez işlemi yapılan jel alınarak UV ışığı altında görüntülenerek incelenmiştir.

Allel gen frekansları her bir örnek için ayrı ayrı sayılarak hesaplanmıştır. Genotip frekanslarının Hardy-Weinberg dengesinde olup olmadığı Ki-kare ($\chi 2$) testiyle incelenmiştir. Popülasyona ait doğum ağırlığı verileri ile genotiplerin ilişkilendirilmesi, SPSS 20.0 software programı kullanılarak varyans analizi ile değerlendirilmiştir. Gerçek süt verimi, 305-gün süt verimi, laktasyon süresi ve günlük süt verimi gibi süt verim özellikleri incelenmiştir. Bu verim özelliklerinde laktasyon sayısı, genotip gibi faktörler üzerinde durulmuştur. Araştırmadaki verim özelliklerine göre aşağıdaki istatistik model kullanılmıştır.

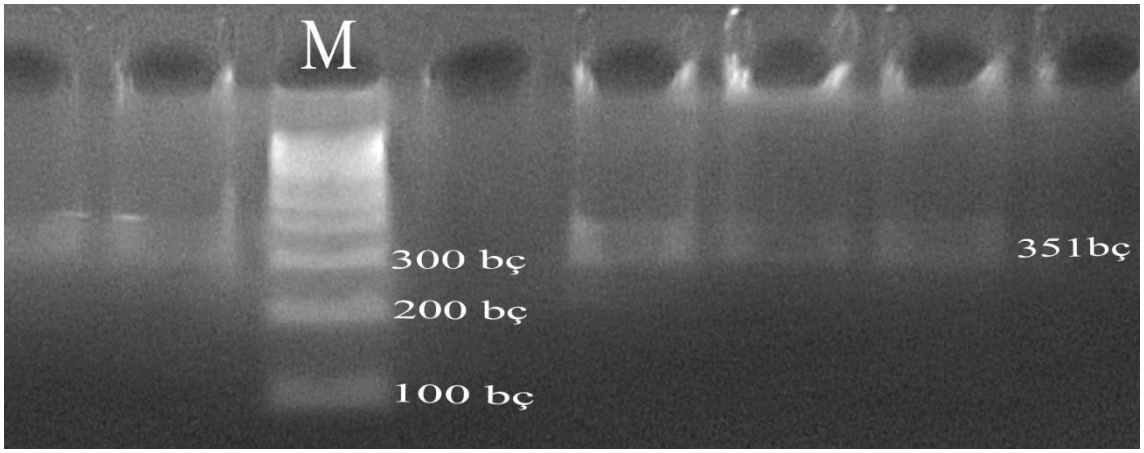
Y_{ijkl} :$\mu+a_i+b_j+c_{kk}+e_{ijkl}$

Y_{ijkl}: Herhangi bir Simmental ineğin ele alınan performans (gerçek süt verimi, 305 günlük süt verimi, laktasyon süresi ve günlük süt verimi) özelliklerinin her hangi biri bakımından değeri; μ: popülasyon ortalaması; a_i : i. Genotip etkisi (AA, AB ve BB); b_j: j. Laktasyon sırasının etkisi (2.-7); c_k: k. Buzağılama mevsiminin etkisi (1: Kış ve ilkbahar, 2: Yaz ve Sonbahar); e_{ijkl}: Hata payı.

Araştırma Bulguları ve Tartışma

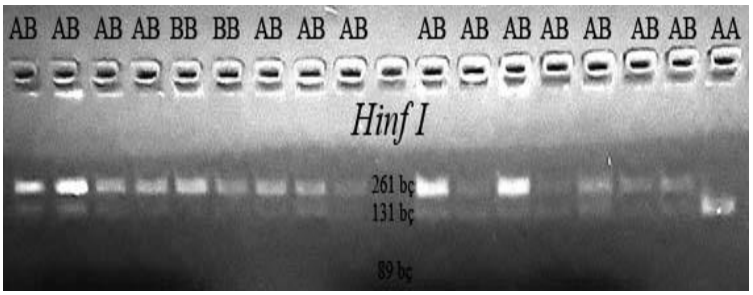
PCR sonuçlarının gözlenmesi

Simmental sığırların kanlarından elde edilen DNA örneklerinin her birine PCR yapılarak %1,2'lik agaroz jelde yürütülmüş ve DNA bantları elde edilmiştir (Şekil 1).



Şekil 1. PCR ürünlerinin agaroz jel görünümü (M: markör, *CSN3*:351 bç)

CSN3-HinfI geni polimorfik bölgeleri genotipleri BB;89-261, AA;89-131-131 ve AB;89-262-31-131-89 bç uzunluğa göre belirlenmiştir.



Şekil 2. *CSN3* genine ait PCR-RFLP kesim bölgeleri: BB;261/89, AA;131/131/89 AB;261/131/131/89 bp

Genotip frekansları, Hardy-Weinberg Genetik Denge Testi ve X^2 bağımsızlık testi yapılmış ve elde edilen sonuçlar Tablo 1'de sunulmuştur.

Tablo 1. *CSN3* genotip frekansları ve Hard-Weinberg genetik denge testi sonuçları

N	Gözlenen			Beklenen			X^2 testi
	AA	**AB**	**BB**	**AA**	**AB**	**BB**	
70	40	23	7	37,89	27,22	4,89	0,1945 ÖS

ÖS: Önemsiz

70 baş Simmental ineğe ait genotip frekansları dağılımının Hardy-Weinberg genetik denge testine göre dengede (P>0.05) olduğu saptanmıştır.

Aynı ırkla daha önce yapılan çalışmalarda Burchberger et al. (1983), Seibert et al. (1987), Akyüz et al. (2013), Akyüz ve Çınar (2014) gibi araştırmacıların bildirdiği Hardy-Weinberg genetik denge testi sonuçları bulduğumuz sonuçlarla uyumludur.

Simmental sığırlarına ait gerçek süt veriminin genel ortalaması 5576±242,0 kg olarak belirlenmiştir. Elde edilen değerlendirmeler sonucunda gerçek süt verimi açısından *CSN3* genotiplerden en yüksek ortalama AB genotipinde (5805±370,3 kg) ve en düşük ortalama AA genotipinde (5151±308,6 kg) belirlenmiştir Bu özellik bakımından varyans analiz tablosu incelendiğinde üzerinde durulan faktörlerden genotip, laktasyon sırası ve buzağılama mevsiminin gerçek süt verimine etkisi önemsiz olduğu gözlenmiştir. *CSN3* genotipi gerçek süt verimini ilişkilendirilmesi ile ilgili Gürcan (2001) ve Demirel (2019) tarafından Siyah Alacalarla yapılan çalışmalarda ve Esmer ve Siyah Alacalarla yapılan diğer çalışmalarda (Doğru ve Dayıoğlu 1996; Özdemir and Doğru, 2005) bu çalışmanın paralelinde gerçek süt verimine *CSN3* genin etkisi önemsiz bulmuştur. Çalışmada bu özellik bakımından en düşük ortalama değer *CSN3* AA genotipinde bulunmuş olup bu sonuç Özdemir ve Doğru (2005)'nun Siyah Alacalar üzerinde yaptığı çalışma ile benzerlik göstermiştir.

Simmental ırkı ineklerden elde edilen 305-günlük süt verimine etkili olduğu düşünülen *CSN3* genotip, laktasyon sırası ve buzağılama mevsimi faktörlerine göre analiz yapılmış sonuç olarak 305-günlük süt verimi genel ortalaması 5852±183,5 kg olarak belirlenmiştir. 305-günlük süt verimi ortalamaları arasında oluşan farklılık önemli bulunmuştur (P<0.05). Diğer taraftan laktasyon sırasının ve buzağılama mevsiminin etkisi önemsiz bulunmuştur.

Yapılan süt protein polimorfizminin genetiği ve süt verimi özellikleriyle ilişkilendirme çalışmalarında 305-günlük süt verimine genotipin etkisini Holstein ırkı ineklerde (Soyudal, 2017) önemli bulurken, Siyah Alaca ve Simmentallerde (Doğru ve Dayıoğlu, 1996; Kaygısız ve Doğan, 1999; Demirel, 2019) çalışmalarımızın aksine önemsiz bulunmuşlardır. Hayvan ıslahında 305-günlük süt verimi esas alındığında *CSN3* genotiplerinden BB genotipi diğer iki genotipe göre daha yüksek ortalama süt verdiği için BB genotipine sahip ineklerin sürüde çoğaltılması gerektiği sonucuna varılmıştır. İncelenen ırkta laktasyon süresi genel ortalaması 301±10,8 gün olarak bulunmuştur. Söz konusu genotipler, laktasyon sırası ve buzağılama mevsimi arasında oluşan ortalamalar arasındaki farklar istatistiki olarak anlamlı bulunmamıştır (p>0.05). Daha önce yapılmış çalışmalarda laktasyon süresinin *CSN3* genotipine etkisini bu çalışmada olduğu gibi Doğru (1996) Esmer, Siyah alaca ve Simmental ırklarında ve Gürcan (2001) Siyah Alaca ırkında önemsiz, çalışmanın aksine Kaygısız (1997) Siyah Alaca ırkında önemli bulmuşlardır(P<0,05).

Tablo 2. Gerçek süt verimi, 305 günlük süt verimi, laktasyon süresi ve günlük süt verimi özelliği bakımından en küçük kareler ortalamaları ve standart hatalar(kg)

GENEL		N	GERÇEK SÜT VERİMİ		305-GÜNLÜK SÜT VERİMİ		LAKTASYON SÜRESİ		GÜNLÜK SÜT VERİMİ	
			X̄ ± Sx̄	Ö.S	X̄ ± Sx̄	Ö.S.	X̄ ± Sx̄	Ö.S.	X̄ ± Sx̄	Ö.S.
		90	5576±242,0		5852±183,5		301±10,8		18,7±0,58	
CSN3 Genotipi	**AA**	49	5151±308,6	ÖS	5313±233,9[b]	*	294±13,7	ÖS	17,9±0,75	ÖS
	AB	29	5805±370,3		5784±280,7[ab]		316±16,5		18,6±0,89	
	BB	12	5772±547,3		6458±414,8[a]		294±24,4		19,6±1,32	
Laktasyon Sırası	**2.**	9	5063±650,0	ÖS	5406±492,7	ÖS	292±29,0	ÖS	17,5±1,57	ÖS
	3.	17	5739±481,6		6046±365,1		288±21,5		19,8±1,16	
	4.	19	5820±468,6		5817±355,2		328±20,		18,2±1,13	
	5.	21	5222±433,5		6114±328,6		269±19,3		19,1±1,05	
	6.	16	5187±489,1		5330±370,7		309±21,8		17,0±1,18	
	7.	8	6426±681,6		6399 ±516,7		321±30,4		20,4±1,65	
Buzağılama Mevsimi	**Kış-İlkbahar**	53	5748±334,8	ÖS	5965±253,8	ÖS	299±14,9	ÖS	19,3±0,81	ÖS
	Yaz-Sonbahar	37	5405±360,4		5739±273,2		304±16,1		18,0±0,87	

ÖS: önemsiz, *: P<0.05; a, b ve c aynı harfle gösterilenler ortalamaları arasındaki fark önemsiz, farklı harfle gösterilenler ortalamaları arasındaki fark önemlidir.

Üzerinde çalışılan sürüde Simmental ineklerinde günlük süt verimlerine ait genel ortalama değer 18,7±0,58 kg olup bu değer *CSN3* genotipi, AA, AB ve BB genotiplerinde sırasıyla 17,9±0,75, 18,6±0,89 ve 19,6±1,32 kg olarak bulunmuştur.istatistiksel analizler sonucunda genotipler arasında genotipler, laktasyon sıraları ve buzağılama mevsimleri arasındaki oluşan günlük ortalama süt verimi farkları önemsiz bulunmuştur (P>0.05). Kaygısız (1997), Gürcan (2001) ve Demirel (2019) tarafından Siyah Alaca ırkı sığırlarla, Doğru ve Dayıoğlu (1996) tarafından Esmer ve Simmental ırkı sığırlarla yaptıkları çalışmada *CSN3* genotipi ile günlük süt verimini ilişkilendirmişlerdir. *CSN3* genotipleri ile süt verimi ilişkisi açısından ortaya koydukları sonuçlar değerlendirildiğinde yaptıkları çalışmalarda bu çalışma sonucuyla uyum içerisinde olduğu görülmüştür.

Sonuç

Sonuç olarak Simmental ırklarından alınan kan örneklerinden PCR-RFLP yöntemi kullanılarak her bir bireye ait *CSN3* genotipleri saptanmıştır. *CSN3* gen polimorfizmi açısından belirlenen genotip ve allel frekansları ırkın genotip çeşitliliğini ortaya çıkarmada yeterli sayılabildiği ve yapılan ilişkilendirme sonucu *CSN3* geni *CSN3* genotipleriyle üzerinde durulan süt verimi performans özelliklerinden sadece 305-günlük süt verimiyle ilişkinin önemli düzeyde anlamlı olduğu sonucuna varılmıştır. Bu tür çalışmalar yapılarak belirlenen polimorfizmin hayvan ıslahında kullanılabilirliğinin ortaya konması ve sonuçların sığır yetiştiriciliğinde kullanılmasına yönelik daha büyük popülasyonlarda çalışılarak ülke hayvancılığının tanımlanmasında ve geliştirilmesinde yeni olanaklar sağlayabileceği düşünülmektedir.

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➢ *ORAL PRESENTATION*

Atkestanesinin (*Aesculus hippocastanum* L.) Antioksidan Özellikleri:Derleme

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Özet

Reaktif oksijenler elektron almak üzere lipidler, proteinler, karbonhidratlar ve DNA ile reaksiyona girmektedirler. Bu oksidantlar fazla miktarda olduğunda membrandaki lipidlerin peroksidasyonuna yol açarak permeabilitenin bozulmasına, dolayısıyla hürce içi iyon dengesizliğine neden olmaktadırlar. Reaktif oksijen türleri, hücre zarında bulunan doymamış yağ asitlerinin peroksidasyonuna neden olarak asıl toksik etkilerini gösterirler.Tüm dünyada olduğu gibi ülkemizde de birçok bitki yıllardan beri halk arasında çeşitli şekillerde tüketilmekte ve çeşitli hastalıkları tedavi etmekamacıyla da kullanılmaktadır.Bitkilerin doğal antioksidan kaynaklar olarak kullanımlarını araştıran çalışmaların sayısı da gün geçtikçe artmaktadır. İran, Kuzey Hindistan, Güneydoğu Avrupa, Balkanlar ve Kafkasları kapsayan birçok bölgede yetişen atkestanesi (*Aesculushippocastanum* L.) tohumları uzun süredir, özellikle varis ve hemoroit gibi venöz hastalıkların yanı sıra artrit, tendonit ve spor yaralanmaları gibi enflamatuar rahatsızlıkları tedavi etmek için kullanılmaktadır. Uzun yıllardır atkestanesiekstresinin başlıca bileşenlerinden olan vetriterpensaponinlerinbir karışımı olan aescininantiexudatif, antiödem ve vazoprotektif etkilere sahipolduğu bilinmektedir. Araştırmalar escinin, enflamasyonmediatörleriniendojen antioksidan kapasitesini artırarak düzenlediğini,flavonoidal yapısından dolayı lipit peroksidasyonunu azaltıphepatoprotektif ve antioksidan savunma sistemini artırarak antioksidan aktivite gösterdiğini ortaya koymuştur. Literatürde fenolikler açısından zengin olan atkestanesi ekstraktlarının önemli bir antioksidan potansiyeline sahip olduğu belirtilmektedir. Farklı kanser türlerine yapılan çalışmalarda aescininkanseri hücrelerinin büyümesini durduğunun ve daha fazla proliferasyonunu engellediğinin gözlemlendiği çalışmalar da bulunmaktadır. Atkestanesinin özellikle antioksidan özelliklerinin daha fazla belirlenmesi için yapılacak araştırmalar bu bitkiden sağlanacak olan biyoyararlanımı artıracaktır.

Anahtar kelimeler: Aesculum hippocastanum L.,escin, antioksidan.

Antioxidant Properties of Horsechestnut (*Aesculus hippocastanum* L.) A Review

Abstract

Reactive oxygen reacts with lipids, proteins, carbohydratesand DNA to donation electrons. These oxidants may cause peroxidation by ausing peroxidation of the lipids in the membrane when there are excess amounts therefore they lead to intracellular ion imbalance. Reactive oxygen species show the actual toxic effects in the cell membrane causing peroxidation of fatty acids. In our country as well as all over the world, many plants have been consumed in various ways among the people for many years, and they are also used to treat various diseases. The number of studies investigating the use of plants as natural antioxidant sources is increasing. Seeds of horsechestnut Aesculum hippocastanum L. growing in Iran, North India, Southeastern Europe, the Balkans and the Caucasus, have long been used to treat inflammatory disorders such as arthritis, tendonit is and sports injuries, as well as venous diseases such as varices and hemorrhoids. It has been known for many years that the escin, which is a major component of horsechestnut seeds extractand is a mixture of triterpenesaponins, has antiexudative, antiedema and vasoprotective effects. Investigations have shown that aescine regulates inflammation mediators by increasing the endogenous antioxidant capacity andantioxidant

activity by decreasing lipid peroxidation and increasing the hepatoprotective andantioxidant defense system due to flavonoidal structure.

In the literature, it is stated that horsechestnut extracts, which are rich in phenolics, have an important antioxidant potential. Studies on different types of cancers have also shown that the aescin has stopped the growth of cancer cells and prevented further proliferation. In addition to the widely known properties of horsechestnut further studies to determine the antioxidant properties will increase the bioavailability of this plant.

Key words: Aesculum hippocastanum L.,escin, antioxidant.

GİRİŞ

Biyolojik sistemlerde oksijenden oluşan ve reaktif oksijen türleri adı verilen (Reactive Oxygen Species, ROS) en önemli serbest radikaller, tüm canlı organizmalarda normal metabolizmanın bir yan ürünü olarak ve çevresel ajanlara maruz kalma sonucu oluşur (Atmaca ve Aksoy, 2009). DNA'ya yapılan ROS saldırısı, modifiye edilmiş bazlar dahil, çok sayıda DNA hasarı ürünü üretir (Burçak ve Andican, 2004). Vücutta doğal metabolik yollarla oluşan serbest radikaller normalde radikal parçalayan antioksidan sistemlerle ortadan kaldırılmaktadır. Ancak, çeşitli nedenlerle reaktif oksijen türlerinin artması ve antioksidan mekanizmaların yetersiz kalması sonucu "oksidatif stres" adı verilen bir dizi patolojik olay meydana gelmektedir. Antioksidanlar, oluşan serbest radikalleri toplayıp, kararlı hale getirerek, zincir kırıcı etki ile serbest radikal üreten kimyasal reaksiyonları durdurarak, baskılayıcı etki ile reaksiyon hızını azaltarak, onarıcı etki ile biyolojik molekülerdeki hasarı onararak, organizmadaki antioksidan enzimler ile enzimatik olmayan antioksidanların sentezini arttırarak etki gösterirler (Dündar ve Aslan 2000). A. Hippocastanum ekstresi veya aescin, büyük ölçüde in vitro ve in vivo testlerle iyi bir şekilde kanıtlanmış olan anti-enflamatuar özelliklerinden dolayı kronik venöz yetmezlik, hemoroid ve postoperatif ödemde klinik olarak oldukça önemli etkiler göstermiştir (Zhang ve Lian, 2010). Bu derlemede atkestanesinin yaygın bilinen ve tedavi amacıyla kullanılan etkilerinden başka antioksidan ve antikarsinojenik etkilerinin araştırıldığı çalışmaların sonuçları değerlendirilmiştir.

Oksidatif Stres ve Antioksidanlar

Vücut hücrelerinin membranına, hücre yapısındaki lipidlere, proteinlere, nükleik asitlere ve DNA'ya zarar vermekte ve bunun sonucunda koroner hastalıklar, diyabet, kanser, karaciğer tahribatı, katarakt gibi çok çeşitli hastalıklara yol açan (Kasnak ve Palamutoğlu, 2015) serbest radikaller, besinlerin oksijen kullanılarak enerjiye dönüşümü sırasında meydana gelen reaktif moleküllerdir. Oksijen molekülleri yaşam için vazgeçilmez olmakla birlikte, metabolizma sırasında serbest radikal kaynağı olarak bilinen ve son derece reaktif olan ara ürünler oluşur. Reaktif oksijen türleri/metaloitleri olarak bilinen bu moleküller lipit, protein ve DNA gibi hücre bileşenlerine zarar verir. Aerobik (oksijen soluyan) organizmalarda serbest radikal oluşumunu kontrol altında tutmak ve bu moleküllerin zararlı etkilerine engel olmak üzere antioksidan savunma sistemleri gelişmiştir. Ancak bazı durumlarda mevcut antioksidan savunma sistemi serbest radikallerin etkisini tamamen önleyemez ve oksidatif stres olarak adlandırılan durum ortaya çıkar (Stres, 2014).

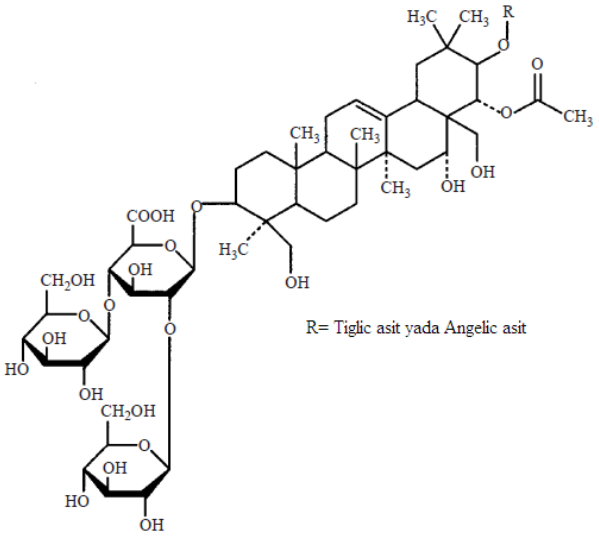
Radikal ürünleri ve reaksiyonlarını inhibe eden bir sisteme sahip olan hücre ve dokularda bu radikallerin otooksidasyon/peroksidasyonun ilerlemesini önleyen maddeler "antioksidan" olarak tanımlanır (Dündar ve Aslan, 1999). Temizleme (scavenging), baskılama (quencher), onarma ve zincir koparma şeklinde olmak üzere dört farklı mekanizma ile oksidanları etkisizleştiren (Meral ve ark., 2012) antioksidanlar (AO) yapılarına, kaynaklarına, çözünürlüklerine ve bulundukları yerlere göre sınıflandırılmaktadırlar. Doğada AO'ların kaynakları olarak; yağlı tohumlar, yapraklar, kökler, baharatlar, tahıl ürünleri, sebzeler, meyveler, çay, su yosunu, hayvansal ürünler ve enzimler gösterilebilmektedirler (Okcu ve Keleş, 2009).

Fitoterapi, (phytos=bitki, therapy=tedavi) bitkisel tedavi anlamında kullanılır (Durusoy ve Ulusal, 2007). Bitkiler, sağlığı korumak ya da geri kazanmak için tarihin her döneminde, her toplum tarafından kullanılmış olan fitoterapinin temelleri çok eski zamanlara kadar dayanmaktadır. Fitoterapi, günümüzde alternatif tıp konuları arasında değerlendirilmekte olup tarih süreci içerisinde birikimi, gelişimi veuygulanışı ile birçok tıp bilimine öncülük etmiştir (Özbek, 2005). Dünya Sağlık Örgütü'nün insanların %80'inin doğal tedaviye inandığını açıklaması bu popüleritenin iyi bir göstergesidir. Bu durum ülkemizde de benzerdir. Ancak yaygın kullanıma rağmen, pekçok bitkisel ilacın etkisi henüz kanıtlanmamış veya bu konuda bilimsel veri eksikliği mevcuttur (Aşçı ve ark., 2007).

Aesculus hippocastanum (Hippocastanaceae) 'ın çevresel koşullara karşı mükemmel dayanıklılığı nedeniyle tüm dünyada park ve bahçelerde ve şehir sokaklarında yaygın olarak kullanılan bir bitkidir.18. yüzyılın başlarından kalma çok sayıda eserdeatkestanesi için tedavi edici özelliklerinden bahsedilmektedir (Sirtori, 2001). Antienflamatuar, antiödematöz ve kapillar koruyucu özelliğinden dolayı atkestanesi tohumları periferal vasküler bozukluklar, varisli damarlar, hemoroid, flebit (damarların iltihabı), ishal, ateş, prostat bezinin büyümesi, romatizma, nevralji gibi durumlar için iyi bir tedavi yöntemi olmasının yanı sıra sellülitisten korunmadave kozmetik sahada yaygın olarak kullanılmaktadır (Yoshikawa ve ark., 1994, Wilkinson ve Brown; 1999). Aesculus L.; kuzey yarımkürede, özellikle doğu Asya ve doğu Kuzey Amerika'da yetişen, bir tür Avrupa'ya ve iki türü batı Kuzey Amerika'ya özgü olmak üzere 12 türü olan yapraklarını döken ağaç ve çalılık içeren Hippocastanaceae familyasının bir cinsidir (Zhang ve ark., 2010). A. hippocastanum'un kabuğu ve yaprakları, böbrek taşlarını geçmek ve mide ağrıları hafifletmek amacıyla ishal ve hemoroitleri, venöz yetmezliği ve postoperatif ödemleri tedavi etmek kullanılırken, tohumları hemoroid semptomlarını hafifletmek için kullanılır (Štajner ve ark.,2014, Patolla ve Rao; 2015).

Atkestanesinde belirlenen en önemli iki bileşen; kumarin türevi olan aesculin ve bir saponin olan aescindir (Şekil 1) (Weiss ve Meuss., 2001, Sirtori, 2001).
Aescin bitkide sodyum ya da potasyum tuzu halinde bulunur. Karışımdaki bileşenler;
- β-escine; C-21 ve C-22 diesterlerin bir karışımıdır.
- kryptoescine: C-21 ve C-28 diesterlerinin bir karışımıdır.
- α- escine : β-escine ve kryptoaescine'nin4:6 oranında bir karışımıdır.
- escinoller: aescinin hidrolizinden elde serbest C-21, C-22 ve C-28 hidroksil gruplarını içeren yapay bir bileşendir (Hostettmann ve Marston, 2005).

A.Hippocastanum'da quersetin ve kaempferolün glikozit türevleri başta olmak üzere astragalin (kaempferol 3-Oglucoside), isoquercitrin (quercetin-3 -glucoside), leucocyanidin (3,3',4,4',5,7 hexahydroxyflavone) ve rutin (quercetin 3-rutinoside) çok sayıda flavonoid tespit edilmiştir. Tohumları ayrıca ağırlıkça % 40-50 nişasta, şekerler, proteinler (özellikle globülin, hipokastan, L- (1) lisin ve L- (1) - riptofan), yağ (oleik, linoleik, linolenik, stearik ve palmitik asitler içeren) ve pürinler (adenozin, adenin ve guanin) içerir (Wilkinson ve Brown; 1999).



Şekil 1. Escinin kimyasal yapısı

Flavonoidler önemli düzeyde antioksidan ve şelatlama özelliklerine sahip difenil propanoidler olup genellikle bitkilerde bulunmakta ve insan vücudunda sentezlenememektedirler. Flavonoidler serbest radikal savar olmaları, enzim aktivitelerini düzenleyici, hücre çoğalmasını inhibe edici, antibiyotik ve antiallerjen özellik taşımaları, ishal, ülser ve iltihabı önleyici ilaç gibi görev almalarından dolayı önem taşımaktadır. Son yıllarda yapılan çalışmalar flavonoidlerin oksidatif DNA zedelenmesini serbest radikal tutulması dışındaki mekanizmalarla da önlediğini göstermektedir. Bu mekanizma vücut tarafından üretilen glutatyon-S-transferaz gibi antioksidanların korunup ve güçlendirilmesi yolu ile de gerçekleştirilebilir. Flavonoidlerin çoğu glutatyon-S-transferazı (GST) aktive etme yeteneğine sahiptir ve istatistiksel olarak anlamlı derecede GST aktivitesini artırarak etkili olur. GST'nin mutajenik potansiyeli bulunan ksenobiyotikleri detoksifiye ederek etkili olduğu

düşünülmektedir. (Çapanoğlu ve Boyacıoğlu, 2009). Bu flavonlara ek olarak, epikateşin ve dimer proantosiyanidin A2 A. hippocastanum'dabulunan bileşikler olarak bildirilmiştir (Wilkinson ve Brown, 1999).

Vücutta üretilen serbest radikallerin ve reaktif oksijen türlerinin kardiyovasküler hastalıklar, kanserler, yaşlanma ve iltihaplanma gibi çeşitli hastalıklar için kritik risk faktörleri olduğu iyi bilinmektedir. Son zamanlarda, serbest radikallerin oluşturdukları hasarların besinler yoluyla önlenmesi amacıyla yapılan çalışmalarda fenolik antioksidanları içeren yapılara olan ilgi giderek artmaktadır. Bunlardan proantosiyanidinler adı verilen polifenolik bileşikler, üzüm, elma, kızılcık, kahverengi soya fasulyesi gibi bitkilerde bulunurlar ve antioksidan aktivitenin yanı sıra diğer biyolojik aktiviteleri sergilerler (Ogawa ve ark., 2008). Son zamanlarda, bir A. Hippocastanum ekstraktının, hem biyokimyasal hem de biyolojik analizler kullanılarak aktif oksijen türlerinin in vitro üretimi ve zarar verici etkilerini azaltmada oldukça etkili olduğu gösterilmiştir (Wilkinson ve Brown, 1999).

Aesculus Hippocastanum L.'nin Antioksidan ve Antikarsinojenik Özellikleri

Atkestanesi ağacı (Hippocastanum) tohumlarından elde edilen önemli bir fito yapıcı olan β-aescin, flavonoidal yapısından dolayı antioksidan savunma sistemini artırarak antioksidan aktivite göstermiştir (Singh ve ark., 2016). Otajagić ve ark., (2012) tarafından yapılan çalışmada A. hippocastanum kabuğu ekstraktlarının güçlü antioksidanlar olarak kabul edilen fenolik bileşikler açısından önemli bir antioksidan potansiyele sahip olduğu gösterilmiştir. Aescin'in enflamasyon mediatörlerini endojen antioksidan kapasiteni artırarak düzenlediği ve ayrıca lipit peroksidasyonunu azaltarak hepatoprotektif aktivite gösterdiği de bilinmektedir (Sagdicoglu Celep ve ark., 2012). Zhang ve ark. (2010) tarafından yapılan aescinin5 hafta boyunca günlük 100 mg / kg dozlarda standart pelet diyeti (SPD) veya HFD ile beslenen erkek farelerde yüksek yağ diyeti (HFD) kaynaklı lipid peroksidasyonunu önlediği ve farelerde karaciğer mimarisi üzerinde koruyucu bir etki gösterdiği bildirilmektedir. Benzer şekilde, A. hippocastanum'un ekstraktı, in vitro olarak hidroksil radikallerinin ve singlet oksijenin salınımını azaltarak, geniş bir aktif oksijen temizleme özellikleri spektrumu sergilemiştir. Bu oksijen türleri, hücresel hasar ve iltihaplanma ile ilişkili olduğundan, atkestanesi fitokimyasallarını içeren kozmetik preparatlar yaşlanma karşıtı bir etki olarak ortaya çıkabilen ve ciltteki hasarı hafifletme potansiyeline sahiptir (Sagdicoglu Celep ve ark., 2012). Yalinkilic ve Enginar (2008) tarafından yapılan ve X ışınlarına maruz kalan sıçanlarda Aesculus hippocastanum L. (tohum) [AHE], Medicagosativa L. [MSE] ve Spinaciaoleracea L. [SOE] ekstrelerinin antioksidatif etkilerinin incelendiği çalışmada bu ekstrelerin serbest radikal kaynaklı lipid peroksidasyon insidansını azalttığı ve antioksidan durumunu arttırdığını ve ayrıca, AHE'nin antioksidan etkisinin, MSE ve SOE uygulanan hayvanlardan daha güçlü olduğu bildirmişlerdir.

Küçükkurt ve ark. (2010) tarafından yapılan bir çalışmada Aesculus hippocastanum tohumundan elde edilen escin karışımının standart pelet diyetinde (SPD) ve yüksek yağlı diyet (HFD) tüketen erkek farelerde kan ve doku antioksidan savunma sistemleri üzerindeki in vivo etkilerini değerlendirilmiş ve escin karışımının, oksidatif stresin yan etkilerini önlediği, hem SPD hem de HFD tüketen erkek farelerde karaciğer dokusu üzerindekoruyucu bir etki gösterdiği ve yüksek yağlı diyetin artırdığı oksidatif stresi dengelemek için antioksidan savunma sistemini aktive ettiği belirtilmektedir. Tohumların antioksidan aktivitesi flavonoidlerin varlığına bağlanmaktadır. Escin'in enflamasyon mediatörlerini endojen antioksidan kapasiteni artırarak düzenlediği ve ayrıca lipit peroksidasyonunu azaltarak hepatoprotektif aktivite gösterdiği de bilinmektedir. (Sagdicoglu Celep ve ark., 2012).

Jiang et al. (2011) tarafından lipopolisakkarit (LPS) enjekte edilerek süperoksit, NO radikali ve sitokinler gibi gibi kimyasal aracıları da kapsayan ve karaciğer yetmezliği ile karakterize edilen karaciğer hasarı oluşturulan farelerde escinin endotoksinin neden olduğu akut karaciğer hasarı üzerindeki etkisini ve altında yatan mekanizmayı araştırmak için yapılan bir çalışmada escinin TNF-α ve diğer enflamatuar ajanların neden olduğu NF- κB aktivasyonunu baskılayarak LPS'nin neden olduğu karaciğer hasarını hafifletebileceği belirtilmiştir. Akut enflamatuar hastalıkları tedavisinde sıklıkla kullanılan glukokortikoidler antiinflamatuar ajanlar olmalarına rağmen glukokortikoid reseptör (GR) gen transkripsiyonunu azaltarak GR sayısının azalmalarına yol açarlarken escinin GR ekspresyon seviyelerini artmasıyla endojen antioksidan kapasiteyi artırarak doku hasarını daha etkili bir şekilde hafifletebilecek glukokortikoid benzeri etkilere sahip olduğu belirtilmektedir.

Streptozotocin ile diyabet oluşturulmuş ve sonrasında dört hafta süreyle 5, 10 ve 20 mg/kg escin uygulanan ratlarda oksidatif stres parametrelerinin de incelendiği bir çalışmada escin; TNF-α ve NF- yolaklarını inhibe ederek antiinflamatuvar ve analjezik etkiye sahip olduğu, motor ve duyusal sinir iletim hızlarını önemli ölçüde geliştirdiği, oksidatif stresin inhibisyonu yoluyla nöroprotektif etkiye sahip olduğu ve antioksidan enzim seviyelerini normalleştirerek siyatik sinirlerdeki oksidatif stresi azalttığı gözlenmiştir (Suryavanshi ve ark., 2020).

Kanser insan sağlığını tehdit eden hastalıkların başında gelmektedir. Yeni kanser ilaç tedavileri arayışında doğal ürünler önemli bir rol oynamaktadır. Genel olarak farmakolojide özellikle de kanser araştırmalarına konu olan tıbbi bitkiler; klinikte kullanılan anti-tümör ajanlarının önemli bir kısmının kökenini oluşturmaktadır (Efferth ve ark., 2007). Zhou et al.(2009) tarafından yapılan çalışmada triterpen saponinlerin bir karışımı olan escin, insan servikal karsinom Hela kanser hücre hattında in vitro hücre büyümesini inhibe edici etkileri ve apoptoz indüksiyonu araştırılmıştır. Metiltiazol difenil tetrazolyum (MTT) hücre canlılığı sonuçları escin'in hücre büyümesinin önemli ölçüde konsantrasyona ve zamana bağlı olarak inhibisyonunu indükleyebildiğini ve escin ile tedavi edilen hücrelerde hücre döngüsünün G1 / S fazında durdurulduğunu gösterdi. Hücrenin inhibisyonu, escin tarafından büyümenin doğrudan inhibe edici etkilerinin G1 / S'de apoptoz ve hücre döngüsü tutulumunun indüksiyonu yoluyla gerçekleştiğini ortaya koydu ve bu etkilerin, konsantrasyona ve zamana bağlı olduğu bildirilmiştir.

β -escinin etki şekli; hücre tipine, uygulama konsantrasyonuna ve tedavi zamanına göre büyük ölçüde değişmekle birlikte β-escin'in bir insan oral mukozal cellline (KB hücreleri) ve fare karaciğer kanseri (H22) ve sarkom (S180) cell line dahil olmak üzere çeşitli cell line türlerinde tümör büyümesini inhibe edebileceğini bildirilmiştir (Patlolla ve ark., 2015). Bu çalışmada β-escin'in, insan kolon kanseri hücre dizileri HT-29 ve HCT-116'da p21 indüksiyonu yoluyla antikanser aktivitesi gösterdiği belirtilmektedir. Bu çalışmaya göre β-Escin; insan kolon kanseri hücrelerinin G1-S fazında büyümesinin durmasına ve daha fazla ilerleme ve proliferasyonun inhibisyonuna yol açacak şekilde fosfo-Rb ve siklin A seviyelerini azaltmaktadır. Beta-escin, akut miyeloid lösemi (HL-60) hücrelerinde doğal bir hücre proliferasyonu inhibitörü ve apoptoz indükleyicisi olduğu ve in vitro ve in vivo olarak hepatoselüler karsinomda antitümör aktivitesi gösterdi bildirilmiştir (Sagdicoglu Celep ve ark., 2012).

Yapılan çalışmalarda A. hippocastanum'dan izole edilen β-escinin, sıçanlarda kimyasal olarak indüklenen kolon karsinogenezini inhibe etti ve in vitro, 30μmol L'de veya daha yüksek konsantrasyonlarda kolon kanseri hücre hatlarında sitotoksisite gösterdiği, 5 μmol / L'deki β-Escin de HT-29 kolon kanseri hücre çoğalmasını inhibe ettiği belirtilmektedir.A. hippocastanum ekstresinin asit hidrolizatlarından 21-O-tigloil-22-O-angeloil-R1-barrigenol ve 21-O-angeloil barringtogenol-C'nin insan nazofarengeal karsinomunda 9 KB hücre kültürü testinde in vitro sitototoksisite gösterdiği saptanmıştır (Zhang ve ark. 2010). Wang ve ark. (2015) tarafından yapılan ve doğal bir triterpen yapısındaki escinin retinal pigment epitel (RPE) hücrelerinde hidrojen perokside karşı (H_2O_2) sitoprotektif potansiyelinin araştırıldığı çalışmada H_2O_2 ile indüklenen ölüm ve apoptozun escin tarafından önemli ölçüde azaltıldığı ve AKT-Nrf2 (nükleer faktör eritroid 2-ilişkili faktör 2) sinyallemesini aktive ederek RPE hücrelerini oksidatif stresten koruduğu belirtilmektedir.

SONUÇ

Aescin'in yaygın olarak bilinen etkilerine ek olarak, farklı çevresel faktörlerin neden olabileceği oksidatif strese karşı antioksidan savunma sistemi üzerindeki etkinliğinin araştırılması, at kestanesi bitkisinin daha etkin kullanılmasını destekleyecektir. Ayrıca yüksek dozda aescinin toksik olmadığını gösteren çalışmaların sonuçları, bu bitkinin kullanım sınırlarını genişletmektedir. Aescin'in gen transkripsiyonunu etkileme ve kötü huylu hücrelerde apoptozu indükleme yeteneği, insan klinik araştırmalarında kullanılabilecek ileri ilaç geliştirme programlarında daha fazla araştırma konusu olmasını gerekli hale getirmektedir. Atkestanesi bitkisinin etken maddesi olan escinin moleküler düzeydeki etkilerinin bilinmesi bu bitkinin daha fazla tanınmasını ve kullanılmasını sağlayacaktır.

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➢ *ORAL PRESENTATION*

Role of voltage-gated sodium channels (Na_v) in mammalian sperm functions

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Abstract

Capacitation is a process that sperm gain fertilizing ability in the female genital tract. Ion channels play an important role during capacitation by controlling plasma membrane potential, intracellular calcium (Ca^{2+}) concentration and intracellular pH. Thus, they trigger physiological events such as hyperactivated motility and acrosome reaction which are essential for successful fertilization. Capacitation is also accompanied by hyperpolarization of plasma membrane potential. Hyperpolarization is associated with increased potassium (K^+) permeability and/or decreased sodium (Na^+) permeability. Sperm contain Ca^{2+}–activated K^+ channels, voltage-gated K^+ channels, two-pore domain K^+ channels, inwardly rectifying K^+ (Kir) channels and sperm specific Slo K^+ channels that regulate intracellular K^+ concentration. Regarding modulation of intracellular Na^+ concentration, participation of epithelial Na^+ channels (ENaC) in capacitation-associated hyperpolarization have been studied extensively. Voltage-gated Na^+ channels (Na_v) are ion channels that play an important role in generating action potentials in excitable cells and participate in the regulation of muscle contraction, cell proliferation and cognitive activities. Interestingly, recent evidence from relatively few studies indicate that Na_v subunits are also expressed in mature sperm of boars and humans. In addition, seven out of nine α-subunits ($Na_v1.1$–$Na_v1.9$) of Na_v channels are present at protein level with specific localization pattern of different subunits. This review focuses on the involvement of Na_v channels in sperm and summarizes recent studies systematically to help understanding of Na_v channels in regulating sperm functions.
Keywords: Sperm, voltage-gated sodium channels, hyperpolarization, motility, capacitation, acrosome reaction

INTRODUCTION

Mammalian sperm are not able to fertilize an oocyte immediately after ejaculation. To achieve fertilization competence, they undergo some biochemical and physiological modifications which are collectively known as capacitation during their passage within the female reproductive tract. Capacitation-associated events include loss of cholesterol from sperm plasma membrane, increase in membrane fluidity, changes in intracellular ion concentrations and intracellular pH, hyperpolarization of sperm plasma membrane potential and tyrosine phosphorylation of sperm proteins. These changes subsequently induce hyperactivation of motility and the ability of sperm to undergo acrosome reaction (Visconti et al., 2011).

Sperm are exposed to different extracellular ion concentrations during transition towards fertilization site in the female reproductive tract from the testis. These changes lead to alterations in the pH and plasma membrane potential. In murine, bovine, equine and human sperm, it has been shown that intracellular concentration of negative charge increases during capacitation subsequently result in plasma membrane hyperpolarization (Puga Molina et al., 2018). Hyperpolarization depends on the activation of the cAMP/PKA pathway and occurs as a result of alteration in fluxes of K^+ and Na^+ ions. Sperm plasma membrane contains various ion channels and transporters which are responsible for these ion permeability changes. Studies demonstrated that Ca^{2+}–activated K^+ channels, voltage-gated K^+ channels, two-pore domain K^+ channels, inwardly rectifying K^+ (Kir) channels and sperm specific Slo K^+ channels are involved in sperm functions (Visconti et al., 2011). Regarding Na^+ channels, epithelial sodium channels (ENaCs) have been studied broadly in sperm of different species and shown to play an important role in the modulation of membrane potential during capacitation (Puga Molina et al., 2018). Moreover, recent evidence indicates that voltage-gated sodium channels (Na_v) are also found in sperm and they participate in regulation of sperm functions.

STRUCTURE OF Na_v CHANNELS

Voltage-gated sodium (Na_v) channels play a crucial role in the electrochemical action potential generation and propagation by regulating Na^+ flow through membranes subsequently triggering membrane depolarization (Xu et al., 2019). Although Na_v channels are found in excitable cells mainly, they are present in non-excitable cells such as fibroblasts, immune cells (Brackenbury et al., 2008), glial cells (Black and Waxman, 2013) and tumor cells (Mao et al., 2019) as well. These channels are involved in maintaining homeostasis and control of muscle contraction, cell proliferation and cognitive activities, and regulation of tumor growth, invasion, and metastasis (Andavan and Lemmens-Gruber, 2011).

Na_v channels are very complex membrane proteins that consist of a central α-subunit and one or more auxiliary β-subunits (Catterall, 2012). To date, nine functional α-subunit isoforms ($Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.4$, $Na_v1.5$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$) have been cloned in mammals. Each isoform of α-subunits is encoded by a different gene and expressed as cell-specific and tissue-specific. $Na_v1.1$, $Na_v1.2$ and $Na_v1.3$ are primarily expressed in the central nervous system (CNS). $Na_v1.4$ and $Na_v1.5$ are predominantly expressed in skeletal muscle and cardiac muscle respectively. $Na_v1.6$ is found in CNS as well as peripheral nervous system (PNS). $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ are mainly expressed in PNS (Xu et al., 2019). Four different mammalian β-subunits have been identified namely β1, β2, β3 and β4. The β-subunits function in regulation of expression and trafficking of the α-subunit and is involved in channel gating. An α-subunit contains an ion-conducting aqueous pore domain and voltage-sensing domains (VSDs) and can function as a Na^+ channel without the β-subunit (Catterall, 2012).

Na_v CHANNELS IN SPERM

The presence of Na_v channels in mammalian sperm was identified for the first time in 2009 by Pinto and co-workers (Pinto et al., 2009). They demonstrated all the mRNAs that encode different α-subunits, and the mRNAs encoding auxiliary subunits β1, β3 and β4 were expressed in capacitated human sperm. At the protein level, all α-subunits except for Nav1.1 and Nav1.3 were present. The localization pattern was specific and different among subunits. Furthermore, they showed that *in vitro* incubation of sperm with Na_v activator veratridine induced progressive motility. The same group focused on $Na_v1.8$ in their next study (Cejudo-Roman et al., 2013). They verified $Na_v1.8$ is present in human sperm and are located in the neck and the principal piece of the flagellum. They also showed that veratridine caused an increase in progressive motility, but did not induce hyperactivated motility or the stimulated acrosome reaction.

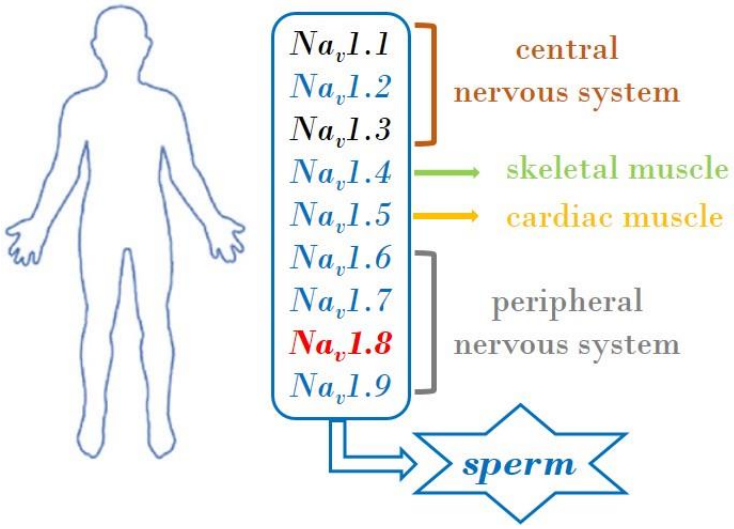

Figure 1. Distribution of α-subunit isoforms of Na_v channels in the human body. mRNAs encoding all isoforms are found in human sperm. Isoforms present in human sperm at the protein level are shown in blue color. Red color ($Na_v1.8$) refers to the most studied isoform in mammalian sperm.

After four years, in 2017, Chauhan and co-workers characterized $Na_v1.8$ in bull sperm (Chauhan et al., 2017). They demonstrated that, $Na_v1.8$ channels are present in head, neck, mid-piece and flagellum of the sperm, with most abundance in neck and mid-piece. They also showed that selective inhibition of $Na_v1.8$ by A-803467 at low concentrations decreased the forward progressive motility in a time-dependent manner while high concentrations lead to faster forward motility in sperm. In addition, activation of $Na_v1.8$ channels by

veratridine treatment for 2h at 10 and 15 mM concentrations induced hyperactivated motility. It was also proposed that inhibition of channel increased the mitochondrial membrane potential, capacitation and acrosome reaction in sperm. In the next study of Chauhan and co-workers (Chauhan et al., 2018) it was revealed that protein tyrosine phosphorylation levels were increased in sperm after treatment with high concentration of veratridine. Five proteins namely p17, p30, p54, p90 and p100 were shown to be phosphorylated in their tyrosine residues while the highest level of phosphorylation was seen in p17.

Candenas and co-workers showed that veratridine-dependent activation of Na_v channels was associated with increased progressive motility of human sperm (Candenas et al., 2018). However, Na_v antagonist lidocaine did not induce hyperactivation of sperm motility. Veratridine treatment results in an increase in protein tyrosine phosphorylation, while lidocaine and tetrodotoxin reversed its effects. In addition, veratridine inhibited progesterone-induced acrosome reaction and led to depolarization of sperm plasma membrane. Concurrently with the previous study, Gakhar and coworkers investigated the effects of different Na_v blockers on human sperm motility and viability in their study (Gakhar et al., 2018). For this aim, they incubated sperm with antiarrhythmic (amiodarone, procainamide and disopyramide) and antiepileptic (carbamazepine, oxcarbazepine, phenytoin, and lamotrigine) drugs. They found that all drugs tested, except oxcarbazepine, inhibited total sperm motility reversibly. Sperm viability also decreased with the treatment of all drugs.

Table 1. Summary of studies on the effect of Na_v channels on sperm functions

Species	Sperm effects	Reference
Human	Na_v channels are present in sperm Activation of channel increases progressive motility	Pinto et al., 2009
Human	Activation of channel increases progressive motility	Cejudo-Roman et al., 2013
Boar	Activation of channel increases progressive motility, reduces membrane integrity. Inhibition of channel increases mitochondrial membrane potential, capacitation and acrosome reaction	Chauhan et al., 2017
Boar	Activation of channel induces protein tyrosine phosphorylation	Chauhan et al., 2018
Human	Activation of channel increases progressive motility and protein tyrosine phosphorylation	Candenas et al., 2018
Human	Antiarrhythmic and antiepileptic Na_v blockers inhibit total motility and decrease viability	Gakhar et al., 2018

CONCLUSION

All the previously published data presented in this review suggest that Na_v channels are expressed in mammalian sperm such as those of human and boar at least (Figure 1) and play an important role in the regulation of sperm functions (Table 1). Na_v channels particularly affect sperm motility. Moreover, they appear to participate in the regulation of other functions such as protein tyrosine phosphorylation, plasma membrane potential, mitochondrial transmembrane potential, membrane integrity, viability and acrosome reaction which are associated with the acquisition of sperm fertilization competence. Further studies are warranted for a better understanding of the role of Na_v channels in sperm functions and male fertility.

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➢ *ORAL PRESENTATION*

Kadmiyum Toksikasyonunda Kafeik Asit Fenetil Ester'in Antioksidan Parametreler ve Karaciğer Histopatolojisi Üzerine Etkisi

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Özet
Kadmiyum ekosistemde en tehlikeli ağır metallerden biri olup, canlı organizmalar için oldukça toksiktir. Kadmiyum çeşitli kaynaklardan çevreye karışarak bitkilere, toprağa ve suya geçmektedir. Böylece kadmiyum insan ve hayvanların sağlığını olumsuz etkilemektedir. Bundan dolayı deneysel kadmiyum toksikasyonunun organizmada meydana getirdiği değişikliklerin histopatolojik ve biyokimyasal parametrelerle belirlenmesi, insan sağlığının korunması ve kadmiyum gibi ağır metallerin olumsuz etkilerinin gösterilmesi açısından önem arz etmektedir. Yapılan geniş literatür taramalarında kadmiyum toksikasyonu, paraoksonaz ve kafeik asit fenetil ester (CAPE) arasında bir ilişkinin olup olmadığını belirten herhangi bir çalışmaya rastlanılmamıştır. Bu çalışmada; deneysel kadmiyum toksikasyonu oluşturulan farelerde CAPE'nin serum Paraoksonaz (PON) aktivitesi, total oksidan / antioksidan kapasite ve lipit profilleri düzeylerini nasıl etkilediğini histopatolojik ve biyokimyasal parametrelerle belirlendi. Ayrıca kadmiyum toksikasyonunda CAPE nin koruyucu etkisinin belirlenmesiyle akut ve kronik ağır metal zehirlenmelerinde tedavi amaçlı kullanılması için yapılacak diğer çalışmalara temel oluşturacaktır.
Anahtar Kelimeler: Kadmiyum toksikasyonu, paraoksonaz, total oksidan/antioksidan kapasite

The Effect of Cafeic Acid Phenethyl Ester on Antioxidant Parameters and Liver Histopathology in Cadmium Toxication

Abstract
Cadmium is one of the most dangerous heavy metals in the ecosystem and is highly toxic to living organisms. Cadmium interferes with the environment through various sources and passes to the soil, soil and water. Thus, cadmium affects the health of people and animals negatively. Therefore, it is important to determine the changes of experimental cadmium toxicity in the organism by histopathological and biochemical parameters in terms of protection of human health and demonstration of adverse effects of heavy metals such as cadmium. In a large literature review, no studies have been conducted to determine whether there is an association between cadmium toxicity, paraoxonase and caffeic acid phenethyl ester. In this study; We will try to determine by histopathological and biochemical parameters how CAPE affects serum Paraoxonase (PON) activity, total oxidant / antioxidant capacity and lipid profiling levels in experimental Cadmium toxicity-induced mice. In addition, the determination of the protective effect of CAPE in cadmium toxicity will form the basis for other studies to be used for therapeutic purposes in acute and chronic heavy metal poisonings.

Keywords: Cadmium toxicity, paraoxonase, total oxidant / antioxidant capacity

GİRİŞ

Kadmiyum (Cd) ekosistemde en tehlikeli ağır metallerden biri olup, canlı organizmalar için oldukça toksiktir (Karbownik M 2001, Prozialeck W.C 2006). Kadmiyumun doku ve organlarda meydana getirdiği toksikasyon organizmada oksidatif strese neden olmaktadır. Oksidatif stres, organizmada antioksidan sistem ve serbest radikaller arasındaki dengenin bozulması sonucu meydana gelmektedir (Gülcen B 2011).

Serbest radikaller; canlı organizmaların hücrelerinde DNA, protein, karbonhidrat ve lipit gibi önemli organik bileşiklerin yapılarını bozarak lipit peroksidasyonuna neden olurlar (El-Sokkary GH 2010, Aydoğdu N

2007). Hücre membranlarında lipit peroksidasyonuna karşı antioksidan etkili olan Paraoksonaz (PON) enziminin lipit peroksidasyonu sırasında önemli derecede aktivite kaybına uğradığı bildirilmektedir (Van, L.B 1995). PON , serumda yüksek dansiteli lipoproteinler (HDL) üzerinde yer alan ve kalsiyuma bağımlı glikoprotein yapısında bir enzimdir. (Costa, L.G 2003, Sorenson, R.C 1999).

Lipit peroksidasyonunun uzun zamandan beri kadmiyum toksikasyonu için birincil mekanizma olduğu düşünüldüğünden dolayı kadmiyumun oluşturduğu oksidatif strese karşı koruyucu ve tedavi amacıyla antioksidan etkili ajanların verilmesi gerektiğine inanılmaktadır (Gülcen B 2011, Casalino E 2002, Lopez E 2006, Shaikh ZA 1999, Santos FW 2005. Bu durum çalışmaları daha çok antioksidan savunma sistemini güçlendirebilecek ve ağır metal zehirlenmelerini engelleyebilecek antioksidan moleküllere yönlendirmiştir. Bu antioksidan etkili moleküllerden biri de kafeik asit fenetil ester (CAPE)'dir. CAPE'nin farmakojik olarak güvenilir bir molekül olduğu ve lipit peroksidasyonunu baskılayarak antioksidan enzimlerin aktivitesini arttırdığı yapılan bir çok çalışmada gösterilmiştir (Deveci HA 2018, Oktem, F 2005, Ozyurt, H 2004, Fadillioğlu 2003).

Yapılan bu çalışmada, önemli bir ağır metal olan kadmiyumun farelerde oluşturulan deneysel toksikasyon modeli ile güçlü antioksidan etkisi olduğu bilinen CAPE'nin organizmanın oksidan/antioksidan dengesinde meydana getirdiği değişikliklerin histopatolojik ve biyokimyasal parametrelerle ortaya konulması amaçlandı.

MATERYAL ve METOD

Çalışmada kullanılan deney hayvanları 3 aylık, 40 adet dişi Swiss albino fareler olup, hayvanlar 15 gün boyunca adaptasyon amacıyla standart fare yemi ve su ile beslendi. Deney ortamı şartları 12 saat ışık ve 12 saat karanlık olmak koşuluyla 25±2 0C ısı ve ortalama %50±5 nem oranı bulunan kafeslerde yetiştirilip deneysel uygulamalara hazır hale getirildi. Hayvanlar, aşağıda belirtildiği gibi gruplara ayrıldı.
Grup I: 10 adet Kontrol grubu
Grup II: 10 adet CAPE grubu
Grup III: 10 adet Kadmiyum grubu
Grup IV: 10 adet Kadmiyum+ CAPE grubu

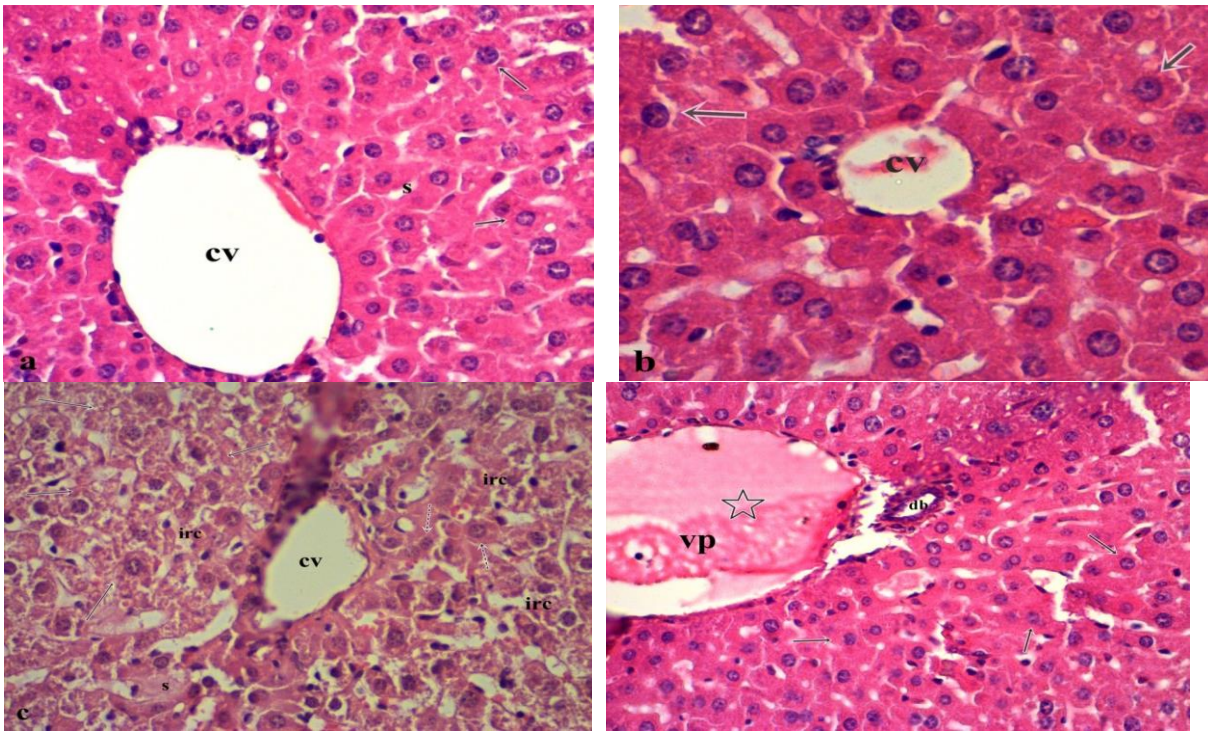
Bu gruplarda bulunan farelerin yumurtalıkları orta hattan yapılan ameliyatla alındı. Ovariektomi işlemini takip eden 20 günlük iyileşme süresi sonunda diğer uygulamalara geçildi. Grup I normal diyet ile beslendi. Gruplar arasında enjeksiyon işlemlerinde meydana gelebilecek stres ve plasebo etkisinden doğacak farklılıkları ortadan kaldırmak için Grup-I'e serum fizyolojik intraperitoneal olarak deney süresince verildi. Diğer gruplarda ise; Grup II'ye normal diyet ve intraperitoneal olarak 10 µmol/kg CAPE , Grup III'e normal diyet + deri altı 1 mg/kg/gün Kadmiyum-klorür 15 gün, Grup-IV'e normal diyet + deri altı 1 mg/kg/gün Kadmiyum-klorür, + intraperitoneal olarak 10 µmol/kg CAPE 15 gün verildi.
Son uygulamalardan 24 saat sonra kan örnekleri heparinli tüplere intrakardiyak olarak alındı ve farelere servikal dislokasyon yöntemi uygulandı. Sistemik nekropsileri yapılarak alınan doku örnekleri % 10'luk formol solüsyonunda tespit edildi. Bilinen rutin işlemlerin ardında hazırlanan parafin bloklarda kesitler alınıp, HE ile boyanarak ve histopatolojik bulgular için ışık mikroskobunda incelendi. Biyokimyasal parametreler için alınan kan örneklerinde PON aktivitesi, HDL ve total oksidan/total antioksidan kapasite ölçümleri spektrofotometrede kitlerle yapıldı.

BULGULAR

Çalışmamızın biyokimyasal parametreleri değerlendirildiğinde, kontrol grubu ve CAPE grubunda biyokimyasal değerler normal düzeylerde iken Cd grubu ve Cd+CAPE gruplarında PON aktivitesi, HDL ve TAK düzeylerinde sırasıyla ($0.001<p$, $0.01<p$, $0.01<p$) önemli bir azalma tespit edildi. CAPE ve Cd+CAPE grubunda biyokimyasal değerler kontrol grubuna yakın düzeylerde iken Cd grubund ise TOK ve OSİ düzeylerinde ise sırasıyla ($0.01<p$, $0.01<p$) önemli bir artış tespit edildi. Çalışmamızda elde edilen biyokimyasal parametreler aşağıda Tablo.1'de verildi.

PARAMETRELER	GRUPLAR				p<
	Kontrol n:10 Mean ± SD	**CAPE** n:10 Mean ± SD	**Cd** n:10 Mean ± SD	**Cd+CAPE** n:10 Mean ± SD	
PON (U/L)	164,67 ± 16,12[a]	166,02 ± 16,42[a]	128,09 ± 14,61[b]	141,28 ± 13,20[b]	0.001
HDL (mg/dl)	47,71 ± 5,02[a]	48,21 ± 4,31[a]	37,54 ± 4,29[b]	42,37 ± 3,68[b]	0.01
TAK (mmol Trolox eq/L)	1,52 ± 0,21[a]	1,57 ± 0,19[a]	1,14 ± 0,14[b]	1,30 ± 0,11[b]	0.01
TOK (µmol H2O2 eq /L)	7,01 ± 0,50[b]	6,92 ± 0,52[b]	8,24 ± 0,47[a]	7,35 ± 0,37[b]	0.01
OSİ (arbitrary birim)	0,05 ± 0,009[b]	0,05 ± 0,009[b]	0,07 ± 0,013[a]	0,06 ± 0,007[b]	0.01

Çalışma sonunda alınan karaciğer dokuları tespit ve doku takibi aşamalarından sonra parafine gömülerek elde edilen bloklardan mikrotom yardımı ile alınan 5 µm'lik seri kesitler hematoksilen-eosin ile boyandıktan sonra ışık mikroskobik düzeyde incelendi. Gruplardan elde edilen kesitlerin histopatolojik incelemeleri sonucu; CAPE ve kontrol grubunda vena centralis ve portal alanın normal görünümde olduğu, hepatosit diziliminin düzenli olduğu gözlenmiştir. Kadmiyum uygulaması yapılan grup kesitlerinde, sentral ve portal vende konjesyon ve fokal nekroz bölgeleri ile dejeneratif alanlar tespit edildi. Kadmiyum ile birlikte verilen CAPE grubunda ise kadmiyum grubuna oranla histolojik değişimlerin şiddetinde ve sıklığında azalmalar gözlenmiştir (Resim 1 a, b, c, d).



TARTIŞMA ve SONUÇ

Kadmiyumun organizmadan atılımı için herhangi bir mekanizma olmadığından, bu ağır metal dokularda birikim eğilimi göstermektedir. Organizmaya alınan kadmiyum öncelikle böbrek ve karaciğer olmak üzere pankreas, kemikler, akciğer ve plesenta gibi organlarda birikmektedir (Brzoska, M.M 2003, Jurczuk, M 2004). Kronik kadmiyum toksikasyonlarında organizmada hepatotoksite ve nefrotoksite gelişmektedir (Friberg, L 1984, Dudley, R.E 1985). Kadmiyuma maruz kalmada artan hastalık risklerinin sistemik

inflamasyon ve indüklenen oksidatif stresten kaynaklanabileceği ileri sürülmektedir (Colacino, JA 2014). Oksidatif stresin lipit peroksidasyonunu arttırdığı, antioksidan enzimlere zarar verdiği ve tiol proteinlerde, enerji metabolizmasında, DNA yapısında, membran fonksiyonunda değişikliklere neden olduğu bildirilmektedir (Jurczuk, M 2004, Friberg, L 1984, Dudley, R.E 1985, Hwang, D.F 2001, Casalino, E 2002, Lopez, E 2006).

Deneysel kadmiyum toksikasyonu yapılan hayvan modeli çalışmalarında kadmiyumun oksidatif strese neden olduğu ve oksidatif stresin de hücre ve dokularda lipit peroksidasyonuna yol açarak organizmanın antioksidan enzim aktivitelerinde azalmaya neden olduğu, lipit peroksidasyon ürünlerinde ise artışa neden olduğunu bildirmişlerdir Jurczuk, M 2004, Casalino, E 2002, Aktay G 1995, Coşan DT 2017, Karoui-Kharrat D 2017, Şimşek 2018, Karapehlivan, M 2014). Yapılan önceki çalışmalara benzer şekilde, bizim yaptığımız bu çalışmada kadmiyum uygulanan farelerde serum TAK düzeyinin önemli derecede azaldığı, TOK düzeyinin ve oksidatif stres indeksinin (OSİ) ise arttığı tespit edildi. CAPE verilen gruplarda ise bu değerlerin kontrol grubuna yaklaştığı gözlendi.

Deney hayvanlarında kadmiyum toksikasyonu ve PON aktivitesi arasında ilişkiyi araştıran herhangi bir çalışmaya rastlamadığımız için fareler üzerinde yaptığımız bu çalışmada, kadmiyum verilen farelerde serum PON aktivitesi ve HDL düzeyinin kontrol grubu farelerden daha düşük olduğu tespit edildi. CAPE verilen gruplarda ise serum PON aktivitesi ve HDL düzeyinin kontrol grubuna yaklaştığı belirlendi. Kadmiyum uygulanan grupta serum PON aktivitesi ve HDL düzeyinin düşük olmasının nedeni hem kadmiyum toksikasyonuna bağlı gelişen oksidatif stresin lipit peroksidasyonuna neden olması hem de kadmiyumun kalsiyum bağımlı paraoksonazın aktivitesini inhibe etmesi olabilir.

Sonuç olarak; yaptığımız bu çalışma ile önemli bir ağır metal olan kadmiyumun organizmaların antioksidan savunma sistemi ve karaciğer histopatolojisi üzerinde önemli değişiklikler meydana getirebileceği, CAPE'nin ise bu değişiklikleri önemli ölçüde önleyebileceği kanaatini taşımaktayız..

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➢ ***ORAL PRESENTATION***

Düşük proteinli L-Lösin ve β-hidroksi-β-metil-bütirat ilave edilmiş başlangıç rasyonlarına etlik piliçlerin tepkisi

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Özet
Bu çalışmada düşük proteinli etlik piliç başlangıç rasyonlarına L-Lösin ve metaboliti olan β-hidroksi-β-metil-bütirat (HMB) ilavesinin performans (canlı ağırlık değişimi, yem tüketimi, yemden yararlanma oranı) ve oransal kas ağırlıkları (göğüs kası ve but kası) üzerine etkisini belirlemek amacıyla 322 adet erkek civciv tesadüfi olarak 4 tekerrür halinde ve tekerrür başına 22 civciv olacak şekilde muamele gruplarına dağıtılmıştır. Deneme planına göre ilk 21 gün boyunca 1. muamele grubuna standart etlik civciv yemi (K; % 22 HP); 2. gruba % 20 HP içerikli etlik civciv yemi (20HP); 3. ve 4. gruplara ise sırasıyla % 0.2 L-Lösin (20L) veya % 0.01 HMB ilaveli (20HMB) % 20 HP içerikli etlik civciv yemi verilmiştir. Toplam deneme süresince canlı ağırlık kazancı en yüksek K grubu hayvanlarında bulunurken bunu sırasıyla 20L ve 20HP grupları izlemiştir. Toplam yem tüketimi 20HP grubunda en düşük bulunurken, YYO sırasıyla K, amino asit grupları ve 20HP hayvanlarında düşük bulunmuştur. Oransal göğüs ve but kası ağırlıkları 20HP grubunda diğer gruplardan daha düşük bulunmuştur. Bu sonuçlara göre kas gelişimini teşvik eden L-Lösin ve metaboliti olan HMB'nin başlangıç yemlerine ilave edilerek rasyon protein oranı indirgenebilir ve böylece hem rasyon maliyeti düşürülüp hem de pazar yaşında satılacak et miktarı artırılabilir. **Anahtar Kelimeler:** Lösin, performans, kas ağırlığı, β-hidroksi-β-metil-bütirat

Response of broilers to L-leucine and β-hydroxy-β-methylbutyrate supplementation of low crude protein starter diets

Abstract
To evaluate the effects of the L-Leucine and its metabolite β-hydroxy-β-methylbutyrate supplementation of a low crude protein starter diet on performance (body weight gain, BWG; feed consumption, FI; feed conversion rate, FCR) and relative muscle weights (pectoralis muscle, PM; iliotibialis muscle, ITM) of broiler chickens, 322 male chicks were randomly allocated into four treatment groups each with four replicates containing 22 birds. Birds were fed dietary treatments for first 21 days of age. Treatments consisted of a control diet (C; 22 % CP), a low crude protein (20CP; 20 % CP) diet, 20CP diet supplemented with L-Leucine (20L; 0.20%) or HMB (20HMB; 0.01%). On day 42, the BWG of C birds was higher than 20L and 20CP groups, respectively. While the FI was lower in 20CP birds compared to other groups, the FCR was lower in the C, amino acid groups and 20CP animals, respectively. The 20CP birds had a lower relative PM and ITM weights compared to other groups. According to these results, the ratio of ration protein can be reduced by adding L-Leucine and its metabolite HMB to the starter feeds, which are promote muscle growth, thus reducing the ration cost and increasing the amount of meat to be sold at market age.
Key words: Leucine, performace, muscle weight, β-hydroxy-β-methylbutyrate

GİRİŞ

Günümüzde kristal ve sentetik amino asitlerin ticari kullanılabilirliği, etlik piliçlerin performansından ödün vermeksizin diyet maliyetlerini düşürmektedir. Etlik piliç yemleri genellikle hayvanların amino asit alımları korunarak minimum ham protein konsantrasyonunda, en düşük maliyetli yazılım kullanılarak formüle edilir. Ticari olarak mevcut sentetik kaynakları kullanarak amino asit sağlama, besleme uzmanlarının etlik piliçlerin performansını ve yüksek et verimini sağlamasına izin vermektedir (Berres ve ark., 2010; Hill ve Kim, 2013; Kobayashi ve ark., 2013; Miranda ve ark., 2014, 2015). Kas kütlesinin programlanmasının tamamlanması için çıkıştan hemen sonra dallanmış zincirli amino asitler (lösin, izolösin ve valin; DZA; Ospina-Rojas ve ark., 2017, 2019; Kop-Bozbay ve Ocak, 2020; Kop-Bozbay ve ark., 2020) ve bu amino asitlerden lösinin metaboliti olan β-hidroksi-β-metil-bütiratın (HMB) kullanılabileceğini gösteren çalışmalar mevcuttur (Nissen ve ark.,

1994; Nissen ve Abumrad, 1997; Moore ve ark., 2005a; Qiao ve ark., 2013). Bu çalışmalarda üretimde ekonomik önemi olan göğüs eti oranın arttırdığı belirlenmiştir.

Kanatlılarda çıkış sonrası erken dönem, kesim yaşındaki göğüs eti kas verimini programlama bakımından kritik dönem olduğundan, çıkıştan hemen sonra yapılacak besleme (Halevy ve ark., 2000; Mozdziak ve ark., 2002; Qiao ve ark., 2013; Kop-Bozbay ve Ocak, 2020; Kop-Bozbay ve ark., 2020), kas kütlesini ve adaptasyon kabiliyeti arttırılabilir. Bir çok çalışmada çıkış sonrası erken dönemde hayvanların besin madde gereksinimlerini karşılamada bazı bireysel veya farklı amino asit karışımlarının yem ile verilmesinin etkileri araştırılmıştır (Moore ve ark., 2005a, b; Berres ve ark., 2010; Miranda ve ark., 2014; Kop-Bozbay ve Ocak, 2020; Kop-Bozbay ve ark., 2020).

Lösin, izolösin ve valin, esansiyel aminoasit ailesinin dallanmış zincirli aminoasitler sınıfını oluşturmaktadır. Özellikle lösin (Panton ve ark., 2000) stres altındaki hayvan kaslarında hem enerji kaynağı, hem de diğer amino asit ve kas proteinlerinin sentezi ve proteoliz miktarını azaltan bir prekursör olarak hizmet etmektedir (Zhenqi ve ark., 2001). β-hidroksi-β-metil-bütirat (HMB), lösin amino asidinin metabolizması sonucu doğal olarak oluşan, biyolojik olarak aktif bir bileşiktir (Tatara, 2008; Qiao ve ark. 2013) ve lösin, HMB oluşumunu artırır (Kornasio ve ark., 2009). Bununla birlikte, lösinin metabolizması esnasında bu metabolit ancak lösin miktarının % 5'i kadar üretilebilmektedir. HMB'nin kaslarda proteoliz miktarını ve stres altında kaslarda oluşabilecek zararları azalttığı gösterilmiştir (Panton ve ark., 2000). Gerçekten de, ilk 3 günlük yaşta uygulanan yüksek ısı veya düşük lisin içerikli karma gibi orta stres şartlarında civcivlerde uydu hücre mitotik aktivitesinin arttığı ve bunun vücut ağırlığını ve oransal göğüs eti verimini artırdığı bildirilmiştir (Pophal ve ark., 2004). Bununla birlikte HMB'nin daha olgun hayvan kaslarında etkili olmadığı gözlemlenmiştir. Bu durum, lösine olan duyarlılığın yaşla birlikte azalmasına bağlanmaktadır (Moore ve ark., 2005a). Bununla birlikte, lösin, sadece HMB oluşumu için bir kaynak olmakla kalmayıp aynı zamanda proteolitik etki oluşturmaktadır. Bu nedenle, lösin, kas protein sentezi üzerinde etkili olduğu ve kas ve kastaki proteoliz miktarını azaltabileceği düşünülmektedir.

Yapılan araştırmalarda ortaya konduğu gibi uydu hücre mitoz aktivitesinin kümes hayvanlarının ilk bir haftalık yaşında yüksek olduğundan dolayı başlangıç yemlerinde ham protein oranı düşürülerek, kas gelişimini teşvik eden dallanmış zincirli amino asitlerden lösini rasyona ilave ederek, hem rasyon maliyetini düşürüp hem de pazar yaşında satılacak et miktarı artırılabilir. Ancak kas programlanması üzerindeki etkinin lösinden mi yoksa metaboliti olan HMB'den mi kaynaklandığının araştırılması gerekmektedir. Lösinin kendi fizyolojik fonksiyonlarını yapması, düşük oranda üretilebilen (% 5) HMB'nin ise organizmada lösinden elde edilmesi yerine, eksojen olarak verilmesi üzerinde durulmalıdır. Bu ifadelerden yola çıkarak, bu çalışmada düşük proteinli etlik civciv rasyonlarına L-Lösin ve metaboliti olan HMB ilavesinin performans ve oransal kas üzerine etkileri araştırılmıştır.

MATERYAL VE METOD

Bu araştırma Eskişehir Osmangazi Üniversitesi Eğitim, Araştırma ve Uygulama Çiftliği Kanatlı Birimi'nde bulunan Etlik Piliç Ünitesi'nde yürütülmüştür. Araştırma Eskişehir Osmangazi Üniversitesi Deney Hayvanları Hayvan Etik Kurulu tarafından onaylanmıştır (2019/703).

Benzer canlı ağırlık ortalamasına sahip toplam 322 adet günlük yaşta erkek civciv (ROSS308), dört tekerrür halinde ve tekerrür başına 22 civciv olacak şekilde tesadüfi olarak dört muamele grubuna dağıtılmıştır. Deneme planına göre ilk 21 gün boyunca 1. muamele grubuna standart etlik civciv yemi (Kontrol, K; %22 HP) ; 2. gruba % 20 HP içerikli etlik civciv yemi (20HP); 3. ve 4. gruplara ise sırasıyla % 0.2 L-Lösin (20L) veya % 0.01 HMB ilaveli (20HMB) % 20 HP içerikli etlik civciv yemi verilmiştir. Denemede yem materyali olarak mısır ve soya ağırlıklı rasyon kullanılmıştır. 0-21. günler arası %22 HP ile beslenen Kontrol grubu standart etlik civciv yemi (%22 HP ve 3050kcal/kg ME) ile beslenirken düşük ham protein alan gruplar (%20 HP) protein içeriği yaklaşık % 9 indirgenmiş etlik civciv yemi ile (%20 HP ve 3050kcal/kg ME) ile beslenmişlerdir. Denemede kullanılacak olan dalanmış zincirli amino asit olan L-Lösin % 0.2 oranında ve lösin metaboliti olan HMB % 0.01 oranında 0-21. günlerde etlik civciv rasyonlarına eklenmiştir (Sigma Alderich). Kullanılacak HMB miktarı literatür önizlemesi sonucunda % 0.01 oranında (Nissen ve ark., 1994) belirlenmiş olup, lösin miktarı, lösin metabolizmasının yaklaşık% 5'i HMB'nin endojen sentezine yol açtığı dikkate alınarak % 0.2 olarak belirlenmiştir. 21-42. günler arası tüm hayvanlara %20 HP ve 3200 kcal/kg ME içeren standart etlik piliç yemi verilmiştir.

Civcivler çevre kontrollü hızar talaşı altlıklı ve floresan lamba ile aydınlatmalı (23 saat aydınlık 1 saat karalık) deneysel bir kümeste barındırılmıştır. Kümesteki her bir bölme 1.25 × 2 m ebatlarında ve her bölmede eşit

miktarda damlalıkla beraber nipel suluk ve bir adet askılı etlik piliç yemliği (tüp tip) mevcut edilmiştir. Kümes sıcaklığı, 32 ± 1°C ve kümes içi nispi nem oranı %60-70 arasında tutulmuştur.

Hayvanlar deneme başında ve sonunda tartılarak canlı ağırlıkları belirlenerek canlı ağırlık değişimleri (CAD) ve yem tartımları yapılarak yem tüketim miktarları (YT) hesaplanmıştır. Yemden yararlanma değeri birim canlı ağırlık artışı için tüketilen yem miktarı olarak ifade edilmiştir. Tartımlarda 1 g hassasiyette terazi kullanılmıştır.

Deneme sonunda tartım sonrası, tekerrür ortalamasına en yakın 4 hayvan (muamele başına toplam 16 hayvan) kesim özelliklerini belirlemek üzere seçilmiştir. Hayvanlar Ziraat Fakültesi Araştırma ve Uygulama Çiftliği'nde bulunan kesimhanede kesilmişler ve kesim sonrası haşlama ve tüy yolma işlemlerinde otomatik ekipman kullanılmıştır. Kesilen hayvanlarda sol göğüs (pectoralis muscle, PM) ve but (ilio tibialis muscle, ITM) kasları ağırlıkları belirlenerek canlı ağırlığa göre standardize edilerek oransal ağırlıkları belirlenmiştir.

Elde edilen veriler SPSS (SPSS Inc. 1999, Release 10_0) istatistik paket programın GLM prosedürüne göre analiz edilmiştir. Canlı ağırlık, yem tüketimi, yemden yararlanma oranı ve kas özellikleri tesadüf parselleri deneme desene göre varyans analizine tabi tutulmuştur. Muameleler arasındaki farklılıklar $P < 0.05$ düzeyinde önemli sayılmıştır.

BULGULAR

Düşük ham protein içeriğine sahip başlangıç yemlerine L-Lösin veya HMB ilave edilmesinin etlik piliçlerin kesim yaşında CAD, YT ve YYO üzerine etkileri Tablo 1'de sunulmuştur. Deneme süresince CAD en yüksek Kontrol (K) grubu hayvanlarında bulunurken bunu sırasıyla 20L ve 20HP grupları izlemiştir (P=0.000). Toplam yem tüketimi 20HP grubunda en düşük bulunurken (P=0.003) YYO sırasıyla kontrol, amino asit grupları ve 20HP hayvanlarında düşük (P=0.000) bulunmuştur.

Tablo 1. Düşük ham protein içeriğine sahip başlangıç yemlerine L-Lösin veya β-hidroksi-β-metil-bütirat (HMB) ilave edilen etlik piliçlerin performans özellikleri

	Kontrol	20HP	20L	20HMB	OSH	P
Canlı ağırlık değişimi, g	2724.52a	2228.50c	2515.91b	2618.32ab	50.842	0.000
Yem tüketimi, g	4448.26a	3933.95b	4282.57a	4446.22a	65.941	0.003
YYO, g yem/g CAA	1.63c	1.77a	1.70b	1.70b	0.014	0.000

Kontrol: %22 ham protein içeren standart etlik piliç başlangıç yemi ile beslenen grup, 20HP: %20 ham protein içeren etlik piliç başlangıç yemi ile beslenen grup, 20L: %20 ham protein içeren etlik piliç başlangıç yemine L-Lösin ilave edilen grup, 20HMB: %20 ham protein içeren etlik piliç başlangıç yemine HMB ilave edilen grup, YYO: yemden yararlanma oranı, CAA: canlı ağırlık artışı, OSH: ortalamanın standart hatası. a, b, c: Farklı harfle gösterilen ortalamalar istatistiki olarak farklıdır ($P < 0.05$).

Kesim yaşında düşük ham protein içeriğine sahip başlangıç yemi ile beslenen etlik piliçlerin oransal PM (P=0.002) ve ITM (P=0.018) ağırlıkları diğer gruplardan daha düşük bulunmuştur (Tablo 2).

Çizelge 3.7. Düşük ham protein içeriğine sahip başlangıç yemlerine L-Lösin veya β-hidroksi-β-metil-bütirat (HMB) ilave edilen etlik piliçlerin oransal göğüs kası (*Pectoralis muscle*, PM) ağırlığı, but kası (*Ilio tibialis muscle,* ITM) ağırlığı

	Kontrol	20HP	20L	20HMB	OSH	P
Göğüs kası, g/100 g canlı ağırlık	35.26a	31.07b	33.54a	33.97a	0.430	0.002
But kası, g/100 g canlı ağırlık	22.76a	20.82b	22.32a	22.17a	0.239	0.018

Kontrol: %22 ham protein içeren standart etlik piliç başlangıç yemi ile beslenen grup, 20HP: %20 ham protein içeren etlik piliç başlangıç yemi ile beslenen grup, 20L: %20 ham protein içeren etlik piliç başlangıç yemine L-Lösin ilave edilen grup, 20HMB: %20 ham protein içeren etlik piliç başlangıç yemine HMB ilave edilen grup, OSH: ortalamanın standart hatası. a, b: Farklı harfle gösterilen ortalamalar istatistiki olarak farklıdır ($P < 0.05$).

TARTIŞMA

Denemenin, performans ile ilgili sonuçları başlangıç yemine L-Lösin veya HMB ilavesi ile karmada % 9'a kadar bir protein azaltmasına gidilebileceğini göstermiştir. Canlı ağırlık, yem tüketimi ve YYO ile ilgili sonuçlarımız ham protein içeriği düşürülmüş rayonlara amino asit ilavesinin performansı iyileştirdiğini bildiren çalışmaları desteklemektedir (Corzo ve ark., 2005; Dean ve ark., 2006). Böyle bir bulgu, yem maliyetini azaltma yanında çevrenin azot kirliliğini de azaltmış olabilir. Diğer taraftan, ölüm oranları, tüm gruplar için normal sınırlar içinde olmasına ve ölümlerin herhangi bir muamele ile ilişkili olmamasına rağmen (Nissen ve ark., 1994), mevcut çalışmada elde edilen gelişimin, ticari etlik piliç yetiştiriciliği standartlarıyla uyumlu olmuştur. Bu durum, karmaların azaltılmış ham protein içerikli hazırlanmasını desteklemektedir.

Düşük proteinli başlangıç yemlerine HMB ilavesinin canlı ağırlık artışı sağlaması önceki çalışmalarla uyumlu bulunmuştur (hindi, ilk 7 gün, Moore ve ark., 2005b; etlik piliç, 42 gün, Qiao ve ark., 2013). HMB ilavesinin proteolizi düşürdüğü, yağsız kas kütlesini (hayvansal üretim açısından yararlı olan yağsız kas kütlesi) artırdığı bir çok araştırmada ispat edilmiştir (Panton ve ark., 2000; Ostaszewski ve ark., 2000). Ayrıca aşırı kas büyüme potansiyeli ve yüksek satalit hücre mitotik aktivitesinden dolayı genç hindi kaslarında HMB'nin daha etkin olduğu belirlenmiştir (Moore ve ark., 2005b). Dolayısıyla mevcut çalışmada lösin amino asidinin bir metaboliti olan HMB'nin etkisi bu etkilerden kaynaklanmış olabilir. L-Lösin ilavesi ile performansın etkilenmediğini (21-42. günler, Erwan ve ark., 2009; 0-21. günler, Chang ve ark., 2015) yada olumsuz etkilendiğini bildiren (21-42. günler, Ospina-Rojas ve ark., 2017, 0-21. günler, 2019) çalışmaların aksine mevcut çalışmada performansta iyileşme sağlanarak kontrol grubu ile aynı seviyeye taşınmıştır. Aradaki farklılıklar kullanılan lösin amino asidinin oranı ve yeme ilave edilme süresi ile ilgili olabilir. Nitekim mevcut çalışmada kullanılan lösin miktarı diğer çalışmalardan daha düşüktür.

Et tipi kanatlılarda göğüs eti, karkasın en büyük ticari oranını temsil eder ve genellikle diğer karkas parçalarına kıyasla daha yüksek bir fiyattan satılır. Tüm vücudun bir parçası olarak, modern etlik piliçlerin göğüs kasları, ticari olarak ilgi çeken diğer karkas parçalarına oranla daha büyük bir allometrik büyüme sağlar (Schmidt ve ark., 2009). Bu nedenle, eğilim yüksek amino asit yoğunluklu diyetler ile beslemektir (Vieira ve Angel, 2012). Bununla birlikte, yüksek amino asit yoğunluklu diyetlerin maliyetlerini artıran soyanın daha büyük oranda kullanılmasını gerektirmektedir. Öte yandan, başlangıç yemlerinde besin yetersizliği, canlı ağırlık değişimi ve göğüs kasında kayıplara neden olduğu gösterilmiştir (Halevy ve ark., 2000). Bu çalışmada, protein oranı azaltılmış başlangıç yemine L-Lösin veya HMB ilavesi ile pazar payı en yüksek karkas parçalarından olan göğüs ve but etinin oransal ağırlıklarının kontrol grubuyla aynı değerlerde olduğu gözlemlenmiştir. Bu etkiler lösin amino asidi ve metaboliti olan HMB'nin proteolitik etkilerinin ispatı olabilir (Nissen ve ark. 1994; Qiao ve ark., 2013; Ospina-Rojas ve ark., 2017). Canlı ağırlık ve oransal kas ağırlıkları incelendiğinde rasyondaki protein içeriğinin düşürülmesinin yaratacağı stresin özellikle stres altındaki hayvan kaslarında hem enerji kaynağı, hem de diğer amino asit ve kas proteinlerinin sentezi ve proteoliz miktarını azaltan bir prekursör olarak hizmet etmelerinden dolayı lösin (Zhenqi ve ark., 2001) ve HMB (Panton ve ark., 2000) ilavesi ile azaltıldığı, böylece performansın kontrol ile aynı seviyeye ulaştığı söylenebilir.

SONUÇ

Bu çalışmada özellikle lösinin kendi fizyolojik fonksiyonlarını yapması ve düşük oranda üretilebilen (%5) HMB'nin ise organizmada lösinden elde edilmesi yerine, eksojen olarak verilmesi üzerinde durulmuştur. Gerçekten de canlı ağırlık ve oransal kas ağırlığı göz önüne alındığında HMB'nin eksojen olarak verilmesinin daha etkili olduğu belirlenmiştir. Aynı zamanda satalit hücre mitotik aktivitesini etkileyen ve karkas verimini artıran bir besin takviyesi olan lösin ve metaboliti HMB'nin başlangıç rasyonlarına ilave edilerek kesim yaşında etlik piliçlerin performansından ödün vermeden rasyon ham protein oranının düşürülerek maliyetin azaltılabileceğini göstermektedir.

TEŞEKKÜR

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➢ ***ORAL PRESENTATION***

Effects of Vital Gluten and Transglutaminase Enzyme on Chemical Characteristics of Pasta Enriched with Lupin Flour and Resistant Starch

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Abstract
In this study, lupin seeds debittered by two different methods (traditional method and ultrasound application) and its flours used in pasta formulation with resistant starch type4 (RS_4) to improve nutritional and functional properties of pasta. The effects of vital gluten, transglutaminase and vital gluten + transglutaminase combination on the chemical properties of pasta containing 15% lupin flour + 10% RS_4 were investigated. Generally, chemical properties of pasta prepared with lupin flour debittered by ultrasound application were found close to pasta containing lupin flour debittered by the traditional method. Compared to control pasta prepared with 100% wheat semolina, pasta containing 15% lupin flour + 10% RS_4 had a higher ash, fat, protein, total dietary fiber and RS content. The addition of vital gluten and vital gluten+transglutaminase provided higher protein content compared to additive-free pasta samples. Other chemical parameters of pasta samples were not affected by additives.
Keywords: Vital gluten, Transglutaminase, Pasta, Lupin, Resistant starch

INTRODUCTION

Lupin (*Lupinus albus* L.) is a leguminous seed with a good source of protein, dietary fiber, minerals, and is an alternative to soybean. Lupin also contains phytochemicals with high antioxidant capacity (Mohammed et al., 2017). Due to its low glycemic index, lupin-based foods can be used in the control of diabetes and obesity (Hall et al., 2005). It has good potential for the development of many low-cost, high-fiber and high-protein food formulations such as bread, pasta, noodles, biscuits, cakes, meat products, dairy products and sauces (Jayasena and Quail, 2004).

Resistant starch (RS), a prebiotic dietary fiber, cannot be digested in the small intestine. It helps to prevent diabetes, cancer, obesity, intestinal diseases and cardiovascular diseases (Asp and Bjorck, 1992; Meyer et al., 2000). RS, which can be used in food formulations as a functional ingredient, provides less technological losses in the product compared to conventional fiber sources and improves the sensory properties of the product (Tharanathan and Mahadevamma, 2003).

Pasta is a cereal product that is cheap, nutritious and easy-to-prepare, that consumed of consumers of all ages. Gluten protein has been known as an important component influencing the quality of pasta. The strength and elasticity of the pasta dough are closely related to the glutenin and gliadin proteins in gluten. The gluten matrix retains the gelatinized starch granules during pasta cooking. Compared to weak gluten of the same protein level, strong gluten wheats provide the less sticky dough and better extrusion and cooking properties (Dexter et al., 1983; Sissons, 2008).

Transglutaminase enzyme catalyzes acyl-transfer reactions in covalent bonds between proteins (Nonaka et al., 1989). The use of transglutaminase enzyme in pasta improves the cooking quality and decreases the cooking loss (Takacs et al., 2008). Sissons et al. (2010) reported that the addition of 0.5% of transglutaminase showed positive effects on dough strength, firmness and stickiness properties of fiber-enriched spaghetti.

This study aimed to investigate the effects of different additives (vital gluten, transglutaminase and vital gluten+ transglutaminase) on the chemical properties of pasta containing 15% lupin flour + 10% RS_4.

MATERIALS AND METHODS

Materials

Durum wheat semolina was obtained from Selva (Konya, Turkey). RS_4 was procured from a commercial manufacturer in Konya, Turkey. Vital gluten was supplied from Vatan Enzyme (İstanbul, Turkey), and transglutaminase enzyme was obtained from SternEnzym GmbH & Co. (Ahrensburg, Germany).

Lupin flours used in this study were obtained in our previous study. For this purpose, two debitttering processes including traditional method (144 h) and ultrasound application (at 25°C for 25 min sonication every 4 h, 60 h in total) were applied to bitter lupin seeds. The debittered seeds were ground into flour and then stabilized by dry roasting method (Yaver, 2020).

Methods

Pasta Preparation

Durum wheat semolina (100%) pasta was prepared using 100:30 semolina:water ratio (w/v) according to Brennan and Tudorica (2007). The mixing, extrusion and cutting processes were carried out using a pilot pasta extruder (La Monferrina Dolly, Moncalieri, Italy) equipped with short cut pasta die. After that, shaped pasta samples were dried in a pilot-scale dryer (La Monferrina EC50, Moncalieri, Italy). In additive-free pasta containing lupin flour and RS_4, semolina was replaced with 15% lupin flour + 10% RS_4. To produce pasta with 15% lupin flour + 10% RS_4 and additives, vital gluten (at the amount of the diluted gluten), transglutaminase (0.5%) and vital gluten+transglutaminase were supplemented into pasta formulations. The same procedure applied for 100% semolina pasta was also employed for these samples.

Analytical Methods

The ash, fat, protein, total dietary fiber and RS contents of pasta samples were determined according to AACC method 08-01, AACC method 30-25, AACC method 46-30, AACC method 32-07 and AACC method 32-40, respectively (AACC, 1990; 2000).

Statistical Analysis

The statistical analysis was performed using TARIST 4.01 (Ege University, İzmir, Turkey) software.

RESULTS AND DISCUSSION

The ash content of pasta samples is shown in Figure 1. Utilization of lupin flour and RS_4 revealed greater ash content compared to 100% semolina pasta. This increase in ash content may be attributed to the high mineral content of lupin (Musco et al., 2017) and the high phosphorus content of cross-linked RS_4 (Xie et al., 2006). Pasta samples containing lupin flour debittered by ultrasound application (LFUA) had a similar ash content to pasta samples prepared with lupin flour debittered by traditional method (LFTM). The additives did not have a negative effect on the ash content of additive-free pasta containing lupin flour and RS_4.

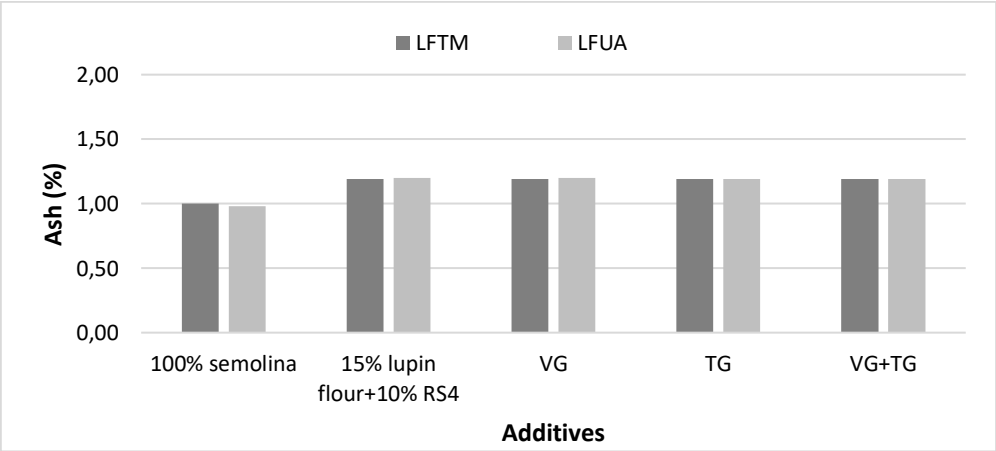

Figure 1. Ash Content of Pasta Samples
(LFTM: Lupin flour debittered by traditional method. LFUA: Lupin flour debittered by ultrasound application. RS4: Resistant starch type4. VG: Vital gluten. TG: Transglutaminase)

The fat content of pasta samples is presented in Figure 2. The lowest fat content was observed in 100% semolina pasta. Usage of 15% LFTM/LFUA + 10% RS_4 in pasta formulation resulted in considerably higher fat content. This could be due to the fact that lupin flour is rich in fat (Awad-Allah and Elkatry, 2013). On the other hand, vital gluten and/or transglutaminase supplementation did not change the fat content of pasta samples compared to additive-free pasta prepared with lupin flour and RS_4. Similar findings were reported by Rosa-Sibakov et al. (2016) with regard to the ash and fat content of faba bean flour pasta supplemented with transglutaminase.

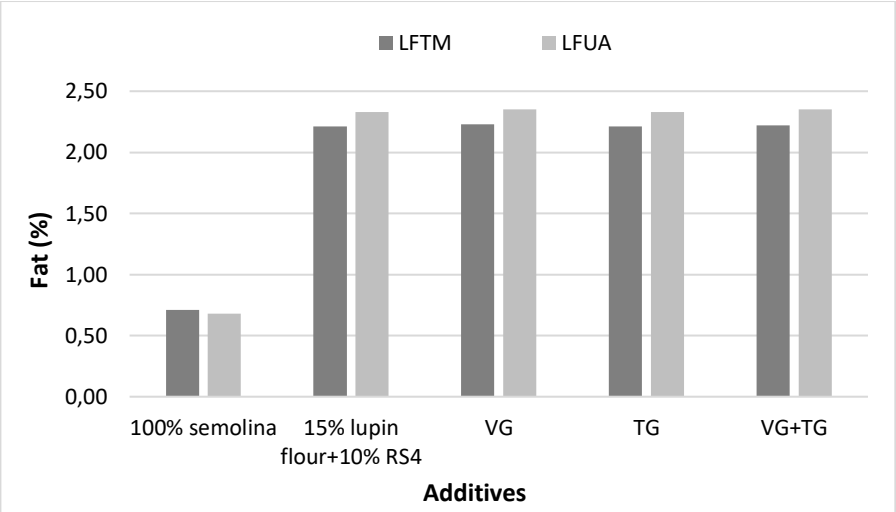

Figure 2. Fat Content of Pasta Samples
(LFTM: Lupin flour debittered by traditional method. LFUA: Lupin flour debittered by ultrasound application. RS4: Resistant starch type4. VG: Vital gluten. TG: Transglutaminase)

The protein content of the samples is given in Figure 3. The protein content of the pasta samples showed an increase with the addition of lupin flour and RS_4. This increase may be related to higher protein content of lupin flour than semolina (Jayasena et al., 2010; Padalino et al., 2014). The protein content of pasta samples containing LFTM was close to pasta samples containing LFUA. While supplementation of pasta samples with transglutaminase did not result in noteworthy differences in the protein content, the addition of vital gluten increased the protein content of samples.

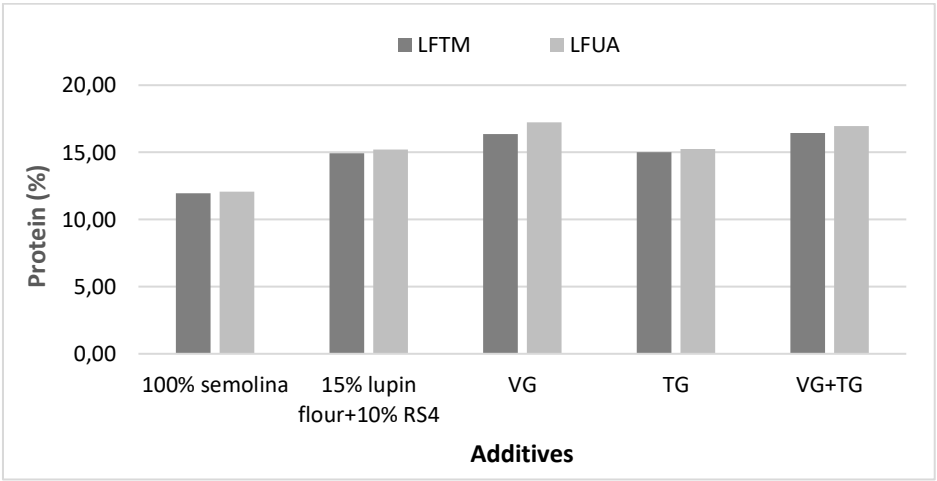

Figure 3. Protein Content of Pasta Samples
(LFTM: Lupin flour debittered by traditional method. LFUA: Lupin flour debittered by ultrasound application. RS4: Resistant starch type4. VG: Vital gluten. TG: Transglutaminase)

The total dietary fiber content of pasta samples is given in Figure 4. The semolina pasta had the lowest total dietary fiber content. A considerable amount of total dietary fiber content increment was obtained with the use of 15% lupin flour + 10% RS_4. Jayasena et al. (2008) reported that the total fiber content of noodles increased from 2.7 g/100 g to 5.2 g/100 g with the addition of 10% lupin flour. Bustos et al. (2011) found that pasta prepared with RS_4 had higher total dietary fiber content than control. The total dietary fiber content of the formulated pasta with additives was close to additive-free pasta containing lupin flour and RS_4.

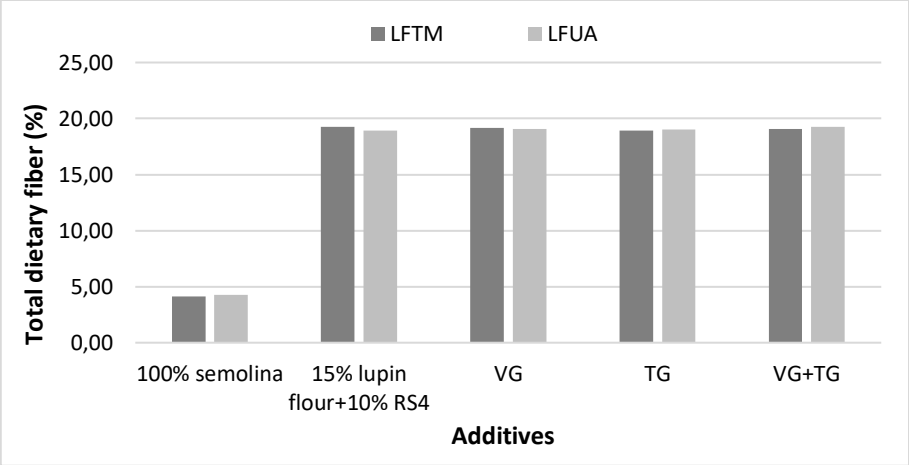

Figure 4. Total Dietary Fiber Content of Pasta Samples
(LFTM: Lupin flour debittered by traditional method. LFUA: Lupin flour debittered by ultrasound application. RS4: Resistant starch type4. VG: Vital gluten. TG: Transglutaminase)

The RS content of pasta samples is shown in Figure 5. Compared to 100% semolina pasta, the RS content of pasta samples increased with the addition of LFTM/LFUA and RS_4. This might be due to the presence of high purity RS_4. Similar results were reported by Aravind et al. (2013). Vital gluten and/or transglutaminase addition did not show a remarkable difference in terms of total dietary fiber content of pasta.

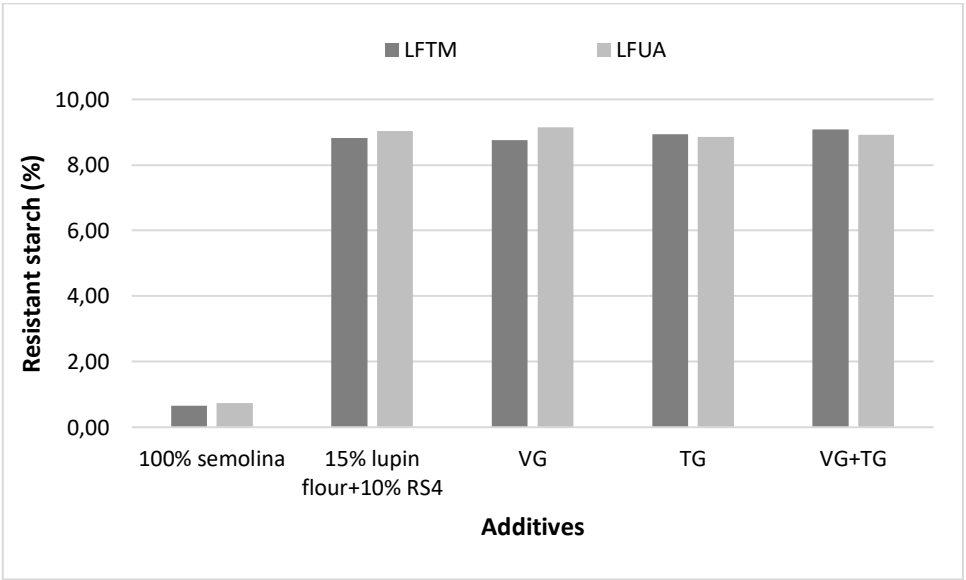

Figure 5. RS Content of Pasta Samples
(LFTM: Lupin flour debittered by traditional method. LFUA: Lupin flour debittered by ultrasound application. RS4: Resistant starch type4. VG: Vital gluten. TG: Transglutaminase)

CONCLUSION

Pasta samples containing 15% lupin flour + 10% RS_4 revealed higher ash, fat, protein, total dietary fiber and RS content compared to 100% semolina pasta. The chemical properties of pasta prepared with LFUA were close to pasta containing LFTM. The use of vital gluten, transglutaminase and vital gluten+transglutaminase did not show a negative effect on the chemical properties of pasta samples. Besides, supplementation of pasta samples with vital gluten increased the protein content compared to additive-free samples.

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➢ ***ORAL PRESENTATION***

Effects of Xylanase Enzyme Treated Wheat By-Products on Cake Batter Properties and Sensory Characteristics of Cake

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Abstract

In this study, wheat milling by-products (bran from debranning system, coarse bran, fine bran and wheat germ) were used in the preparation of cake at different ratios (0, 10, 20 and 30%) after being treated with xylanase enzyme. The pH and density of the cake batter, color values of cake crumb and sensory properties (taste, odor, pore structure, crumb wetness and overall acceptability) of cake samples were determined. The wheat by-product type, by-product ratio and xylanase addition factors were not significant ($p>0.05$) on the cake dough properties. Increasing the by-product ratio in cake formulation or xylanase treatment decreased the crumb L^* and b^* values of cake samples. It has been determined that the use of 30% wheat by-products in cake production reduced the overall acceptability value of all cake samples, and 10-20% usage ratio of wheat germ revealed similar or higher overall acceptability scores than the control cake.

Keywords: Wheat by-products, Xylanase, Bran, Wheat germ, Cake.

INTRODUCTION

Wheat is an important energy source in human diet which is mainly related to its carbohydrate content. After the milling process, about 25% of the whole wheat grain separates as by-products (bran and germ). Most of the wheat by-products, rich in protein, dietary fiber, vitamins, minerals and phytochemicals, are used as an energy component in the feed industry (Petrofsky, 2012).

Wheat bran consists of pericarp, testa, aleurone, germ and part of the starchy endosperm. It is a natural source of dietary fibers and is also rich in protein, vitamins, minerals and essential fatty acids. On the other hand, bran has a great amount of phytic acid. Nowadays, bran can be used as a source of dietary fiber in food formulations that can bring health benefits against most of the chronic diseases (Duta et al., 2018).

Wheat germ contains embryo and scutellum, represents 2-3% of the whole grain (Sakhare et al., 2017). The germ is an important source of essential amino acids, unsaturated fatty acids, dietary fibers, minerals, vitamins and phytochemicals (Marti et al., 2014). Besides, wheat germ has some antinutritional factors such as phytic acid, raffinose and wheat germ agglutinin, resulting in reduced digestibility of minerals and proteins (Nortey et al., 2007). Consumption of wheat germ can reduce the risk of coronary heart diseases, obesity, cholesterol and some cancer types (Priya, 2014).

Xylanase enzyme catalyzes the hydrolysis of arabinoxylans and changes their physicochemical properties by turning water insoluble arabinoxylans to soluble (Lebesi and Tzia, 2012). It has a positive effect on technologic properties such as baking, specific volume, shape and texture of bakery products (Courtin and Delcour, 2002; Harada et al., 2005).

The main aim of this study was to investigate the effects of the wheat milling by-products (bran obtained by debranning (BOD), coarse bran, fine bran and wheat germ) at different ratios (0, 10, 20 and 30%) on the cake batter properties and sensory quality of cakes.

MATERIALS AND METHODS

Materials

Wheat flour, sugar, whole chicken eggs, shortening, salt, skimmed milk powder, corn starch and baking powder were purchased from a local market in Konya, Turkey. Wheat milling by-products (BOD, coarse bran, fine bran and wheat germ) were obtained from a flour mill factory in Konya (Turkey). Xylanase enzyme was supplied from Kerry Inc. (Kildare, Ireland).

Methods

Pre-treatment of the By-products with Xylanase

For the preparation of xylanase-treated by-product mixtures, the by-products (35%) and distilled water (65%) were mixed. Then, the xylanase enzyme (0.5%) was added to mixture and rested at 40°C for 60 min. The treated by-products were added into the cake batter as described below (Lebesi and Tzia, 2012).

Cake Preparation

Control cake sample was prepared with 100 g wheat flour, 75 g sugar, 75 g whole eggs, 75 g shortening, 10 g corn starch, 5 g skimmed milk powder, 4.5 g baking powder and 0.5 g salt. Firstly, eggs and sugar were whisked in a mixer (Kenwood Kmix, Woking, UK) for 5 min. Following this, shortening was added to foam and mixed. After, the other ingredients were included in to the batter and mixed for 1 min. The cake batter was poured into an aluminum, fat coated pan and was baked in an oven (Beko BK-MF5, İstanbul, Turkey) at 160°C for 50 min. Cakes containing the by-products were produced by substituting wheat flour with xylanase-treated or non-treated BOD/coarse bran/fine bran/wheat germ at 10, 20 and 30% ratios. The procedure used was the same as the cake preparation procedure applied for the control.

Analytical methods

Farinograph (AACC 54-21) and extensograph (AACC 54-10) measurements of wheat flour were determined according to AACC (2002).

pH value of cake batter was determined using a digital pH meter ((WTW-315, GmbH, Weilheim, Germany). The density of batter was measured according to Mercan (1998).

Color *L**, *a** and *b** values were determined using a Konica Minolta CR 400 (Osaka, Japan). SI ($[a^{*2} + b^{*2}]^{1/2}$) and hue (a>0 and b>0, hue = arctan [b*/a*]; a<0 and b>0, hue = arctan [b*/a*] + 180°) values were calculated.

Sensory Analysis

Sensory properties (taste, odor, pore structure, crumb wetness and overall acceptability) of cake samples were evaluated by 25 panelists using a 1-7 scale.

Statistical Analysis

Statistical analysis of the results was performed using JMP (SAS Institute Inc., North Carolina, USA) software.

RESULTS AND DISCUSSION

Farinograph values of wheat flour are presented in Table 1. The water absorption, development time, stability and softening degree of wheat flour were 61.6%, 2.7 min, 18.7 min and 21 FU, respectively. Our results are in accordance with earlier study of Atalay et al. (2013).

Table 1. Farinograph Values of Wheat Flour

Water absorption (%)	61.6
Development time (min)	2.7
Stability (min)	18.7
Softening degree (FU)	21

Extensograph values of wheat flour are given in Table 2. The energy, resistance, extensibility and maximum resistance values of wheat flour were 136 cm^2, 604 BU, 134 mm and 780 BU, respectively.

Table 2. Extensograph Values (at 135th min) of Wheat Flour

Energy (cm^2)	136
Resistance (BU)	604
Extensibility (mm)	134
Maximum resistance (BU)	780

The pH and density values of cake samples are presented in Table 3. The pH values of samples changed between 6.72-6.87. The density values of cakes have varied in the range of 0.96 and 1.04 g/cm^3. According to Duncan's multiple comparison test results, the by-product types did not significantly ($p>0.05$) change the pH value of cakes (Table 4). The increase in the ratio of by-products resulted in statistically ($p>0.05$) similar pH values in cake samples. Xylanase enzyme treatment did not show a significant ($p>0.05$) difference in pH values of cakes. Fondroy et al. (1989) reported that pH values of cakes containing oat fiber changed between 6.62 and 6.66. They observed that there was no significant ($p>0.05$) difference in terms of pH value between cake

samples. None of the three sources of variance (by-product type, by-product ratio and xylanase addition) significantly ($p>0.05$) changed the density of the cakes.

Table 3. pH and Density Values of Cake Samples

Xylanase addition (%)	*By-product type*	*By-product ratio (%)*	pH	Density (g/cm^3)
0	BOD[1]	0	6.75±0.30	0.98±0.03
		10	6.75±0.34	1.00±0.01
		20	6.83±0.25	0.99±0.01
		30	6.86±0.30	1.00±0.07
	CB[2]	0	6.78±0.38	0.98±0.03
		10	6.78±0.38	0.99±0.01
		20	6.81±0.41	0.96±0.06
		30	6.82±0.44	1.03±0.04
	FB[3]	0	6.78±0.38	0.97±0.03
		10	6.78±0.33	0.96±0.01
		20	6.78±0.27	0.97±0.01
		30	6.80±0.40	1.03±0.04
	WG[4]	0	6.75±0.42	0.97±0.03
		10	6.75±0.38	0.97±0.07
		20	6.80±0.41	0.99±0.03
		30	6.83±0.34	0.99±0.04
0.5	BOD	0	6.74±0.31	0.98±0.03
		10	6.76±0.32	1.03±0.07
		20	6.81±0.35	1.04±0.10
		30	6.87±0.49	1.04±0.08
	CB	0	6.75±0.33	0.97±0.03
		10	6.73±0.24	0.96±0.01
		20	6.78±0.34	0.98±0.06
		30	6.80±0.47	1.00±0.14
	FB	0	6.73±0.31	0.97±0.03
		10	6.72±0.18	0.99±0.06
		20	6.73±0.40	1.00±0.07
		30	6.75±0.42	1.00±0.08
	WG	0	6.73±0.27	0.98±0.03
		10	6.73±0.30	0.97±0.04
		20	6.77±0.30	0.98±0.03
		30	6.85±0.43	0.99±0.07
Mean			6.78	0.99
Minimum			6.72	0.96
Maximum			6.87	1.04

[1] BOD: Bran obtained by debranning. [2] CB: Coarse bran. [3] FB: Fine bran. [4] WG: Wheat germ.

Table 4. Duncan's Multiple Comparison Test Results of pH and Density Values of Cake Samples[1]

Factor	n	pH	Density (g/cm^3)
By-product type			
BOD[2]	16	6.80±0.25^a	1.01±0.04^a
CB[3]	16	6.78±0.28^a	0.98±0.04^a
FB[4]	16	6.76±0.25^a	0.99±0.04^a
WG[5]	16	6.78±0.27^a	0.98±0.04^a
By-product ratio (%)			
0	16	6.75±0.25^a	0.98±0.02^a
10	16	6.75±0.23^a	0.98±0.04^a
20	16	6.79±0.25^a	0.99±0.05^a
30	16	6.82±0.31^a	1.01±0.05^a
Xylanase addition (%)			
0	32	6.79±0.26^a	0.98±0.03^a
0.5	32	6.77±0.26^a	0.99±0.05^a

[1] Means followed by the same letter within a column are not significantly ($P < 0.05$) different. Duncan's multiple comparison test according to three ways analysis of variance. Values are the average of triplicate measurements on the duplicate samples. [2] BOD: Bran obtained by debranning. [3] CB: Coarse bran. [4] FB: Fine bran. [5] WG: Wheat germ.

Crumb color (*L**, *a**, *b**, *SI* and *hue*) values of cake samples are shown in Figure 1. The increasing xylanase treated/non-treated by-products ratios from 0% to 30% notably decreased the *L** values, but increased *a** values of cakes. The natural dark colors of the by-products may have affected the color parameters of cakes. Compared to the control (0% of by-products), addition of non-treated BOD and fine bran decreased the *b** values of samples. The *b** value of cake containing 10% wheat germ was higher than the control. *Hue* values of cakes decreased as the by-product addition ratio increased.

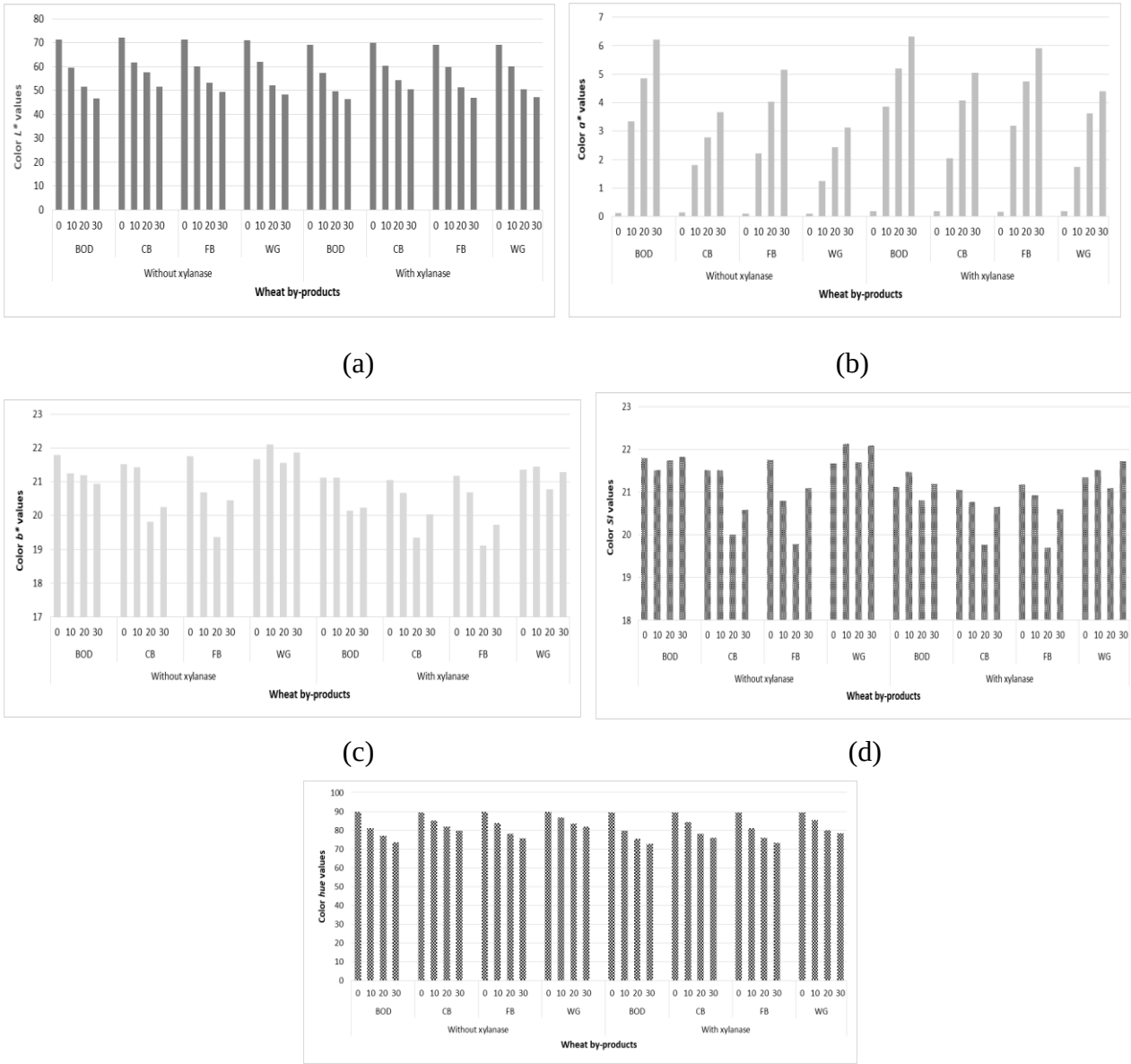

Figure 1. Crumb Color *L** (a), *a** (b), *b** (c), *SI* (d) and *Hue* (e) Values of the Cake Samples

The sensory scores of cake samples containing xylanase treated by-products are shown in Figure 2. Taste and odor scores of wheat germ cakes were higher than control sample. Usage of the by-products caused a negative effect on the pore structure parameter of cakes compared to control. Generally, crumb wetness values decreased with increasing ratios of the by-products. The highest overall acceptability score was obtained in cake containing 10% wheat germ.

CONCLUSION

The results of this study showed that the wheat by-product type, by-product ratio and xylanase addition had no significant ($p>0.05$) effects on the cake batter pH and density. While the addition of BOD revealed higher *a** values than the other by-products, cake samples containing wheat germ demonstrated greater *b** values. Cakes containing 10-20% wheat germ gave similar or higher overall acceptability scores than the control. High utilization ratios of by-products decreased overall acceptability scores of cakes.

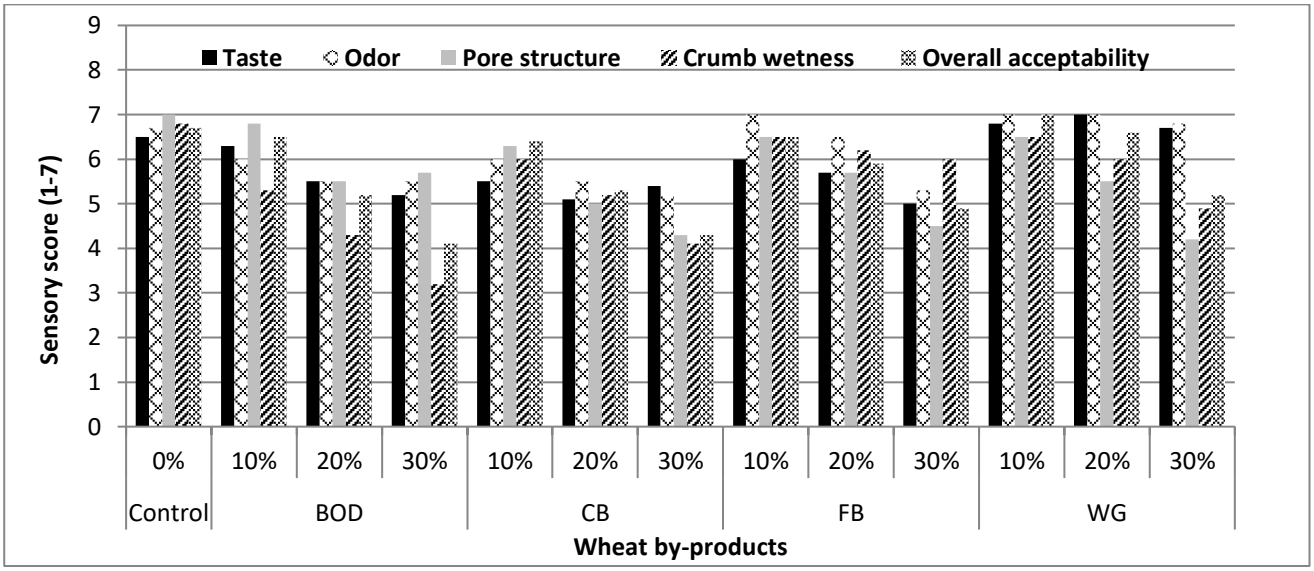

Figure 2. Sensory Scores of the Cake Samples

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➢ ***ORAL PRESENTATION***

Otlu peynirlerin mineral madde içeriği

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Özet
Otlu peynir, geleneksel peynir çeşitlerimiz arasında kendine has tat ve kokusu ile en nadide örneklerdendir. Otlu peynir, yaz aylarında yaylalarda otlatılan koyunların sütleri ve yine bu yaylalardan toplanan eşsiz otlarla birleştirilmesiyle üretilir. Özellikle geleneksel olarak üretilen Otlu peynir örneklerinin oldukça yüksek değerlerdeki kuru madde içeriği zengin besin içeriğini yansıtmaktadır. Beslenme açısından oldukça önemli olan mineral maddeler fizyolojik olarak devamlı tüketilmesi gereken bileşiklerdir. Otlu peynir, içerisinde sütten gelen minerallerin yanında üretim sırasında katılan otlar sayesinde oldukça zengin bir mineral madde kaynağı olmaktadır. Yapılan çalışmalar ile mineral madde içeriği hakkında fikir sahibi olduğumuz Otlu peynirler, aynı zamanda beslenme yönünden mineral madde ihtiyacımızı karşılamada önemli bir gıda olarak karşımıza çıkmaktadır.
Anahtar Kelimeler: Otlu peynir, mineral madde, beslenme.

Mineral content of herby (Otlu) cheeses

Abstract
Herby (Otlu) cheese is one of the rarest examples of our traditional cheese varieties with its unique taste and smell. Herby (Otlu) cheese is produced by combining the milk of sheep grazing in the highlands during the summer and the unique herbs collected from these highlands. Especially the high dry matter content of the traditional herb cheese samples reflects the rich nutritional content. Mineral substances, which are very important in terms of nutrition, are compounds that should be consumed continuously physiologically. In addition to the minerals coming from milk, the herby (Otlu) cheese increases the mineral substance content thanks to herbs added during production. Herby (Otlu) cheeses, which we have an idea about the mineral substance content with the studies carried out, also emerge as an important food in meeting our mineral substance needs in terms of nutrition.
Keywords: Herby cheese, mineral substance, nutrition

GİRİŞ

Otlu Peynir, ülkemizin doğu ve güneydoğu bölgelerinde yaygın olarak ve bilenen kadarıyla 200 yılı aşkın süredir üretilmekte ve tüketilmektedir (Coşkun ve Tunçtürk, 2000; Hayaloglu ve Fox, 2008). Otlu peynir, sıcak yaz aylarında yaylalarda otlatılan koyunların sütleri yine bu yaylalardan toplanan kimi endemik olan otlarla birleştirilerek üretilmektedir.

Otlu peynir oldukça tuzludur ve genellikle sarımsak ve kekik aromasına sahiptir (Coşkun ve Tunçtürk, 2000). Endüstriyel üretim ile ülkemizin hemen her bölgesinde ulaşılır hale gelmiştir. Ancak geleneksel yöntemlerle ve özellikle koyun sütünden üretilen Otlu peynirin sahip olduğu lezzet ve aroma kendisini vazgeçilmez bir gıda ürünü haline getirmiştir. Geleneksel olarak üretilen bu peynirlerin sahip oldukları üstün tat tüketicilerinin hemen her öğünde Otlu peynir görmek istemesinin en büyük nedenidir.

Otlu peynirlere tadını veren geleneksel üretim metotları içerisinde koyun sütü ile üretilmiş olmasının yanında peynire katılan otların büyük bir yeri vardır. Otlu peynir sahip olduğu sarımsak ve kekik aromasını bu otlardan almaktadır. Otlu peynir üretiminde en çok tercih edilen otlar *Liliaceae (Allium sp.), Apiaceae (Ferula sp., Anthriscus nemorosa) ve Lamiaceae (Thymus migricus)* ailelerine aittir (Ocak ve ark., 2015; Tarakci ve Akyuz, 2009).

Otlu Peynirlerin Beslenmedeki Önemi

Peynirler beslenmemizde önemli bir yer tutar. Bunun başlıca sebepleri yüksek değerli hayvansal protein ve yağ içeriğinin fazlalığıdır. Esansiyel amino asitleri bünyesinde barındırması, B grubu vitaminler, yağda çözünen vitaminler ve Ca ile P varlığı peynirleri vazgeçilmez bir gıdaya dönüştürmektedir.

Tarakçi ve ark.,(2004) Van piyasasından topladıkları 20 adet taze ve 20 adet olgun Otlu peynir örneği üzerinde yaptıkları çalışma ile Otlu peynirlerin besin içeriğine dair genel bilgiler edinmemizi sağlamışlardır (Tablo 1). Taze ve olgun peynir içerikleri doğal olarak farklılık göstermektedir. Ancak bilindiği gibi geleneksel peynirlerin genellikle olgun halde tüketilmesi tercih edilir. Olgun peynirler oldukça yüksek değerlerdeki kuru madde içeriğine sahiptir. Dolayısıyla daha fazla yağ ve daha fazla protein miktarı, zengin besin içeriğini beraberinde getirmektedir.

Tablo 1. Van il piyasasından temin edilen taze ve olgun Otlu peynirlere ait bazı özellikler (Tarakçi ve ark., 2004).

	Taze Peynir (N=20)		Olgun Peynir (N=20)	
Özellikler	Aralık	Ortalama	Aralık	Ortalama
Kuru madde (%)	40.04-56.15	45.80±4.458	50.54-66.05	55.41±4.454
Yağ (%)	14.50-24.50	17.83±2.715	18.50-31.50	24.37±3.697
Protein (%)	16.59-26.02	21.37±3.626	18.01-25.98	21.22±1.964
Tuz (%)	3.86-6.40	5.19±0.812	4.80-9.07	6.64±1.190
pH	4.90-5.96	5.52±0.275	4.01-5.40	4.55±0.314
Titrasyon asitliği (% Laktik asit)	0.27-0.71	0.48±0,124	0.82-2.35	1.84±0.374

Mineral maddeler

Doğada 90 çeşit element bulunmakta ancak 25 tanesi hücrelerde bulunmakta çünkü bunlar yaşam için elzemdir. Tüketicilerin gıdalardan elde ettiği temel elementler potasyum, sodyum, kalsiyum, magnezyum, klor, kükürt ve fosfordur (Saldamlı, 2007). Mineral maddelerin vücutta üstlendiği bazı önemli görevler;

- Hücrelerin ozmotik basınçlarının dengede kalmasını sağlamak,
- Metabolizma için asit ve baz dengesini sağlamak,
- Enzimlerin yapı ve çalışmalarında görev almak,
- Kemik ve diş yapısında yer almak,
- Kas ve sinir sisteminin uyarılması işlevinde bulunmak (Tayar vd., 2011).

Şekil 1. Van il piyasasında satışa sunulan Otlu peynir örnekleri.

İşte bu noktada otlu peynirlerin önemi ortaya çıkmaktadır. İklim şartları nedeniyle uzun süren kış şartları insanların taze otlara ve yeşil yapraklı bitkilere ulaşması mümkün olmamaktadır. Ancak taze olarak toplanıp peynire işlenen bu bitkiler kış dönemi boyunca Otlu peynir ile tüketilebilmektedir. Dolayısıyla bitkilerin sağlıklı beslenme üzerine olan etkileri görülmeye devam etmektedir.

Otlu Peynir Mineral Madde İçeriği İle İlgili Çalışmalar

Ocak ve Köse, (2015), Van piyasasında satılan Otlu peynirlerin mineral madde içeriğini tespit etmek amacıyla 26 adet Otlu peynir örneğini piyasadan temin etmiş ve kalsiyum, magnezyum, potasyum, çinko, mangan demir ve bakır içeriği belirlemişlerdir. Kuru yakma metodu ile hazırlanan örneklerin mineral içeriği Atomik Absorbsiyon Spektrofotometresi kullanılarak tespit edilmiştir. Peynir örneklerindeki Ca, Mg, K miktarlarını sırasıyla 268.7-678.7, 26.3-80.8, 84.6-163.2 mg / 100g, Zn, Mn, Fe ve Cu miktarlarının değişim aralığını ise sırasıyla 8.13-25.94, 0.38-2.23, 3.14-29.25, 0.29-2.60 mg / kg olarak belirlemişlerdir. Tespit edilen değerler arasındaki farklılıkları peynir üretiminde kullanılan sütün elde edildiği hayvanın cinsi ve beslenme şartlarına, üretimde farklı türlerde ve miktarlarda ot kullanımına ve farklı üretim kaynaklarının etkisine bağlamışlardır.

Yapılan bir başka çalışmada (Vural ve ark., 2008) Güneydoğu Anadolu bölgesinden toplanan 50 adet Otlu peynir örneği atomik adsorpsiyon spektrofotometresi ile analize tabi tutulmuştur. Elde edilen verilere göre kurşun, bakır, kobalt, nikel, krom, kadmiyum ve demir gibi eser metal oranları sırasıyla 1.5-10.7, 1.1-11.9, 0.8-2.1, 0.8-4.8, 1.9-8.7, 0.1-0.6 ve 5.0-119.8 µg / g aralığında tespit edilmiştir. Çalışmadan elde edilen sonuçlar literatür ile karşılaştırılmış, toplanan Otlu peynir örneklerinde kurşun, bakır, kobalt, nikel, krom ve kadmiyum seviyelerinin önemli derecede yüksek olduğu bildirilmiştir. Otlu peynir örneklerinin demir seviyeleri ise literatür değerleri içerisinde yer aldığı ifade edilmiştir.

Sağun ve ark., (2005) ürettikleri Otlu peynir örneklerinin 90 günlük depolama süresi boyunca mineral madde değişimini takip etmişlerdir. Olgunlaşma süresince peynirlerdeki sodyum miktarının artış gösterdiğini ($P < 0.05$), kalsiyum, magnezyum, çinko, demir, mangan, krom ve nikel miktarlarının azaldığı ($P < 0.05$) ve fosfor, bakır, kobalt ve kadmiyum miktarlarının ise önemli bir değişiklik göstermediğini ($P > 0.05$) belirlemişlerdir. 1 ve 90. günlere ait kalsiyum, fosfor, sodyum ve magnezyum değerleri sırasıyla 904.05-755.45, 457.40-438.70, 2628.5-3379.5, 32.75-25.35 mg / 100g seviyelerinde, çinko, bakır, demir, mangan, kobalt, krom, nikel ve kadmiyum değerlerini sırasıyla 35.08-33.63, 9.54-9.37, 27.94-25.41, 3.55-3.51, 0.05-0.07, 0.19-0.11, 0.19-0.17, 0.17-0.16 mg / kg seviyelerinde tespit edilmiştir.

Kılıçel ve ark., (2004), Otlu lor örneklerinin mineral madde ve ağır metal içeriğini sade lorlar ile karşılaştırdığı çalışmalarında, Van'da üretilen 30 adet Otlu lora ait mineral içerikleri: kalsiyum (Ca) 638.43±101.04 mg / 100g, sodyum (Na) 3212.96±218.97 mg / 100g, fosfor (P) 422.66±34.76 mg / 100g, magnezyum (Mg) 38.50±11.42 mg / 100g; çinko (Zn) 29.19±3.45 mg / kg, bakır (Cu) 8.18±1.32 mg / kg, demir (Fe) 74.77±13.54 mg / kg, mangan (Mn) 6.93±0.83 mg / kg, kobalt (Co) 0.29±0.13 mg / kg, krom (Cr) 0.25±0.15 mg / kg, nikel (Ni) 0.30±0.11 mg / kg ve kadmiyum (Cd) 0.20±0.08 mg / kg olarak belirlemiştir. Otlu lorların sade lorlara göre kül, tuz, kalsiyum, sodyum, demir, kobalt ve kadmiyum yönünden farklılık gösterdiği belirlenmiştir.

SONUÇ

Geleneksel gıdaların nadide parçalarından biri olan Otlu peynir, hayvansal protein ve yağ açısından zengin bir ürün olmasının yanında fizyolojik faaliyetler için elzem olan mineral maddeler bakımından da oldukça zengin olduğu anlaşılmaktadır. Miktar olarak fazla mineral madde içermenin yanında çeşitli mineral maddeler içerdiği yapılan çalışmalarla belirlenmiştir. Otlu peynirleri mineral madde yönünden diğer peynirlerden farklı kılan içeriğine katılan çeşitli otlardır. Hemen her öğünde tüketilen Otlu peynirin içerdiği hayvansal protein ve yağ ile birlikte sahip olduğu mineral maddeler ile çok besleyici bir ürün olduğu anlaşılmıştır.

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➢ ***ORAL PRESENTATION***

Otlu peynirlerin antioksidan ve antimikrobiyal özellikleri

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Özet
Geleneksel gıdalarımızdan Otlu peynir, içerdiği otlar ile binlerce peynir çeşidi arasından sıyrılarak nadide bir peynir olduğu göstermektedir. Otlu peynir, yaylalardan toplanan taze otlar ile burada otlayan koyunların sütü birleştirilerek üretilir. Otlu peynir üretiminde onlarca farklı ot kullanılmaktadır. Bu otlar sahip oldukları bazı özellikleri beraberinde peynire taşımaktadır. Bu özelliklerden en önemlileri de antioksidan ve antimikrobiyal aktivite özellikleridir. Otlu peynire işlenen otların bu özellikleri akademik çalışmalar ile tespit edilmiştir.
Anahtar Kelimeler: Otlu peynir, antioksidan, antimikrobiyal

Antioxidant and antimicrobial properties of herby (Otlu) cheeses

Abstract
Herby (Otlu) cheese, one of our traditional foods, stands out from thousands of cheese varieties and shows that it is a rare cheese. Herby (Otlu) cheese is produced by combining the fresh herbs collected from the highlands and the milk of the sheep grazing here. Dozens of different herbs are used in the production of herby (Otlu) cheese. These herbs carry some of their properties to cheese. The most important of these properties are antioxidant and antimicrobial activity properties. These properties of herbs processed on herby (Otlu) cheese have been determined by academic studies.
Keywords: Herby (Otlu) cheese, antioxidant, antimicrobial

GİRİŞ

Geleneksel gıdalar yüzyıllar içinde gelişen, toplumun en önemli kültür meyveleridir. Geleneksel gıdaların ortaya çıkma hikâyelerinde en önemli etken yaşam şartlarıdır. Bölgenin coğrafik özelliklerinin belirlediği yaşam şartları tüketilen gıdalara son şeklini vermektedir. Afrika'nın çöllerinde tüketilen geleneksel gıdalar ile tropik orman şartlarında gelişen gelenekler arasındaki farklar bu gerçekliğin yansımasıdır. Ülkemiz doğu bölgelerinin geleneksel gıdalarından biri Otlu peynirdir. Otlu peynir, sıcak yaz aylarında yaylalarda otlatılan koyunların sütleri ve yine bu yaylalardan toplanan kimi endemik olan otlar ile birleştirilerek üretilmektedir. Nitekim literatürde Otlu peynir, ülkemizin doğu ve güneydoğu bölgelerinde yaygın olarak ve bilenen kadarıyla 200 yılı aşkın süredir üretilmekte ve tüketilmekte olduğu bildirilmektedir (Coşkun ve Tunçtürk, 2000; Hayaloglu ve Fox, 2008).

Beslenme ilkeleri açısından önemli olan yeşil yapraklı sebzelerin tüketimi Doğu Anadolu illerinde oldukça düşük sevilerde kalmaktadır. Ancak yaz aylarında taze olarak toplanan ve Otlu peynir üretiminde kullanılan otlar uzun kış mevsimleri boyunca yüzyıllardır bölge halkının sağlıklı beslenmesinde etkili olmaktadır.

Otlu peynir üretiminde en çok tercih edilen otlar *Liliaceae (Allium sp.), Apiaceae (Ferula sp., Anthriscus nemorosa) ve Lamiaceae (Thymus migricus)* ailelerine aittir (Ocak ve ark., 2015; Tarakci ve Akyuz, 2009). Bu otların peynire katılması ile Otlu peynirlerin bazı fonksiyonel özellikler kazandığı görülmüştür. Yapılan çalışmalarda bu otların peynirlere antioksidan ve antimikrobiyal özellikler kazandırıldığı belirlenmiştir. Otlu peynir yapımında farklı bölgelerde bulunan birçok türe ve cinse ait otlar kullanılabilmektedir (Tablo 1) (Şekil 1).

Gıda bakımından antioksidanlar, oksijen ile reaksiyona girerek gıdalarda oksidatif bozulmayı önleyen veya geciktiren bileşiklerdir. Bu bileşikler oksidatif ve otooksidatif işlemlerin başlangıcında etki göstererek oksidasyonu ve buna bağlı olarak oluşan istenmeyen reaksiyon ürünlerinin (kötü koku ve lezzet)oluşumunu engelleyebilmektedir (Altuğ, 2001). Antimikrobiyal maddeler ise gıdalarda istenmeyen, ancak bir nedenle bulunabilen bakteri, küf ve mayaları, patojen olan veya olmayan her türlü mikroorganizmayı ortamdan yok etmek, çoğalma veya faaliyetini önlemek için kullanılan bileşiklerdir (Demirci ve Alparslan, 1994). Günümüzde doğal antioksidan ve doğal antimikrobiyal maddelere olan ilgi her geçen gün artmıştır.

Tablo 1. Van Otlu peynirine katılan bazı otların Latince ve yöresel isimleri ile peynirde kullanılan kısımları (Ocak ve Köse, 2015).

Latince Adı	Familyası	Yöresel Adı	Kullanılan Kısmı
Allium sp.	*Liliaceae*	Sirmo	Yaprak ve sap
Chaerophyllum macropodum Boiss	*Apiaceae*	Mendo, Mendi	Yaprak ve sap
Prongos ferulaceae (L.) Lindl.	*Apiaceae*	Heliz	Yaprak ve sap
Ferula rigidula DC	*Apiaceae*	Siyabu, Siyabo	Yaprak ve sap
Anethum graveolens L.	*Apiaceae*	Dere otu	Yaprak ve sap
Mentha spicata	*Lamiaceae*	Nane	Yaprak
Thymus migricus	*Lamiaceae*	Kekik	Yaprak

Yapılan çalışmalar

Sagun ve ark., (2006) gerçekleştirdikleri çalışmada Otlu peynir üretiminde kullanılan bazı otlara (*Allium vineale, Chaerophyllum macropodum* ve *Prongos ferulaceae*) ait ekstraktların *Listeria monocytogenes serovars* 1/2b, 4b, 4ab türleri üzerindeki antimikrobiyal aktivitelerini araştırmışlardır. Metanol, etanol ve n-hegzan ekstraktların (metanol ekstaktı daha yüksek olmak üzere) mikroorganizma üzerinde farklı düzeylerde antimikrobiyal etki sergilediği belirlenmiştir. Su ekstaraktının antimikrobiyal etki göstermediği görülmüştür. Bu otlara ait ekstreaktlara karşı direnci en düşük olan *Listeria monocytogenes* 4ab türüdür. Çalışılan otlar arasından *Allium vineale, Listeria monocytogenes* türlerine karşı en güçlü antimikrobiyal etkiyi sergilemiştir.

Gerçekleştirilen bir diğer çalışmada (Çelik ve ark., 2008) *Allium sp., Chaerophyllum macropodum, Prongos ferulaceae, Ferula rigidula DC* ve *Thymus* türlerine ait 16 adet ot üzerinde antioksidan aktivite değerleri incelenmiştir. Uygulanan farklı analiz metodlarından (CUPRAC, ABTS, FRAP ve Folin metodu) en yüksek antioksidan kapasite değerleri Folin metodu ile tespit edildi. CUPRAC metodu ile tespit edilen antioksidan değerleri (troloks eşdeğeri olarak) thymus sp. > chaerophyllum sp. > allium sp. > prangos sp. ≥ ferula sp., Folin bulgularına göre ise thymus sp. > allium sp. > chaerophyllum sp. > ferula sp. ≥ prangos sp. şeklinde tespit edilmiştir. *Thymus sp.* Türleri her iki analiz metodunda en yüksek değerleri ortaya koymuştur.

Dagdelen ve ark., (2014) Otlu peynir üretiminde daha fazla tercih edilen otlardan Sirmo (*Allium schoenoprasum* [*Liliaceae*]), Mendi (*Chaerophyllum macropodum* [*Apiaceae*]), Siyabo (*Silene vulgaris* [*Caryophyllaceae*]), Yarpuz (*Mentha spicata* [*Lamiaceae*]) ve Heliz'in (*Prangos ferulacea* [*Apiaceae*]) antioksidan ve antimikrobiyal kapasitelerini araştırmışlardır. İncelenen otlardan en yüksek DPPH ve ABTS aktivitesini Heliz otunun metanol ekstraktının gösterdiği, peynire ot katılmasının da örneklerin antioksidan potansiyelini arttırdığını ortaya koymuşlar. Otlardan kaynaklanan antimikrobiyal etkinin geniş bir spektrumda olmadığı, gram-pozitif bakterilerin gelişiminin otların antimikrobiyal etkisiyle inhibe edildiği tespit etmişlerdir.

Köse ve Ocak (2018) tarafından, Otlu peynirde kullanılan Sirmo, Mendi ve Siyabo otlarının taze ve salamura hallerinin antioksidan ve antibakteriyel özellikleri incelenmiştir. Otların metanol, etanol ve aseton ekstraktları *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* ve *Yersinia enterocolitica* üzerinde antibakteriyel etki gösterdiği belirlenmiştir. Otların taze hallerinin Toplam Fenolik bileşikler, DPPH ve ABTS değerleri, salamura hallerinden elde edilen değerlerden daha yüksek sonuçlar verdiğini tespit etmişlerdir. Fonolik bileşikler, antioksidan ve antibakteriyel etkilerin metanol ve etanol ekstraktlarında asetona göre çok daha yüksek bulunduğu bildirilmiştir. Sirmo, Mendi ve Siyabo ekstraktlarının gıda işletmelerinde doğal antimikrobiyaller ve antioksidanlar olarak kullanılabileceği ifade edilmiştir.

SONUÇ

Geleneksel gıdalar üzerinde yapılan çalışmalar ile birçok fonksiyonel özellik ortaya çıkarılmaktadır. Otlu peynirin diğer peynir çeşitlerine göre farkı üretiminde kullanılan otlardır. Bu otların peynire fonksiyonel özellikler katmaktadır. İncelenen çalışmalarda antioksidan ve antimikrobiyal etkisi belirlenen otların, peynire işlendiğinde bu özellikleri peynire kazandırmaktadır. Peynir ortamında gerçekleştirilecek çalışmalar ile daha doğru sonuçlara ulaşılabilir. Otlu peynir üzerinde yapılacak yeni çalışmalar ile farklı özelliklerin gün yüzüne çıkacağı düşünülmektedir.

Şekil 1. Satışa sunulan Otlu peynir örnekleri.

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➢ *ORAL PRESENTATION*

***Liquidambar orientalis* var. *orientalis* ve *Liquidambar orientalis* var. *integribola* yapraklarının çeşitli ekstrelerinin üreaz enzim inhibisyon aktiviteleri**

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Özet

Türkiye zengin bir bitki florasına sahiptir ve bu floranın önemli bir kısmını tıbbi bitkiler oluşturmaktadır. Ülkemizin tıbbi doğal kaynaklarından olan ve 65 milyon yıldır Anadolu'da varlığını sürdüren ve geleneksel tıpta yaygın olarak kullanılan endemik sığla ağacı, Hamamelidaceae (Altingiaceae) familyasının bir üyesi olan Liquidambar cinsinin bir türüdür. *Liquidambar orientalis*, Türkiye'nin Güneybatısında doğal olarak yetişmekte olup *L. orientalis* var. *orientalis* ve *L. orientalis* var. *integribola* ve *Liquidambar orientalis* var. *suber* adlarıyla 3 varyetesi bulunmaktadır. Binlerce yıldır, Güneybatı Anadolu'da sığla reçinesi ve yaprakları halk arasında astım, bronşit gibi üst solunum yolu hastalıklarında, reflü, gastrit ve ülser gibi mide rahatsızlıklarında, yanık ve yara tedavisinde, mantar ve diğer deri hastalıklarının tedavisinde ve bağırsak rahatsızlıklarında kullanılmaktadır. Bu çalışmada, *Liquidambar orientalis* var. *orientalis* ve *L. orientalis* var. *integriloba* yapraklarının sırasıyla hekzan, etilasetat, metanol ve su ekstrelerinin üreaz enzim inhibisyon aktiviteleri araştırıldı. 200 µg/ml Konsantrasyonlar dikkate alındığında; her iki varyetenin yapraklarının metanol ekstreleri (sırasıyla; %63,31± 0,72 ve %65,92±0,93) diğer ekstreler arasında daha yüksek üreaz enzim inhibisyon aktivitesi göstermektedir

Anahtar Kelimeler: *Liquidambar orientalis,* sığla, üreaz enzim inhibisyonu, ülser

Urease enzyme inhibition activities of various extracts of *Liquidambar orientalis* var. *orientalis* and *Liquidambar orientalis* var. *integribola* leaves

Abstract

Turkey has a rich flora and flora constitutes an important part of medicinal plants. The endemic sweetgum tree, which is one of the medical natural resources of our country and has existed in Anatolia for 65 million years and is widely used in traditional medicine, is a species of the *Liquidambar* genus, a member of the Hamamelidaceae (Altingiaceae) family. *Liquidambar orientalis*, is naturally grown in Turkey's southwest. There are 3 different varieties (*L. orientalis* var. *orientalis, L. orientalis* var. *integribola* and *L. orientalis* var. *suber).* For thousands of years, resin and its leaves of *L. orientalis* have been used by the local people, in upper respiratory tract diseases such as asthma, bronchitis, stomach ailments such as reflux, gastritis and ulcers, in the treatment of burns and wounds, in the treatment of fungal and other skin diseases, and intestinal disorders. The urease enzyme inhibition activities of the hexane, ethylacetate, methanol and water extracts of, *Liquidambar orientalis* var. *orientalis* and *L. orientalis* var. *integriloba* leaves, respectively, were investigated. Methanol extracts of leaves of both varieties (63.31% ± 0.72 and 65.92 ± 0.93%, respectively at 200 µg/ml concentrations) show higher urease enzyme inhibition activity among other extracts.

Keywords: *Liquidambar orientalis,* sweetgum, urease enzyme inhibition, ulcer

GİRİŞ

Türkiye zengin bir bitki florasına sahiptir ve bu floranın önemli bir kısmını tıbbi bitkiler oluşturur (Baytop, 1999). 65 milyon yıldır varlığını sürdürmekte olan ve geleneksel tıpta kullanılan sığla ağacı, Hamamelidaceae

(Altingiaceae) familyasının bir üyesi olan Liquidambar cinsinin bir türüdür. *Liquidambar orientalis* (LO), Türkiye'nin güney batısında yetişen endemik bir türdür (Aydıngöz ve Bulut, 2014). Türkiye'de *L. orientalis* türüne ait *L. orientalis* var. *orientalis*, *L. orientalis* var. *integribola* ve *L. orientalis* var. *suber* olmak üzere 3 farklı varyete mevcuttur (Duru ve ark., 2002; Karadeniz, 2011). *Liquidambar orientalis* Türkiye'de halk arasında "Sığla ağacı" veya "Günlük ağacı" olarak bilinir. *L. orientalis*, tıbbi ve kozmetik özelliklere sahip olduğu bilinen çok yıllık bir bitkidir ve Akdeniz bölgesinde fitoterapide yaygın olarak kullanılır. *L. orientalis*'in gövdesini zedeleyerek üretilen reçine (storax) iyi bir antiseptik özelliğe sahiptir (Fernandez, 2005). Buna ilaveten Anadolu'da halk arasında parazit düşürücü, balgam söktürücü ve bazı cilt hastalıklarının tedavisi için kullanılır. Storax kozmetikte geniş bir uygulama alanına sahiptir (Hafizoğlu, 1982). *Liquidambar* türlerinin reçineleri, Geleneksel Çin Tıbbında tedavide kullanılmıştır. Çinliler halk arasında bu bitkinin yapraklarını hemostazı tedavi etmek için, kabuklarını ishali önlemek için, meyvelerini karaciğeri koruyucu olarak ve köklerini de enflamasyon, diyare ve hazımsızlık tedavisinde kullanmaktadırlar (Anonim, 1999). *Liquidambar orientalis* var orientalis yapraklarının etanol ekstresinin in vitro olarak yüksek antioksidan aktiviteye sahip olduğunu ve kolay, erişilebilir bir doğal antioksidan kaynağı olabileceği rapor edilmiştir (Saraç vd., 2014). Karbon tetraklorür ile karaciğeri hasarlanmış sıçanlara, *Liquidambar styraciflua* ağacının yapraklarının metanol ekstresi uygulanmış ve in vivo olarak hepatoprotektif ve antioksidan aktiviteleri araştırılmış ve *L. styraciflua* yapraklarının metanol ekstresinden yedi fenolik bileşik elde edilmiştir (Konno ve ark., 1988; Eid ve ark., 2015). Benzer şekilde L. orientalis reçinesi üzerine yapılan bir araştırmada, reçine kullanılan deneklerdeki HDSB seviyelerinin CCl4 grubuna kıyasla belirgin azalmalara neden olduğu ve reçine katılan diyetin CCl4 ile indüklenen MDA ve ADS'yi kontrol altına almasını sağladığı rapor edilmiştir (Süzek vd., 2016).

MATERYAL VE METOD

Bu çalışmaya konu olan *Liquidambar orientalis* var. *orientalis* ve *Liquidambar orientalis* var. *integriloba* yaprakları, Muğla Köyceğiz-Marmaris sığla orman sahalarında Muğla Orman Bölge Müdürlüğü'nün kontrolünde toplandı. *Liquidambar orientalis*'in her iki varyetesinin gölgede kurutulan yaprakları toz haline getirildi. Her bir materyalin kuru miktarının 5 katı kadar çözücü ile oda şartlarında sırasıyla n-hekzan, etil asetat, metanol ve sıcak su ile ekstraksiyonları yapıldı. Böylece her bir drogun artan polaritelerdeki çözücülerle yaptığımız ekstraksiyonlar sonucunda toplam 4 farklı ekstre (n-hekzan, etil asetat, metanol ve su ekstreleri) elde edildi.

Üreaz Enzim İnhibisyon Aktivite Yöntemi

Farklı konsantrasyonlardaki ekstrelerin üreaz enzim inhibisyon aktivitesi indofenol metodu kullanılarak yapıldı. Substrat olarak üre kullanıldı. Reaksiyon sonucunda oluşan amonyağın ölçümü spektroskopik olarak belirlendi (Weatherburn, 1967). 96 kuyucuklu mikroplakanın her bir kuyucuğuna farklı konsantrasyonlardaki örneklerden 10 µL, üreaz enzimi çözeltisinden 25 µL ve substrat olarak kullanılan üreden ise 50 µL ilave edildi. Kontrol olarak 10 µL saf su kullanıldı. Hazırlanan mikroplaka, 30°C'de 15 dk boyunca inkübasyona bırakıldı. Ardından 45 µL fenol ve sodyumnitropurissit içeren fenol reaktifinden; 70 µL sodyumhidroksit ve sodyumhipokloröz içeren alkali reaktifinden eklendi. Plate 50 dakika inkubasyona bırakıldıktan sonra 630 nm'de absorbansı ölçüldü.

BULGULAR ve TARTIŞMA

L. orientalis 'in her iki varyetesinin yapraklarının üzerine çalıştığımız ekstrelerinin üreaz enzim inhibisyon aktiviteleri incelendiğinde; *L. orientalis* var *orientalis* ve *L. orientalis* v*ar integriloba* 'nın yapraklarının metanol ekstrelerinin 200µg/ml konsantrasyonda, sırasıyla; %63,31± 0,72 ve %65,92±0,93 inhibisyon gösterdiği, bunu etil asetat ekstresinin (200µg/ml konsantrasyonda sırasıyla; %33,98±0.55 ve % 35,75±1.21) takip ettiği Tablo 1 den anlaşılmaktadır. Elde ettiğimiz verilere göre, her iki varyetenin yapraklarının hekzan ve su ekstrelerinin üreaz inhibisyon aktivitesinin düşük olduğu görüldü.

SONUÇ

Sonuçlar olarak *L. orientalis* var *orientalis* ve *L. orientalis* v*ar integriloba* yapraklarının 4 farklı ekstrelerinin üreaz enzim inhibisyonları karşılaştığında her iki varyetenin yaklaşık olarak benzer sonuçlar gösterdiği belirlendi. Standart olarak kullanılan tiyoüre ile karşılaştırıldığında; 200µg/ml konsantrasyonda her iki metanol ekstresinin 50µg/ml tiyoüre ile yarıştığı görülmektedir. Bu sonuç *L. orientalis* yapraklarının metanol ekstresinde doğal üreaz inhibitörlerinin bulunma olasılığını ve bu nedenle de metanol ekstresinin bileşenlerinin üreaz enzim inhibisyon aktivite kontrollü araştırması gerektiği kanaatini oluşturmuştur. Ayrıca,

L. orientalis'in çeşitli droglarının ülser başta olmak üzere gastrointestinal rahatsızlıklarda halk arasında binlerce yıldır kullanılmasının haklı gerekçelerden birisi olduğunu da göstermektedir.

Tablo 1. *L. orientalis* var *orientalis* ve *L. orientalis* var *integriloba* 'nın yapraklarının çeşitli ekstrelerinin üreaz enzim inhibisyonu aktivitesi (% inhibisyon)

Bitki Türü	Ekstre	25µg/ml	50µg/ml	100µg/ml	200µg/ml
L. orientalis Var orientalis	Hekzan	-	6.89±0.24	13.80±0.13	24.55±0.30
	Etil asetat	-	10.47±0.97	19.05±0.88	33.98±0.55
	Metanol	**18.79±0.52**	**34.80±0.96**	**51.02±1.09**	**63.31±0.72**
	Su	-	9.60±1.11	15.82±0.34	26.63±0.21
L. orientalis Var integriloba	Hekzan	-	8.91±0.35	14.65±0.21	23.87±0.45
	Etil asetat	6.03±0.16	11.56±0.71	20.81±0.70	35.75±1.21
	Metanol	**21.90±0.40**	**36.77±0.56**	**52.41±0.80**	**65.92±0.93**
	Su	-	9.10±0.56	14.79±0.45	25.18±1.15
Standart	Tiyoüre	51,19±0,19	63,05±1,21	72,32±0,75	83,56±1,05

TEŞEKKÜR

Bu çalışma TÜBİTAK 118Z417 nolu proje ile desteklenmiştir. Desteklerinden dolayı TUBİTAK'a teşekkür ederiz.

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➢ *ORAL PRESENTATION*

Türk sığla reçinesinin çeşitli ekstrelerinin antiinflamatuar aktivitesi

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Özet

Halk arasında "Günlük ve Sığla ağacı" olarak bilinen *Liquidambar orientalis* Mill. ülkemizin endemik tıbbi bitki türlerindendir ve geleneksel tıpta binlerce yıldır yaygın olarak kullanılmaktadır. Sığla ağacı jeolojik dönemlerden kalmış olup reçine veren ve reçinesinin tıbbi özelliklerinden dolayı Fenikeliler zamanından beri ticarete konu olan tıbbi bir bitkidir. Halk arasında sığla yağı olarak bilinen sığla reçinesinde; sinnamik asit, stacin, styrol, stresinol ve styroganin gibi maddeleri içermesiyle ilaç, parfümeri, sabun ve çeşitli kimya endüstrilerinde kullanılmaktadır. Tıbbi bitkiler antik çağlardan beri bitkilerin çeşitli drogları antiinflamatuar (iltihap, ağrı, yaralanmalara karşı vücudun gösterdiği tepki) etkiden dolayı kullanılmaktadır. Sığla reçinesi halk arasında uzun yıllardır antiinflamatuar etkisinin yanısıra antiseptic, parazit söktürücü, yara iyileştirici ve ülsere karşı kullanılmaktadır. Bu çalışmada, geleneksel olarak inflamasyon gidermek amacıyla kullanılan sığla reçinesinin antiinflamatuar etkisi siklooksijenaz-1 (COX-1) ve siklooksijenaz-2 (COX-2) enzimlerine karşı *in vitro* koşullarda araştırıldı. Reçineden elde edilen 4 farklı ekstrenin antiinflamatuar etkileri değerlendirildiğinde; Etil asetat ekstresinin 200 µg/mL konsantrasyonda hem COX-1 (% 61,14) hem de COX-2 (%38,16) enzimleri bakımından diğer ekstrelerden daha yüksek inhibisyon gösterdiği belirlendi.

Anahtar Kelimeler: *Liquidambar orientalis,* reçine, antiinflamatuar, COX-1, COX-2

Anti-inflammatory activity of various extracts of Turkish sweetgum

Abstract

Liquidambar orientalis Mill, popularly known as "Storax and Sweetgum Tree", is one of the endemic medicinal plant species of our country and has been widely used in traditional medicine for thousands of years. The sweetgum tree is a medicinal plant that has remained from geological times and has been traded since the time of the Phoenicians because of the medicinal properties of its resin. Sweetgum resin, popularly known as sweetgum oil, is used in pharmaceutical, perfumery, soap and various chemical industries as it contains substances such as cinnamic acid, stacin, styrol, stressinol and styroganin.. Medicinal plants have been used since ancient times for their anti-inflammatory effects (inflammation, pain, body's reaction to injuries). Sweetgum resin has been used by the local people for many years, as well as its anti-inflammatory effect, as an antiseptic, parasite remover, wound healing and ulcer. In this study, the anti-inflammatory effect of sweetgum resin, was investigated against COX-1 and COX-2 enzymes *in vitro* conditions. It was determined that ethylacetate extract showed higher inhibition than other extracts in terms of COX-1 (61.14%) and COX-2 (38.16%) enzymes at 200 µg / mL concentration.

Keywords: *Liquidambar orientalis*, resin, anti-inflammatory, COX-1, COX-2

GİRİŞ

Halk arasında Günlük/Sığla ağacı olarak bilinen *Liquidambar orientalis* Mill. ülkemizin endemik tıbbi bitki türlerindendir. 65 milyon yıldır Anadolu'da varlığını sürdüren ve geleneksel tıpta yaygın olarak kullanılan sığla ağacı, *Hamamelidaceae* (*Altingiaceae*) familyasının bir üyesi olan *Liquidambar* cinsinin bir türüdür. Türkiye'de *Liquidambar orientalis* türüne ait *Liquidambar orientalis* var. orientalis, *Liquidambar* orientalis var. integribola ve *Liquidambar orientalis* var. *suber* olmak üzere 3 farklı varyete mevcuttur (Duru ve ark., 2002; Karadeniz, 2011). *Liquidambar*, Latince *liquidus*, Arapça *amber* sözcüklerinin birleşmesinden meydana gelmiştir ve güzel kokulu sıvı demektir (Önal ve Özer 1985). Sığla ağacı; Amber ağacı, günlük ağacı, buhur ağacı, Mia pelesengi, Miai sail, Revgani suğla olarak da adlandırılmaktadır. *L. orientalis*, ekolojik ve biyocoğrafik öneme sahip olduğu kadar, sığla yağı (Styrax storax, Styrax liquids, Orientalis sweet gum, Levant styrax) adı verilen bir balzamın elde edilmesi nedeniyle ekonomik açıdan da çok önemli bir türdür. Sığla yağı ülkemizde bulunan *L. orientalis*'in dışında sadece Orta Amerika'da bulunan *L. styraciflua*'dan elde edilmektedir. Amerikan storax (Sweet gum, Red gum, Styrax Americanus, White Peru Balsam) olarak bilinen bu balzamın üretimi Honduras ve Guatemala'da yapılmaktadır (Acar ve ark., 1993; Aydıngöz ve Bulut,2014). Sığla yağı çok eski zamanlardan beri tanınır. Eski Mısırlılar sığla yağını mumyaların hazırlanmasında kullanmışlardır. Kleopatranın güzellik iksiri olarak da bilinen sığla yağı, Hipokrates (MÖ 460-377) döneminden başlayarak ilaç olarak kullanıldığı bilinmektedir. Sığla balzamının yöre halkı tarafından çok eski zamanlardan günümüze kadar ve günümüzde de tıbbi tedavi başta olmak üzere değişik amaçlar için kullanıldığı bilinmektedir. Batık Fenike gemilerinden çıkan içinde sığla balzamı bulunan amorflardan dolayı eskiden Akdeniz'de ticaretinin yapıldığı düşünülmektedir. Halk arasında sığla balzamı, balgam söktürücü, astım, bronşit ve akciğer hastalıklarında kullanılmaktadır (Top ve ark.,2007; Fıçıoğlu, 1988). Sığla balzamı (reçinesi) halk arasında yatıştırıcı ve analjezik özelliği olduğuna inanıldığından dolayı romatizma ağrılarını azaltmakta kullanılmıştır. Yine parazit kovucu özelliğinden dolayı mantar, uyuz gibi deri hastalıklarının tedavisinde ve özellikle inflamasyon giderici olarak da kullanılmıştır. Antibakteriyel, antiseptik etkisinden dolayı yaraların iyileşmesinde pomat olarak kullanılmış, dişetlerini güçlendirmek amacıyla ağızda çiğnenmiştir. Mide ülseri başta olmak üzere, mide hastalıklarında şekerle ya da balla karıştırılarak kullanılmıştır (Aureli ve ark., 1992, Baytop, 1984). Günümüzde sığla yağı parfüm sanayisinde sabitleyici olarak da kullanılır (Aureli ve ark., 1992; Gürbüz ve ark., 2013; Aydıngöz ve Bulut,2014).

MATERYAL VE METOD

Bu çalışmaya konu olan sığla reçinesi Muğla Köyceğiz-Marmaris sığla orman sahalarında Muğla Orman Bölge Müdürlüğü'nün kontrolünde toplandı. Reçinenin, oda şartlarında sırasıyla n-hekzan, etil asetat, metanol ve sıcak su ile ekstraksiyonları yapıldı. Böylece reçineden artan polaritelerdeki çözücülerle yaptığımız ekstraksiyonlar sonucunda toplam 4 ekstre (n-hekzan, etil asetat, metanol ve su ekstreleri) elde edildi. Ekstrelerin anti-inflamatuar aktiviteleri *in vitro* olarak test edildi.

Anti-inflamatuar Aktivitenin Belirlenmesi

Elde edilen *n*-Hekzan, etil asetat, metanol ve su ile ekstreleri sırası ile dimetilsülfoksit ve saf su ile çözüldükten sonra anti-inflamatuar aktivite testleri Muğla Sıtkı Koçman Üniversitesi, Fen Fakültesi, Kimya Bölümü, Organik Kimya Araştırma Laboratuvarında yapıldı. Drogların anti-inflamatuar aktiviteleri, COX-1 (Cyclooxygenase-1) ve COX-2 (Cyclooxygenase-2) enzim inhibisyon aktivite yöntemlerine göre yapıldı. Anti-inflamatuar aktivite testi için, kolorimetrik COX (ovine) inhibitor deney kiti (Cayman, No. 760111) kullanılarak spektroskopik olarak ölçüm yapıldı. Bu çalışmada ibuprofen pozitif kontrol olarak kullanıldı. Örnekler DMSO, distile su veya etanol ile çözülerek farklı konsantrasyonlarda çözeltiler hazırlandı ve kuyucuklara her birinden 10 µL eklendi. Daha sonra 20 µL kolorimetrik test substratı çözeltisi TMPD'den eklendi ve üzerine 20 µl araşidonik asit ilave edildi. Hazırlanan mikroplaka, 5 dakika boyunca oda koşullarında sürekli olarak karıştırılarak inkübe edildi. İnkübasyonun sonunda mikroplakanın 590 nm'de absorbansı ölçüldü (Ochieng ve ark., 2017).

BULGULAR ve TARTIŞMA

Üzerine çalıştığımız ekstrelerin anti-inflamatuar aktivite sonuçları Tablo 1'de verilmektedir. Tablo 1 incelendiğinde; sığla reçinesinin etil asetat ekstresi 200 µg/mL konsantrasyonda hem COX- 1 (%61,14) hem de COX-2 (%38,16) enzimi bakımından yüksek inhibisyon gösterdiği belirlendi. Reçinenin hekzan ekstresi ise 200 µg/mL konsantrasyonda COX-1 (%47,92) enzimine karşı metanol ekstresinin ardından ikinci sırada aktivite göstermektedir.

Tablo 1. Sığla reçinesinin çeşitli ekstrelerinin anti-inflamatuar aktiviteleri

Bitkisel Materyal	**Ekstreler**	**COX-1 (200 µg/mL konsantrasyonda % inhibisyon)**	**COX-2 (200 µg/mL konsantrasyonda % inhibisyon)**
Reçine	Hekzan	47.92	25.09
	Etilasetat	**61.14**	**38.16**
	Metanol	38.19	21.55
	Su	36,33	20,66

SONUÇ

Sonuçlar olarak sığla reçinesinin etil asetat ekstresi diğer ekstrelere göre hem COX-1 hem de COX- 2 enzimi bakımından yüksek anti-inflamatuar etki gösterdiği, reçinenin diğer tıbbi etkilerinin yanısıra antiinflamatuar etkisinin de kayda değer düzeyde olduğu söylenebilir. *İn vitro* koşullarda elde edilen bu sonuçlar dikkate alındığında, reçinenin etil asetat ekstresinin antiinflamatuar etkiye sahip olan bileşiklerinin izole edilerek belirlenmesi ve hayvan deneylerinde reçinenin bu özellikleri ile araştırılmasının ihtiyaç olduğu kanaati oluşmuştur.

TEŞEKKÜR

Bu çalışma TÜBİTAK 118Z417 no'lu proje ile desteklenmektedir. Desteklerinden dolayı TUBİTAK'a teşekkür ederiz.

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➢ *ORAL PRESENTATION*

Development of an Ankle Rehabilitation Robot

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Abstract
This study explains the design and control of one degree of freedom therapeutic exercise robot for the ankle of children with cerebral palsy, patients who need a rehabilitation after a stroke, muscle disorder, or a surgical operation. The robot can perform active and passive exercises and maintain therapeutic rehabilitation with high accuracy, precision and repeatability unlike manual therapy by physiotherapist (PT). Impedance control is used as control method in this robot since it is very efficient, reliable and widely used in human-machine interactive systems. Knowledge and experiences of PT is used to develop the control algorithm of the system. Since the patient's data of measurement and exercise results are saved in the database, a PT can monitor the patient's data remotely via internet and it is possible to be chosen correct treatment exercise by the PT. With the development of this system, it is aimed that patients will be able to have physiotherapy in their home environment where they are comfortable and have a higher motivation. In this way, difficulty of transportation to treatment centers of patients and the problem of having less physical therapists compared to the number of patients will be solved. Besides, in this COVID-19 pandemic, patients who use the developed ankle rehabilitation robot will be away from places where they may be exposed to the coronavirus.
Keywords: rehabilitation robots, ankle rehabilitation, biomechatronics, telemedicine, impedance control.

1. INTRODUCTION

Rehabilitation is a set of interventions designed to optimize functioning and reduce disability in individuals with health conditions in interaction with their environment. Patients who had stroke, surgical operation or have muscle disorder and children who have cerebral palsy need rehabilitation to regain their mobility (Akdogan and Adli, 2011). The need for rehabilitation worldwide is increasing with the changes in health and characteristics of people who are living longer, but with more disability and chronic disease. According to World Health Organization, at present, the rehabilitation services are not enough and in some low/middle-income countries, more than half of people do not receive the rehabilitation services they require. Moreover, the existing rehabilitation services in 60-70% of countries have been disrupted because of the COVID-19 pandemic. According to estimations, need for rehabilitation is going to increase in the next years. Considering the increasing number of people who need rehabilitation, it is required to find new methods or develop new systems to fill the gap and fulfil the needs of the rehabilitation services.

Rehabilitation consists of two main sections: therapeutic modalities and therapeutic exercises. Therapeutic modalities (e.g. hot and cold packs, electrical stimulation, ultrasound) are tools which PTs might use to decrease pain, swelling, muscle spasms and to help generate healing. However, although they might have some benefits, recent researches do not confirm the therapeutic modalities as a way to achieve therapeutic goals. For total rehabilitation, therapeutic exercise should also be used to achieve the ultimate goal of rehabilitation which is to recover complete muscle function and painless mobility. Therapeutic exercises include strengthening, endurance, stretching and balance-coordination exercises. In robotic rehabilitation, therapeutic exercises can be classified as passive exercise (passive stretching) to improve range of motion (ROM), active-assistive exercise, active-resistive exercise and other exercises which are isotonic, isometric and isokinetic exercises (Akdogan and Aktan, 2019). Traditionally, PTs used to perform therapeutic exercises manually. However, since functional recovery is a long and laborious process which takes time and needs dedication to work, it

was seen that there was a need for rehabilitation robots. Therefore, scientists have studied on rehabilitation robots and it has been proven that robotic systems might provide many advantages both for patients and PTs. From the perspective of patients, the use of rehabilitation robots in the physical therapy will solve the transportation problem of patients to far treatment centers, prevent the lack of PT and decrease treatment costs. From the point of view of PT, it will reduce their workload, help them in exercises which require muscle power. Moreover, since robotic sensors have high accuracy and precision, measurement errors will be avoided. Therapeutic exercises by robots can be performed with high precision and accuracy repeatedly. These are not possible with manual therapy of PT. Since measurement and evaluation data are saved in the database, PT will be able to access these data remotely. Analysis of these data will help to improve treatment process for other patients as well. For these reasons, many studies have been carried out in the field of rehabilitation robots in the last 15 years and some of them have turned into commercial products (Sobh and Xiong, 2012). With the COVID-19 pandemic, it is also seen that there is a need for home care robots to receive treatment services away from crowded health centers.

In this context, Continuous Passive Motion devices have been developed and used for rehabilitation in health centers. However, since these devices cannot respond instantly and appropriately to the forces applied by the patient during exercise, they might cause excessive load on the patient's muscles and damage their muscles and tendons (Akdogan and Adli, 2011). In this respect, these devices are not safe and suitable solution for rehabilitation.

In order to prevent such problems, it is necessary to develop systems which use intelligent control algorithms to apply appropriate treatment to the patient and there is a need for control methods which are compatible with human-machine interaction.

One of the types of rehabilitation robots are therapeutic exercise robots that assist PT for passive, active, active assistive and resistive exercises.

Various therapeutic exercise robots have been developed for ankle rehabilitation. These robots differ according to the types of therapeutic exercises they can apply, their mechanical designs and control methods. Lin et al. (2008) have developed an ankle rehabilitation robot with a single degree of freedom. It performs passive and isotonic exercise types. Dorsiflexion and plantar flexion movements are performed. The control structure consists of fuzzy logic, force and position control. Saglia et al. (2009) have developed a system with two degrees of freedom. Dorsiflexion, plantar flexion, eversion and inversion movements are performed. The system works with an electric motor and consists of a position and admittance control structure. Ayas et al. (2017) have developed a fuzzy logic based adaptive admittance controlled ankle rehabilitation robot with two degrees of freedom. The system can perform passive exercises. Jamwal et al. (2014) have developed a pneumatic actuator driven system with three degrees of freedom, capable of dorsiflexion-plantar flexion, inversion-eversion and adduction-abduction. It performs passive movements with force feedback and adaptive fuzzy logic control structure. Yoon et al. (2006) have developed a robot that works with pneumatic actuators with two degrees of freedom. Force and position control is done by using pressure sensor. The system performs passive and isotonic exercise types. The patient can do the exercises with visual feedback.

Zhou et al. (2015) have designed a single degree of freedom robot. The system works with an electric motor and the platforms can be adjusted according to the limbs. Passive and game-based isometric exercises are performed with EMG data, torque and angle values. The main mechanical disadvantages of these systems are that they are physically large and not suitable for home use.

In this study, it is proposed to develop a wearable ankle rehabilitation robot for home use which can perform passive and active exercises for the ankle joint. For this purpose, Section 2 explains the theory of ankle rehabilitation briefly, Section 3 includes system description, mechanical design and Section 4 contains control method of the proposed ankle rehabilitation robot.

2. THEORY OF ANKLE REHABILITATION

In order to determine the appropriate exercise type for the patient, first of all, muscle tone should be determined according to Modified Ashworth Scale (Table 1).

Table 1. Modified Ashworth Scale (Bohannon and Smith, 1987)

Grade	Description
0	No increase in muscle tone
1	Slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the ROM when the affected part is moved in flexion or extension
1+	Slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the remainder (less than half) of the ROM
2	More marked increase in muscle tone through most of the ROM, but affected part easily moved
3	Considerable increase in muscle tone, passive movement difficult
4	Affected part rigid in flexion or extension

2.1 Movements of ankle exercises

There are basically three types of ankle rehabilitation exercises: dorsiflexion-plantar flexion, abduction-adduction, and inversion-eversion (Figure 1). The robot proposed in this study can perform dorsiflexion and plantar flexion movements.

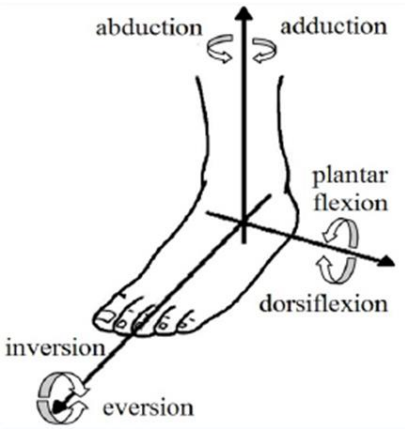


Figure 9. Movement types of ankle

2.2 Therapeutic exercise types

Passive (Stretching) or ROM exercise: This exercise is performed by PT or rehabilition device. It is usually applied for patients without muscle strength. It can be used to improve ROM of patients with spasticity.

Active assistive exercise: Active exercises begin when the patient is able to move the limb with her own muscle power. In this exercise, necessary and sufficient support is provided for the patient's limb to move to the desired position. This can be performed by the PT manually or with a counterweight. It is possible to increase the patient's muscle strength with this exercise.

Active resistive exercise: When the patient can move her limb comfortably in the ROM, a certain level of resistance can be applied to develop muscle strength.

Isotonic exercise: It is performed by moving the limb under a constant resistance in the ROM.

Isometric exercise: In this exercise, while the position of the limb remains constant, the amount of resistance applied, ie muscle contraction, is variable.

Isokinetic exercise: In this type of exercise, while the movement speed of the patient's limb is constant, maximum muscle contraction is applied.

Manual exercise: These are all active and passive exercises performed by PT.

3. SYSTEM DESCRIPTION

There are control system and human-machine interface (HMI) at the center of the ankle rehabilitation system (Figure 2). The PT enters information such as age, weight, height, limb length of the patient through this graphical interface and determines the type of exercise to be applied. The patient performs certain active exercises according to instructions and biological feedback on this screen.

Position, joint torque and impedance parameters are calculated according to the sensor data (force, position, EMG signals) and the robot manipulator is controlled by the HMI. Thus, the force or position of the robot manipulator can be changed.

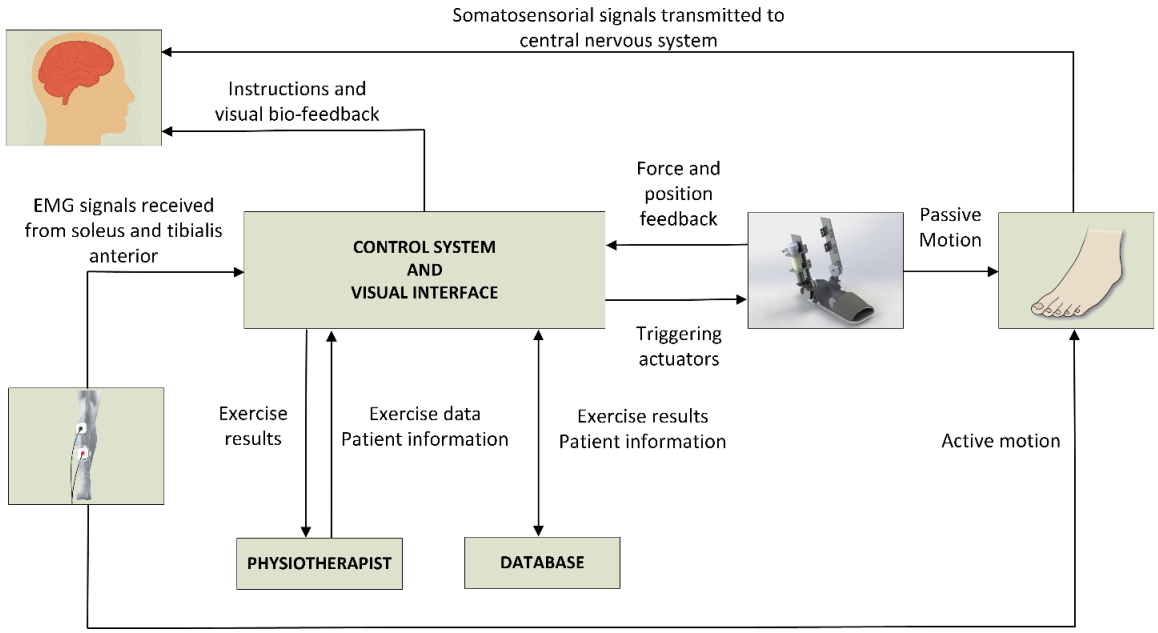


Figure 2. System of the ankle rehabilitation robot

3.1 Robot manipulator and hardware configuration

3.1.1 Robot manipulator

3.1.1.1 Design requirements

Design requirements are determined according to the ankle rehabilitation theory. In order to meet these requirements, an appropriate mechanism has been designed, the hardware has been selected, and suitable control methods have been used to control the mechanism. The following describes the design requirements:

- The main exercises are dorsiflexion and plantar flexion. Therefore, the mechanism should consist of a single degree of freedom which allows dorsiflexion and plantar flexion movements.
- The rehabilitation robot should be used for patients with different physical characteristics. Therefore, it should have a mechanism that can be adjusted according to the limb size and should be able to apply to sufficient torque according to patients with different masses and muscle strength.
- The mechanism should be compact, wearable, and reasonably sized to be suitable for home use. In order to be portable and safe, it should be made of light and durable material.
- The mechanism should be able to measure the reaction forces applied by the patient with sensors.
- The sensors should be able to receive real-time, accurate data and record them properly. Because the proper measurement, evaluation and recording of interaction forces are very important in rehabilitation. In addition, the controller must be able to react against the patient's instant reactions.
- Safety is a very important factor in human-machine interaction. For this reason, necessary safety precautions should be taken in terms of both software and hardware.

- In order to perform the therapeutic exercises required for ankle rehabilitation, the developed system should have an appropriate hardware to control the position and force of the robot.

The mechanism and suitable hardware components designed in accordance with these requirements are shown in Figure 5.

3.1.1.2 Mechanism specifications

- The mechanism has one degree of freedom.
- It can perform dorsiflexion-plantar flexion movements around an axis.
- There is a servo motor mounted next to the mechanicsm. Bevel gear mechanism with 3:1 ratio is designed to rotate the movement axis and increase torque.
- The range of motion of the robot manipulator has been determined in accordance with the physical properties of the ankle and exercise movements.
- The mechanism can be adjusted according to the patient's foot.
- It is appropriate to use duralumin material to make the mechanism durable and light.
- Mechanical safety precautions are also taken in addition to the software and hardware safety.

The wearable ankle rehabilitation mechanism designed in accordance with these features can be seen in Figures 3 and 4.

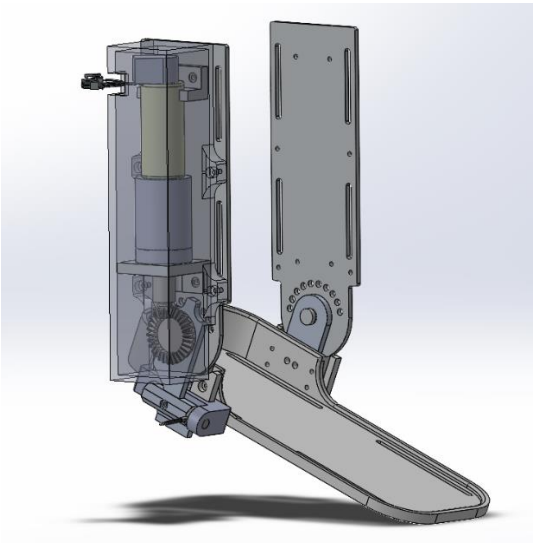


Figure 3. General view of the mechanism

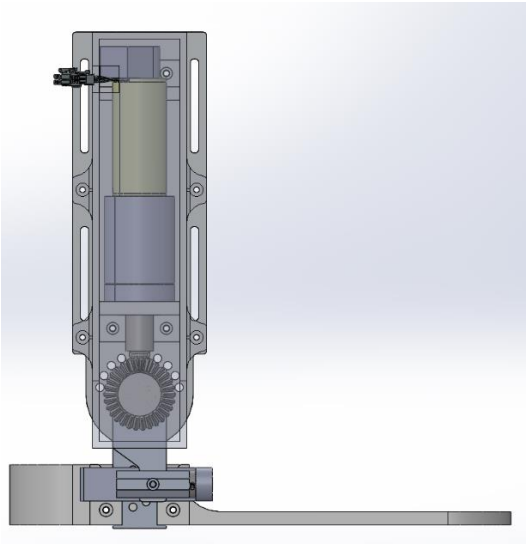


Figure 4. Side view of the mechanism

3.1.2 The hardware configuration

The block diagram of the system hardware is shown below and detailed explanations for the hardware components are made (Figure 5).

3.1.2.1 Actuator (servo motor), driver, encoder, gearbox and bevel gear

The system has a servo motor (Maxon EC-max 40 120 Watt), a servo driver (Maxon Escon 50/5), an encoder adet (Maxon HEDL5540), a gearbox (Maxon GP52C).

A bevel gear set with a ratio of 3:1 is designed to turn the axis of motion 90 degrees. Ankle rehabilitation exercises can be performed for patients up to 100 kg with the chosen hardware.

3.1.2.2 Position and force feedback

The system has encoder and hall sensor for position feedback. Thus, position data can be obtained with encoder emulation provided by the servo drive. Load cell (Futek LCM200) placed in the mechanism is used for force feedback. This load cell can measure compression and tensile loads up to 113 kg. Thus, the forces applied by the patient or applied to the patient can be measured.

3.1.2.3 Data acquisition

USB based Real-Time Control & Data Acquisition System (Simkotek V-DAQ) which is compatible with MATLAB is used during developing the control algorithm. After the control algorithm is developed on MATLAB, the software will be embedded in the Development Kit (TI LAUNCHXL-F28379D) which is compatible with MATLAB Embedded Codder.

3.1.2.4 Safety

Safety precautions have been taken both in terms of hardware and software. There are mechanical limits in the mechanism so that the patient does not exceed the allowable range of motion, and the control software does not allow the angle limits to be exceeded during exercise. The currents sent to the motors are limited by software in order not to exceed the specified torque limits.

3.2 Human-Machine Interface

A HMI consisting of a Raspberry PI 4 and a touch screen has been proposed to enable interaction between PT-robot and patient-robot. The characteristics of the patient (name, surname, age, gender, height, weight, extremity lengths) can be entered on the touch screen by PT. Before starting the treatment, the patient's ankle ROM and muscle strength are measured with the choice of PT. The tone of the ankle plantarflexor and dorsiflexor muscles is then scored according to the Ashworth scale. As a result of the precise measurement made by the sensors, the robotic system will be able to suggest appropriate treatment methods to the patient, and the appropriate exercise method will be finally selected by PT. EMG signals are received from the patient's tibialis anterior and soleus muscles with surface electrodes, torque values and joint angles information are collected via sensors and graphically displayed on the touch screen. Thus, the patient can perform active game-based exercises with graphical bio feedback. Game-based exercises help children and even adults to participate more voluntarily in rehabilitation process. Thanks to the game-based active exercises, improvement of muscle strength and motor control is provided.

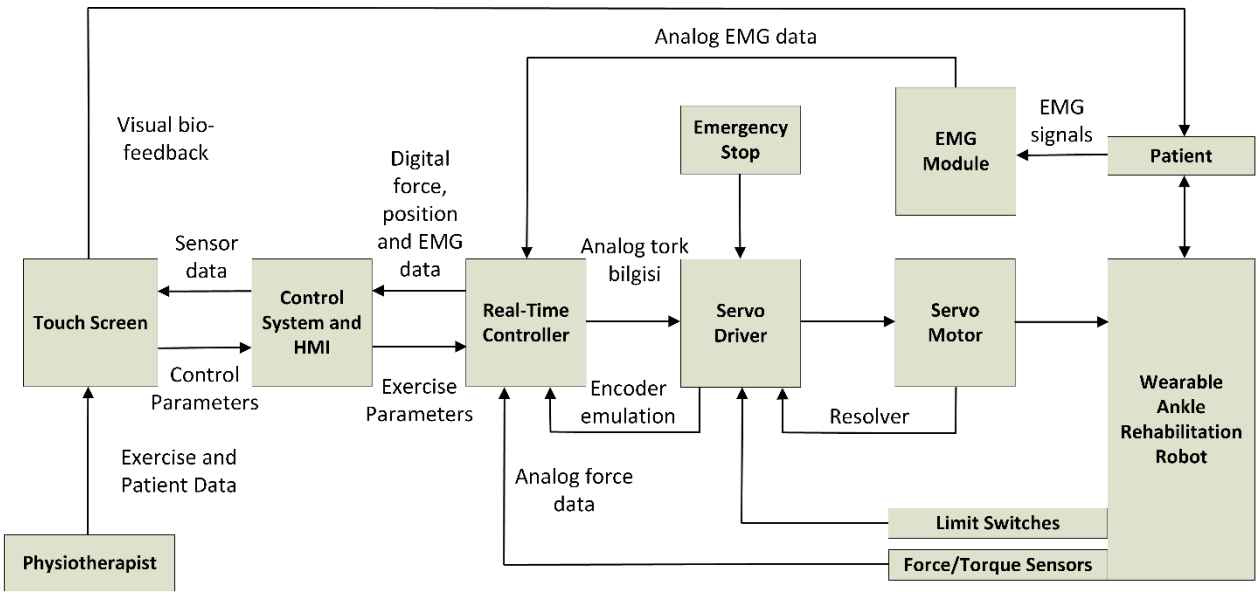


Figure 5. System hardware

4. CONTROL TECHNIQUE

During manual rehabilitation therapy, a PT acts according to the physical characteristics of the patient and simultaneously provides the adequate support the patient needs. In this process, there is a continuous dynamic interaction between the PT and the patient. Control of this dynamic interaction can be maintained by impedance control in robotic systems. With the impedance control, the resistance of the robot against the environment can be adjusted (Hogan, 1985). Adaptive impedance control shows a high performance during rehabilitation tasks due to its ability to detect the patient's capacity and to adjust parameters (Kwakkel et al., 2008; Marchal-Crespo and Reinkensmeyer, 2009). Impedance control establishes a real-time dynamic relationship between robot position and interaction forces. This allows the active participation of the patient during the exercise. Determining impedance parameters is one of the important elements of impedance control. Impedance parameters should be updated periodically in accordance with the patient's capacity and improvement, as the patient's mobility and energy changes throughout the exercise sessions and duration (Koopman et al., 2013).

4.1 Dynamic model of the ankle rehabilitation robot

In order to test the control methods suitable for ankle rehabilitation exercises and to simulate how speed, angle and torque of the robot changes according to the change of impedance parameters, the dynamic equations of the system (including inertia, gravity and friction effects) are created and a dynamic model is created by using these equations.

General dynamic equation of a robot manipulator is given in Equation (1) (Schilling, 2003).

$$\tau = D(q)\ddot{q} + c(q,\dot{q}) + h(q) + b(\dot{q}) \tag{1}$$

Equation (1) includes the joint variables q and the actuator torque τ for a robot with n degrees of freedom. D represents inertia matrix, c represents interaxis velocity coupling due to Coriolis and centrifugal forces, h represents loading due to gravity, and b represents the effects of friction (Schilling, 2003). Since the wearable ankle rehabilitation robot has only one degree of freedom, the joint variables matrix q contains only the variable θ. Therefore, the angle variable θ can be used instead of the q matrix. θ, $\dot{\theta}$ and $\ddot{\theta}$ represent the joint angle, the angular velocity, and the angular acceleration of the joint, respectively. Since robots with a single degree of freedom do not have velocity couplings, c equals zero. D includes the inertia of the robot mechanism (I_{mec}) and the inertia of the foot (I_L) placed on the mechanism and the inertia the rotor of the motor (I_R) and the inertia of the gear (I_r). While inertia of the mechanism (D) is calculated, the rotor inertia of the motor is multiplied by the square of the total reduction ratio (N) and the inertia of the gear is multiplied by the square of the gear reduction ratio (N_2). The total reduction ratio N is equal to the product of the motor reduction ratio (N_1) and the gear reduction ratio (N_2). Accordingly, the inertia term D is obtained as in Equation (2).

$$D = N^2 I_R + N_2^2 I_r + I_{mec} + I_L \tag{2}$$

h refers to the gravitional load torque due to the mass of the mechanism (m_{mec}) and the patient's foot (m_{foot}). The distance of the center of mass of the mechanism to the axis of rotation of the joint is L_{mec}, the distance from the center of mass of the foot to the axis of rotation of the joint is L_{foot}. b refers to the torque imposed against movement caused by friction. This term is found empirically. h term which includes the joint angle is given as in Equation (3).

$$h(\theta) = (m_{mec}L_{mec} + m_{foot}L_{foot})\cos(\theta) \tag{3}$$

Thus, with the combination of Equations (1), (2), and (3), the dynamic equation of the system is given as in Equation (4).

$$\tau - \tau_L = D\ddot{\theta} + h(\theta) + b(\dot{\theta}) \quad (4)$$

By creating a dynamic model in MATLAB Simulink, the output can be observed according to the input torques of the system, and the system performance can be evaluated with various control methods.

4.2 Position-based impedance control

Position-based impedance control is used in passive exercises that require position control and active-assistive exercises which require force + position control. In this study, the position-based impedance control model developed by Yoshikawa (1990) has been simplified to one degree of freedom. Accordingly, interaction torque (τ_{ext}) is given according to desired impedance parameters as follows:

$$M_d\ddot{\theta} + B_d\dot{\theta}_e + K_d\theta_e = \tau_{ext} \quad (5)$$

M_d, B_d ve K_d are impedance parameters representing the desired inertia, damping and stiffness, respectively. θ represents the actual position and θ_d represents the desired position. $\theta_e = \theta - \theta_d$ is given. By adapting Equation (4) according to this model, the dynamic equation of the robot manipulator interacting with its environment is given as in Equation (6). The dynamic equation is defined in the joint space. M represents the inertia of the robot manipulator.

$$M\ddot{\theta} + h(\theta) = \tau + \tau_{ext} \quad (6)$$

By the combination of Equation (5) and (6), the position based impedance control model is given as follows:

$$\frac{M}{M_d}\left(-B_d\dot{\theta}_e - K_d\theta_e\right) + \left(\frac{M}{M_d} - 1\right)\tau_{ext} + h(\theta) = \tau \quad (7)$$

4.3 Force-based impedance control

For the force-based impedance control, the dynamic behavior of the system according to desired torque (τ_d) is given in Equation (8). In this study, the desired force (F_d) has been adapted to the desired torque (τ_d).

$$M_d\ddot{\theta} + B_d\dot{\theta} + \tau_d = \tau_{ext} \quad (8)$$

The force (torque) based impedance control model is obtained from Equations (6) and (8) as follows:

$$\frac{M}{M_d}\left(-\tau_d - B_d\dot{\theta}\right) + \left(\frac{M}{M_d} - 1\right)\tau_{ext} + h(\theta) = \tau \quad (9)$$

4.4 Hybrid impedance control

The hybrid impedance control method was first developed by Anderson and Spong (1988). This method provides a useful structure by combining position and force based impedance control under a single control

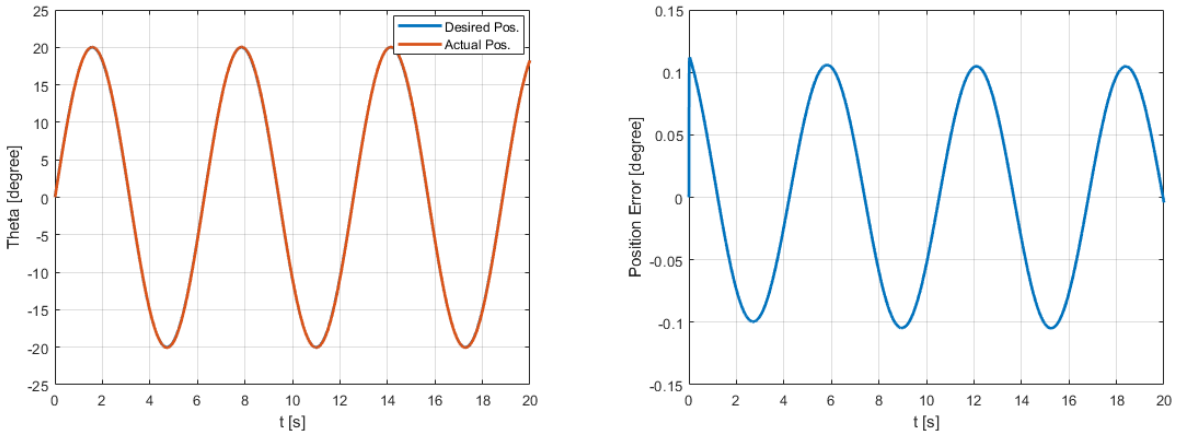
rule. Thus, this method can be used in exercises that require both position and force control. Switching between position and force based impedance control is possible by changing the selection coefficient (S). If S is set to 0, the system works in force-based impedance control, if S is set to 1, the system works in position-based impedance control. The desired system dynamics consisting of the combination of Equations (7) and (9), and including the S selection coefficient (S) is given as follows:

$$\frac{M}{M_d}\left((S\text{-}1)B_d\dot{\theta} + SB_d(\dot{\theta}\text{-}\dot{\theta}_d) + SK_d(\theta\text{-}\theta_d) + (S\text{-}1)\tau_d\right) + \left(\frac{M}{M_d}\text{-}1\right)\tau_{ext} + h(\theta) = \tau \qquad (10)$$

5. SIMULATION RESULTS and DISCUSSION

The system was tested by using dynamic model of the ankle rehabilitation robot in MATLAB Simulink and the dorsiflexion-plantar flexion movements were simulated by using impedance control method. The mechanism was modeled using SolidWorks software. By using this model, physical properties such as the inertia and kinematic relationships required for the dynamic model of the robot were provided.

Two scenarios were considered while testing the position based impedance control mode. In the first scenario, the mechanism was simulated to perform dorsiflexion and plantar flexion movements in sinusoidal trajectory between -20 and +20 degrees. It was assumed that the resistance force of the patient during movement was constant. Impedance parameters were determined as $M_d = M$, $B_d = 200$, $K_d = 100$. Sinusoidal position trajectory was used as the desired position. After applying the position-based impedance control, it was seen that the robot manipulator followed the desired trajectory accurately. The position error was measured less than 0.12 degrees. The simulation results of the position control mode are shown in Figure 6.



(a) (b)

Figure 6. Simulation results of position-based impedance control at the first scenario (a) Desired and actual positions (b) Position error

In the second scenario, it was assumed that a sudden reaction force was exerted from the patient during movement from 0 degrees to 20 degrees. Accordingly, response of the system was simulated. Impedance parameters were determined as $M_d = M$, $B_d = 900$, $K_d = 500$. The fixed position trajectory (20 degree) was used as the desired position. While applying the position-based impedance control mode, it was assumed that the patient applied 10 Nm torque between 4th and 7th seconds. Accordingly, the simulation results are shown in Figure 7.

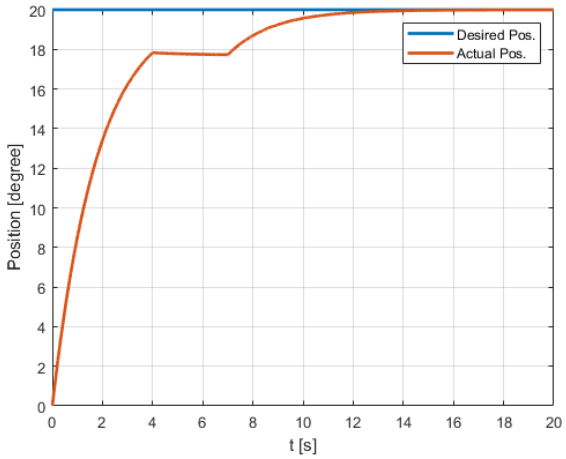


Figure 7. Simulation results of position-based impedance control at the second scenario

As seen in the first scenario, appropriate impedance parameters were determined and the desired position trajectory was followed with high accuracy and position-based impedance control was successfully applied (Figure 6). Thus, it was shown that rehabilitation exercises in which ankle dorsiflexion and plantar flexion movements are performed in certain trajectories can be successfully performed. In the second scenario, it was simulated that during an exercise, a sudden reaction force was exerted from the patient or a contraction occurred in their dorsiflexor/plantarflexor muscles. If PID control method was used here, the robot manipulator would increase its torque at the point where it meets resistance to reach the desired position and would force the patient's ankle to move to that position. This might cause several damage to the ankle or muscles of the patient. However, by using of the impedance control method, the system has damped this reaction torque and paused the movement when a resistance exerted from the patient (Figure 7). When the resistance torque was removed, the movement continued. Thus, it can be seen that impedance control is the most suitable and effective control method in human-robot interactive systems.

In this study, the studies in the literature for ankle rehabilitation were reviewed, and it was seen that the designed mechanisms were large and bulky, so a mechanism that can be worn at home and adjustable according to the physical characteristics of the person was developed.

By using rehabilitation robots to perform rehabilitation exercises, human-induced errors in measurement and treatment will be prevented. In addition, since patients can use this device at home, transportation and cost problems will be solved. And PTs will have time for more patients and the robot will help them in exercises which require muscle strength. Since the exercise results and patient data will be saved on a database, PTs will be able to access these data remotely. These data will be used to evaluate recovery of the patient by PT. By evaluating these data, more effective treatment methods will be provided to other patients as well.

CONCLUSION

A compact and wearable mechanical design and suitable control method approach for a single degree of freedom ankle rehabilitation robot was presented in this study. The ankle rehabilitation theory was explained briefly. The design requirements of the system were explained. Mechanical and electronic hardware of the system were presented. Equations for impedance control for a single degree of freedom were derived and this control metod was simulated for dorsiflexion and plantar flexion movements in two scenarios. From simulation results, it was seen that impedance control is suitable for rehabilitation exercises which contains human-machine interaction. As a future work, the wearable ankle rehabilitation robot mechanism will be produced and the proposed control method will be tested in the real system. After that, experimental studies will be done with real subjects and real patients.

ACKNOWLEDGEMENTS

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➢ *ORAL PRESENTATION*

Probiyotik bakterilerin mikroenkapsülasyon yöntemi ile Balık yemlerine ilave edilmesi

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Özet
İnsanların ve hayvanların sağlıklı bir hayat yaşamaları için sağlıklı bağırsak sistemine ihtiyaçları vardır. Bağırsak sisteminin sağlığını güçlendirmek amacıyla probiyotik ve prebiyotik takviyeleri kullanmaları gerekmektedir. Probiyotik hücrelerin vücuttaki etkileri, bağırsak pH'ını dengeler, antimikrobiyal etkisi ile patojen bakterilerin üremesini engeller, bağlanma noktarı ve besin maddeleri için patojen bakteriler ile yarışırlar. Probiyotik hücrelerin faydalı etkileri gösterebilmesi için 10^6-10^7 kob/ml veya daha fazla sayıda vücuda alınmaları gerekmektedir. Probiyotikler üretim, işleme ve depolama sırasında canlılıklarını koruyamadıkları için bağırsak sistemine ulaştıklarında olumlu sonuç gözlenememektedir. Probiyotiklerin canlılığını kaybetmeden bağırsak sistemine ulaşması için mikroenkapsülasyon (ME) yöntemi uygulanmaya başlanmıştır. Mikroenkapsülasyon bir tutuklama yöntemidir. Probiyotik hücreye uygun kaplama yöntemi ve kaplama materyali seçilerek hücrenin kapsüllenmesi yapılır. Böylece hücreye fiziksel bir bariyer sağlanmış olur. Günümüzde en çok kullanılan mikroenkapsülasyon yöntemi kalsiyum-aljinat jel kapsülü oluşumu esasına dayalı ekstrüzyon kaplama yöntemidir. ME yöntemi, ilaçlardan aroma maddelerine kadar çok geniş bir alanda kullanılmaktadır. Probiyotik hücreler çiftlik hayvanları, kümes hayvanları ve balıklarda yem katkı maddesi olarak kullanılabilir. Yetiştiricilikte hastalıklara dayanım, büyüme ve yem etkinliğinin geliştirilmesi için probiyotik hücrelerin kullanımı artmıştır. Su ürünlerinde kullanılan probiyotik hücreler antibiyotik direncini ortadan kaldırmada önemli bir alternatiftir. Probiyotik hücreler hayvanlara çeşitli yollarla verilebilir. Bunlar pelet şeklinde rasyona katılabileceği gibi hazırlanış itibariyle kapsül, granül veya toz halinde de direkt hayvanlara yedirilebilir. Bu derlemede probiyotik bakterilerin mikroenkapsülasyon yöntemi ile kapsüllenmesi ve bu kapsüllerin balık yemlerine ilave edilmesi konusu çalışılmıştır.
Anahtar Kelimeler: Probiyotik, Mikroenkapsülasyon, Balık Yemi

Addition Of Probiotic Bacteria To Fish Feed By Microencapsulation Method

Abstract
Humans and animals need a healthy intestinal system to lead a healthy life. They need to use probiotic and prebiotic supplements to strengthen the health of the intestinal system. The effects of probiotic cells in the body balance the intestinal pH, prevent the growth of pathogenic bacteria with its antimicrobial effect, compete with pathogenic bacteria for binding point and nutrients. In order for probiotic cells to exert beneficial effects, they need to be taken into the body at 106-107 cfu / ml or more. Since probiotics cannot maintain their vitality during production, processing and storage, a positive result cannot be observed when they reach the intestinal system. Microencapsulation (ME) method has been applied to ensure that probiotics reach the intestinal system without losing their vitality. Microencapsulation is an arrest method. Encapsulation of the cell is performed by selecting the coating method and coating material suitable for the probiotic cell. Thus, a physical barrier is provided to the cell. Today, the most used microencapsulation method is the extrusion coating method based on calcium-alginate gel capsule formation. The ME method is used in a wide range of areas from drugs to flavorings. Probiotic cells can be used as feed additives in livestock, poultry and fish. In breeding, the use of probiotic cells has increased to improve disease resistance, growth and feed efficiency. Probiotic cells used in aquaculture are an important alternative in eliminating antibiotic resistance. Probiotic cells can be administered to animals in a variety of ways. These can be added to the ration in the form of pellets or can be fed directly to animals as capsules, granules or powder as of preparation. In this review, the encapsulation of probiotic bacteria by microencapsulation method and the addition of these capsules to fish feed were studied.
Keywords: Probiotic, Microencapsulation, Fish Feed

GİRİŞ

Probiyotikler; konak canlıyı patojenlere karşı koruyarak ve bağışıklık sistemini güçlendiren yararlı bakterilerdir. Aynı zamanda hem yoğurt, kefir, lahana turşusu, bitter çikolata gibi çeşitli gıdalarda hem de insan mikrobiyotasında doğal olarak bulunmaktadır. Ayrıca sağlığa olan yararlı etkilerinin çokluğu sebebiyle son zamanlarda tüketimi oldukça artmıştır. Bunun yanı sıra en önemli özellikleri toksik üretmemeleri ve sağlığa zarar vermemeleri olmalıdır. Probiyotik olarak kullanılacak mikroorganizmanın en önemli özellikleri, antimikrobiyel madde üretmesi, antibiyotik kullanımından etkilenmemesi, .yan etkilere sebebiyet vermemesi ve düşük pH ve safra tuzları gibi ekstrem koşullardan etkilenmemesidir (Küçüköner ve ark.,2003). Probiyotik kültürlerin olumsuz koşullardan etkilenmemesi, konakta sağladığı yararı arttırmak ve stabilitesini sağlamak için mikroenkapsülasyon yöntemi üzerinde yoğunlaşılmıştır (Geniş,2019).

Mikroenkapsülasyon (ME)**;** katı, sıvı veya gaz halindeki gıda bileşenlerinin, enzimlerin, hücre ve diğer maddelerin, mikroorganizmaların protein veya karbonhidrat esaslı bir kaplama materyaliyle kaplanması şeklinde tanımlanabilmektedir (Cho ve ark.,2003). Mikroenkapsülasyon bir tutuklama yöntemidir. Mikroenkapsülasyon işlemi farklı yöntemler uygulanarak yapılsada genel olarak üç aşamadan oluşmaktadır. İlk aşamada katı veya sıvı matriks içerisine biyoaktif bileşen ilave edilir. İkinci aşamada sıvı matriks dispersiyonu sağlanır. Son aşamada ise fiziksel (buharlaştırma, katılaştırma vb.), kimyasal (polimerizasyon) veya fizikokimyasal (jelifikasyon) bir yöntemle stabilize edilerek enkapsülasyon işlemi gerçekleştirilir (Sarao ve Arora, 2017).Mikroenkapsülasyon işleminden sonra oluşan mikrokapsüllerin genellikle çapları 0.01 ve 1.000 µm aralığında iken, aktif malzemenin üzerini kaplayan çeperin kalınlığı ise 0.5-150 µm olmaktadır (Peanparkdee vd., 2016). Mikroenkapsülasyon sonucu oluşan yapılar mikropartiküller olarak adlandırılır. Mikroenkapsülasyon yöntemi günümüzde ilaç (%68), gıda (%13), kozmetik (%8), tekstil (%5), biyomedikal (%3), tarım (%2) ve elektronik (%1) gibi farklı alanlarda kullanılmaktadır (Paulo ve Santos, 2017). Kaplanacak aktif materyalde kullanılan teknik ve kaplama materyali mikroenkapsülasyonun başarısını belirler. Probiyotik mikroorganizmaların kaplanmasında yaygın olarak kullanılan kaplama materyalleri aljinatlar, peynir altı suyu proteinleri, nişasta, karregenan, selüloz asetat fitalat (CAP), kitosan, jellan ve ksantan gam, jelatin ve nohut proteinleridir. Bu kaplama materyalleri ekstrüzyon, emülsiyon, akışkan yatak, rennet ile jelleştirilmiş protein, dondurarak kurutma, püskürtmeli kurutma, hibridizasyon, çarpışmalı aerosol teknolojisi ve elektrodöndürme yöntemleri ile probiyotik mikroorganizmalara uygulanabilmektedir (Martín vd., 2015; Kavitake vd., 2018).

Probiyotik Mikroorganizmaların Mikroenkapsülasyon Tekniği ile Uygulamaları

Probiyotik mikroorganizmaların türüne göre kaplama materyali ve kaplama yöntemi değişiklik göstermektedir.

Mikroenkapsülasyonda kaplama materyalinin seçimi çok önemlidir. Kullanılacak olan kaplama materyali toksik özellikte olmamalı, güvenli kabul edilen özellikte olmalı, işlem sırasında kolay işlenebilir olmalı, inert olmalı, aktif materyal ile uygulama esnasında ve uygulama sonunda materyalin özelliklerini etkileyecek şekilde reaksiyona girmemeli, stabiliteyi arttırmalı, çevresel etkilerden (fiziksel etkiler) koruyabilmeli, beklenen salınım zamanında salınımı kolay olmalı ve ucuz olmalıdır (Azagheswari vd., 2015; Giro-Paloma vd., 2016; Atak vd., 2017; Başyigit vd., 2017; Suganya ve Anuradha, 2017; Ramani ve Ramani, 2018).

Ekstrüzyon tekniği en eski ve en yaygın kullanılan tekniktir. Ekstrüzyon tekniğinde ilk önce hidrokolloit solüsyonu hazırlanır ve hücre bu solüsyon içerisine katılır. Hücre ekstrüzyon şeklinde şırınga ile boncuk halinde düşerek sertleşir. Boncuğun büyüklüğü ve şekli şırınganın başlığının çapı ve düşme mesafesine bağlıdır. Ekstrüzyon tekniğinde kaplama materyali olarak genellikle aljinat kullanılır (Krasaekoopt ve ark. 2003).

Probiyotiklerin mikroenkapsülasyonu için sprey kurutma yöntemi yaygın olarak kullanılır. Polimerik çözelti içindeki hücre süspansiyonunun sıcak kuru hava içine atomizasyonunu kapsar. Bu aşamadan sonra suyun hızlı bir şekilde evaporasyon işlemi gerçekleştirilir. Mikrokapsüle edilmiş hücre siklon içinde taşıyıcı hava yardımıyla kuru toz haline getirilir.

Dondurarak kurutma yönteminin çalışma prensibi ise kurutulacak hücre ilk önce dondurulur. Ardından yüksek vakumda süblimasyonu yapılarak kurutulması esasına dayanır (Martín vd., 2015).

Yine bir çalışmada, oranda japon balıklarında (Carassius auratus, L. 1758) yeme ilave edilen probiyotiklerin büyüme performansı üzerindeki etkilerini araştırmayı amaçlamaktadır. Bu amaçla biri kontrol grubu olmak

üzere dört gruptan oluşan deneme düzeninde 0 ml kg-1(Kontrol), 1 ml kg-1, 2 ml kg-1 ve 3 ml kg-1 konsantrasyonlarında probiyotik karışımı (Saccharomyces cerevisae, Bacillus subtilis, Lactobacillus plantarum, Lactobacillus casei) ticari havuz balığı yemine ilave edilmiştir. 60 günlük deneme sonunda kontrol ve muamele grupları arasında büyüme performansı ve spesifik büyüme oranı bakımından önemli bir fark gözlenmemiştir. Yem dönüşüm oranı ve hepatosomatik indeks bakımından Grup II (2 ml kg-1) tüm gruplardan daha iyi sonuç verirken bu fark istatistiki olarak da önemli bulunmuştur. Elde edilen sonuçlar, 2 ml kg yem-1 düzeyinde probiyotik ilavesinin japon balıklarının sağlıklı büyümesine olumlu katkısı olduğunu ortaya koymuştur (Doğankaya 2017).

Çizelge 1: Laktik asit bakterileri ve probiyotik bakterilerin enkapsülasyonunda kullanılan kaplama materyalleri ve enkapsülasyon yöntemleri

Bakteri	Kaplama materyalleri	Enkapsülasyon yöntemi	Kaynak
Lactobacillus acidophillus (La-05)	1-Selüloz asetat fitalat 2-Kalsiyum aljinat	1-Püskürtmeli kurutma 2-Ekstrüzyon	1-Fávaro-Trindade ve Grosso (2002) 2-Mirzaei ve ark.(2012)
Bifidobacterium lactis (Bb-12) *Bifidocaterium brevi* R070 *Bifidobacterium longum* R023	Peynir altı suyu proteinleri	Püskürtmeli kurutma	Picot ve Lacroix (2004)
Bifidobacterium bifidum	Kuersetin, aljinat, kitosan	Ekstrüzyon	Chavarri ve ark. (2010)
Saccharomyces boulardi	Jelatin, peynir altı suyu protein konsatresi, modifiye nişasta, maltodekstrin, bezelye proteini, gam Arabik	Püskürtmeli kurutma	Arslan vd. (2015)
Lactobacillus plantarum 299v	Sodyum aljinat ve peynir altı suyu proteinleri	Liyofilizasyon	Gbassi vd. (2010)
Laktik asit bakterileri	Akasya gam	Sprey kurutma	Perez-Chabela ve ark. (2013)
Bifidobacterium lactis, Lactobacillus acidophilus	Palm ve kernel yağı	Dondurarak kurutma	Pedroso ve ark. (2012)

Krasaekoopt ve ark. (2004), *L. acidophilus, B. bifidum ve L. casei* probiyotik bakterilerini kalsiyum aljinat ile enkapsüle etmiştir. Aynı bakterileri 3 tip kaplama materyali ile (kitosan, sodyum aljinat ve aljinat-polilisin kombinasyonu) tekrar kaplamıştır. Sonradan kullanılan kaplama materyallerinin, ilk kapsüllerin sıkılığını artırdığını tespit etmişlerdir. Araştırmacılar ayrıca kaplanan bakterileri çeşitli özellikler yönünden ve mide özsuyunda canlılık düzeyleri bakımından da incelemişler. Enkapsüle edildikten sonra kaplanan *B. bifidum*'un mide özsuyunun asidik ortamında canlılığını yitirdiği sonucuna ulaşmışlardır. Krasaekoopt ve Watcharapoka (2014), prebiyotik olan inülin ve galaktooligosakkarit (GOS) kullanarak probiyotik bakterileri (*L. casei* ve *L.*

acidophilus) sodyum aljinatın içerisine enkapsüle edip daha sonra bu mikrokapsülleri kitosan ile kaplamışlardır. Bu mikrokapsülleri yapay sindirim sisteminde, yoğurt ve meyve suyu üretiminde kullanarak izlemişlerdir. Araştırma sonuçlarına göre prebiyotik ilavesi oluşan mikrokapsül boyutunu % 3.8 oranında arttırmıştır. Yoğurtta 4 haftalık depolama periyodu içerisinde GOS ilaveli mikrokapsüllerdeki canlı hücrelerin, ilavesiz mikrokapsüllerdeki hücrelere göre daha fazla sayıda olduğu gözlenmiştir. Sultana ve ark. (2000) *B. bifidum* ve *L. acidophilus* cinsi probiyotik bakterileri iki farklı kaplama materyali (mısır nişastası ve aljinat) ile ayrı ayrı enkapsüle etmişler. Bu probiyotik bakterilerin yapay sindirim sistemi ve yoğurt içerisindeki yaşamsal faaliyetlerini incelemişlerdir. Çalışmada gliserol ve aljinat karışımının -20^{o}C'de bakterilerin yaşayabilme özelliklerini arttırdığı gözlemlenmiştir. Bununla birlikte yoğurtta 8 haftalık periyotta enkapsüle edilmiş bakterilerin sayısında 0.5 log'lık bir azalma gözlenirken, enkapsüle edilmeden katılmış bakteri kültüründe 1 log'lık azalma olduğu bildirilmiştir.

SONUÇ

İnsan sağlığı açısından önemli olan probiyotikler üretim şartları, muhafaza koşulları, kullanılacak olan gıdanın üretimi ve sindirim sisteminde canlı kalabilmesi için mikroenkapsülasyon yöntemi önemli avantajlar sağlamaktadır. Mikroenkapsülasyon yöntemi maliyeti düşük bir yöntemdir. Kullanılan yöntem ve kaplama materyali kapsüle edilen probiyotiği çevre koşullarına karşı korumalı, konak canlıya yüksek derecede biyoyarar sağlamalı ve istenilen zamanda salınımını etkilemelidir. Kullanılan yöntemler ve kaplama materyalleri bazı dezavantajlarından dolayı mikrokapsülasyon yöntemi iler üretilen ürünler günlük yaşantımızda istenilen düzeyde yerini alamamıştır. Yeni yöntemlerin geliştirilmesi, geliştirilen bu yöntemlere uygun kaplama materyalleri veya karışımların araştırılması bu yöntemin etkinliğini arttıracaktır.

Kültür balıkçılığında da özellikle antimikrobiyal maddelerin kullanımı gündeme gelerek tartışma ve yasaklamalara konu olmaktadır. Probiyotik ve prebiyotikler son yıllarda giderek daha fazla ilgi çeken ve insanlardan balıklara kadar kullanım alanı genişleyen doğal ve umut vadeden maddelerdir. Çeşitli su canlılarında araştırmalara konu olmaya devam eden probiyotik ve prebiyotiklerin etki mekanizmalarının tam olarak anlaşılması ve bunlardan sağlanacak faydanın maksimize edilebilmesi için biyolojik, histolojik ve mikrobiyolojik çalışmaların genişletilerek devam ettirilmesine ihtiyaç duyulmaktadır.

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➢ *ORAL PRESENTATION*

Antibacterial effects of green iron nanoparticles (GINPs)

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Abstract

This study was aimed to investigate the antimicrobial effect of green iron nanoparticles (GINPs) produced by green tea (*Camellia sinensis*) leaf extract. Synthesized GINPs were characterized using SEM, XRD, FTIR, EDX and Zeta Sizer techniques. Antibacterial activity of GINPs was assessed against Gram negative, *Klebsiella pneumoniae*, *Escherichia coli* and Gram positive, *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus faecalis*. This study showed that the average particle size of GINPs is in the range of 10-100 nm. The prepared nanoparticles have amorph structure and uniform particle size distribution and mostly zero-oxidation state. The prepared GINPs showed high antibacterial activity against gram-negative and gram-positive bacteria.

Keywords: *Camellia sinensis*, green synthesis, iron nanoparticles, antibacterial activity

INTRODUCTION

Nano-materials are defined as structured components with dimensions ranging from approximately 1–100 nanometers. Green synthesis has multiple advantages over physical and classical methods. For instance, it is cost effective, eco–friendly and does not require high pressure, energy, temperature or the use of toxic chemical reagents.

Nano-sized iron in particular has many applications in the medical, bio-monitoring, food, energy and environmental fields because of its versatile properties and high level of catalytic activity. The green synthesis of nano-scale iron materials, especially from plants, has gained substantial attention due to easy applicability, eco-friendly nature and low cost. Researchers and scientists have investigated the synthesis of iron nano-materials and the effectiveness of iron synthesized as such for the removal of environmental pollutants [1-3].

Iron nanoparticles (INPs) have been used to remove various pollutants in the environmental engineering due to their reducing properties recently [4,5]. INPs are more preferred than micro-sized iron particles due to highly reactive surface area-to-mass ratio of their nano-sized features [6]. Application of nano-sized iron materials for the removal of heavy metals, metal ions, dyes, chlorinated organic compounds, bacteria, and other non-biodegradable pollutants Nitrate, phosphate, COD and TOC present in industrial wastewater, surface water and groundwater [1-3]. In order to improve the performance of water and wastewater treatment processes, green iron nano particles (GINPs) can be added to the systems [4, 5]. Environmentally, green manufacturing technologies reducing chemical usage, waste and wastewater have developed to produce green iron nano-particles (GINPs) instead of advanced nano technologic processes with high-cost and toxic substance consumption [7].

The most recent method of green iron nano-particle synthesis is using of vegetable, fruit, and organic waste extracts by mixing certain proportion of an iron compound without the energy and any toxic chemical requirements [8]. The green iron nanoparticles production method occurs with special reaction, which are defined as the color change from pale green to dark green or black color, between iron compound with polyphenols found in plant extract in very short time at room temperature [9]. GINPs are produced by this method with 10 to 100 nm size distribution [10]. Camellia sinensis is a rich source of polyphenols and its bio-molecules act as reducing and capping agents during the synthesis of metal NPs. In comparison to published reports on the green production of NPs and physical and chemical properties, very limited information is available on the antibacterial properties of metal NPs.

In literature, GINPs can be produced from various plants, such as tea [3, 9, 11], coffee [3], mint [6, 10], moss [7], banana [12], eucalyptus, pineapple and mango, extracts containing polyphenols obtained by different methods [13]. Various iron compound are mixed different plant extracts by determined volumetric ratio to produce green iron nanoparticles. Iron compounds used in the synthesis of green iron nanoparticles are iron (III) chloride [11, 14-17], iron (II) sulphate [17-19] and iron (III) nitrate [9, 19, 20]. The percentage of iron compound in total GInP solution depends on polyphenols fraction in plant extract, and thus the volumetric ratio of iron solution and plant extract must be investigated every plant extract to achieve nano-sized iron particle. Application of nano-sized iron materials for the removal of heavy metals, metal ions, dyes, chlorinated organic compounds, bacteria, and other non-biodegradable pollutants Nitrate, phosphate, COD and TOC present in industrial wastewater, surface water and groundwater [1-3]. In order to improve the performance of water and wastewater treatment processes, green iron nano particles (GInPs) can be added to the systems.

The present study was carried out for synthesis and characterization of green iron nanoparticles using green leaves extracts. The antibacterial activity of GNPs was evaluated for the water and wastewater treatment. This study will be the first comprehensive report on the detailed production of GINPs and their antimicrobial activity for the water and wastewater treatment.

MATERIALS AND METHODS

Synthesis of GINPs

In this study, GINP synthesis with green tea (Camellia sinensis) leaf extract was investigated to be used water and wastewater treatment. 0.1 M $FeCI_3.6H_2O$ (Merck) solution was used as a source of iron. 5 g leaf was extracted in 100 mL water at 80 °C during 15 minutes at autoclave before adding 0.1 M $FeCI_3.6H_2O$ (Merck) as a source of iron. Same trials were made for GINP solution with different volumetric ratio of iron solution and leaf extract to achieve nano-sized iron particle efficiently.

Characterization of GINPs

Characterization of the produced iron nanoparticles was performed by Zeta Sizer, SEM, EDX, XRD and FT-IR analyses to explain their structure and physical and chemical properties.

In order to determine size distribution of iron particle Zetasizer (Malvern 500210) analysis was performed for each trial. The refractive index was chosen 2.65 for iron compound in GINP solution. In size analysis, GINP solutions were prepared freshly and these solutions were sonicated in ultrasonic bath at 45 kHz frequency.

In order to characterization of GINPs, SEM (Zeiss EVO 40) and EDX (Bruker) analyses were performed. Each solution was sampled on to different glass piece and every samples were dried at 80 °C for 1 hour. Gold-palladium coating was made for these samples before SEM-EDX and XRD analysis. Final GInPs product were analyzed with FT-IR (SHIMADZU IRTracer-100 Model) and ATR (Pike Tech).

Antibacterial activity of GINPS

The antimicrobial activity of synthesized GINPs was determined by the disc diffusion method. Antibacterial studies were performed in Hereous KSP-18 class II sterile cabinet in order to prevent external microbiological contaminations in experiment conditions and all equipment have been sterilized within the Nuve 40 autoclave for a period of 20 min at 121°C. For antibacterial testing, Gram negative (*Klebsiella pneumoniae, Escherichia coli)* and Gram positive (*Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus faecalis)* bacterial strains were used. Firstly, the 5 mg GINPs samples were located the hole in the previously prepared plate count agar media (PCA, Merck). Approximately 150 -200 µL bacterial solution of each bacterial solution with $2x10^6$ CFU/mL initial bacteria concentration was inoculated to these plates. After inoculation of the samples, the plates were incubated at 37°C in Innova-42 shaker series incubator with 18–24 h. Lastly, the dimension of the bacterial inhibition zone occurred in the surface of plates was measured for the investigation of antibacterial activity. Three independent experiments were performed and the average results were given to illustrate the antibacterial activity.

RESULTS and DISCUSSION

The size distribution analysis was given in the Figure 1. As a results of size distribution analysis, it was determined that size of produced GnIPs ranged from 10-100 nm in the case of 10 % (v/v) iron compound in total solution. Also, when GnIP solution prepared freshly, the agglomeration occurred in the solution quickly and created the flocks sized nearly 1000 nm.

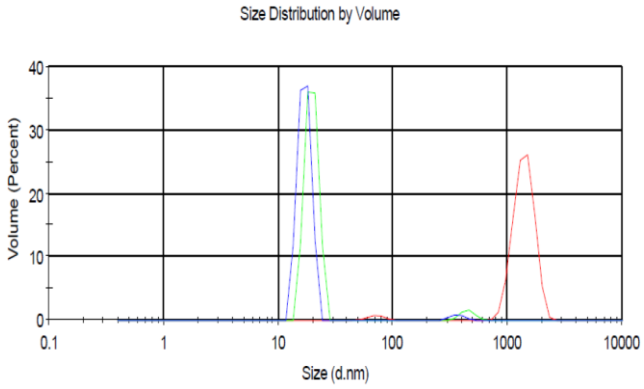

Figure 10. Size distribution analysis

XRD spectrum of green iron nanoparticle was given in the Figure 2. The nature and phase composition of GINPs were identified by X-ray powder diffractometer with Bragg's angle ranging from 10∘ to 90∘. XRD spectrum images demonstrated that the prepared GINPs have amorph structure and uniform particle size distribution and mostly zero-oxidation state. Kanagasubbulakshmi and Kadirvelu [27] studied the green synthesis of iron oxide nanoparticles using *Lagenaria siceraria* and evaluation of its antimicrobial activity. In their study, the leaves extract was found to be capable in green synthesis of iron oxide nanoparticles and their characteristics were studied by using UV-visible spectrophotometer, SEM, EDX, XRD, Zeta sizer, and FT-IR. They reported that the synthesized Fe3O4-NPs were naturally stabilized, cubic shaped and in the size range of 30 mm-100 nm.

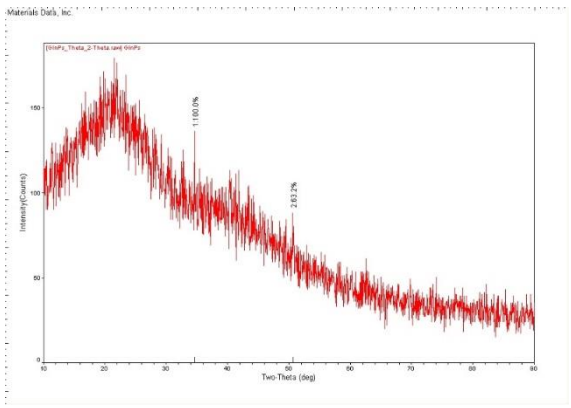

Figure 2. XRD spectrum of GINPs

Kanagasubbulakshmi and Kadirvelu [27] studied the X-ray diffraction patterns obtained for the INPs synthesized, and they found that the exits strong diffraction peaks with 2θ values of 28.26º, 32.28º corresponding to the hkl value of 220,222, and the average crystallite sizes of the produced INPs with 14 nm - 18 nm size. Their results indicated that all the nanoparticles had spinel structure with face-centered cubic phase. In the other study, Naseem and Farrukh [28] investigated the presence of Fe nanoparticles synthesized by *Gardenia* leave extract was confirmed by a series of reflection angles (2θ) at 44.34∘ and 64.43∘ having hkl values (111), (200) and (202), respectively, with cubic plane of Fe. Also, Mahdy et. al investigated the antimicrobial activity of zero-valent iron nanoparticles. Their results indicated that the synthesized NZVI particles showed distributed spherical particles approximately 2µm in size. Their XRD spectrum had two major diffraction intensity peaks at 2θ = 36.08° and 41.01°. These peaks were identified to originate from the (111) and (200) planes of FeO respectively [29].

SEM images of green iron nanoparticle at 20 kV EHT voltage and 11 mm working distance with different magnification were given in Figure 3. Also, EDX spectrum of GINPs was given in the Figure 4. Final GINPs product was analyzed with FT-IR as shown in the Figure 5.

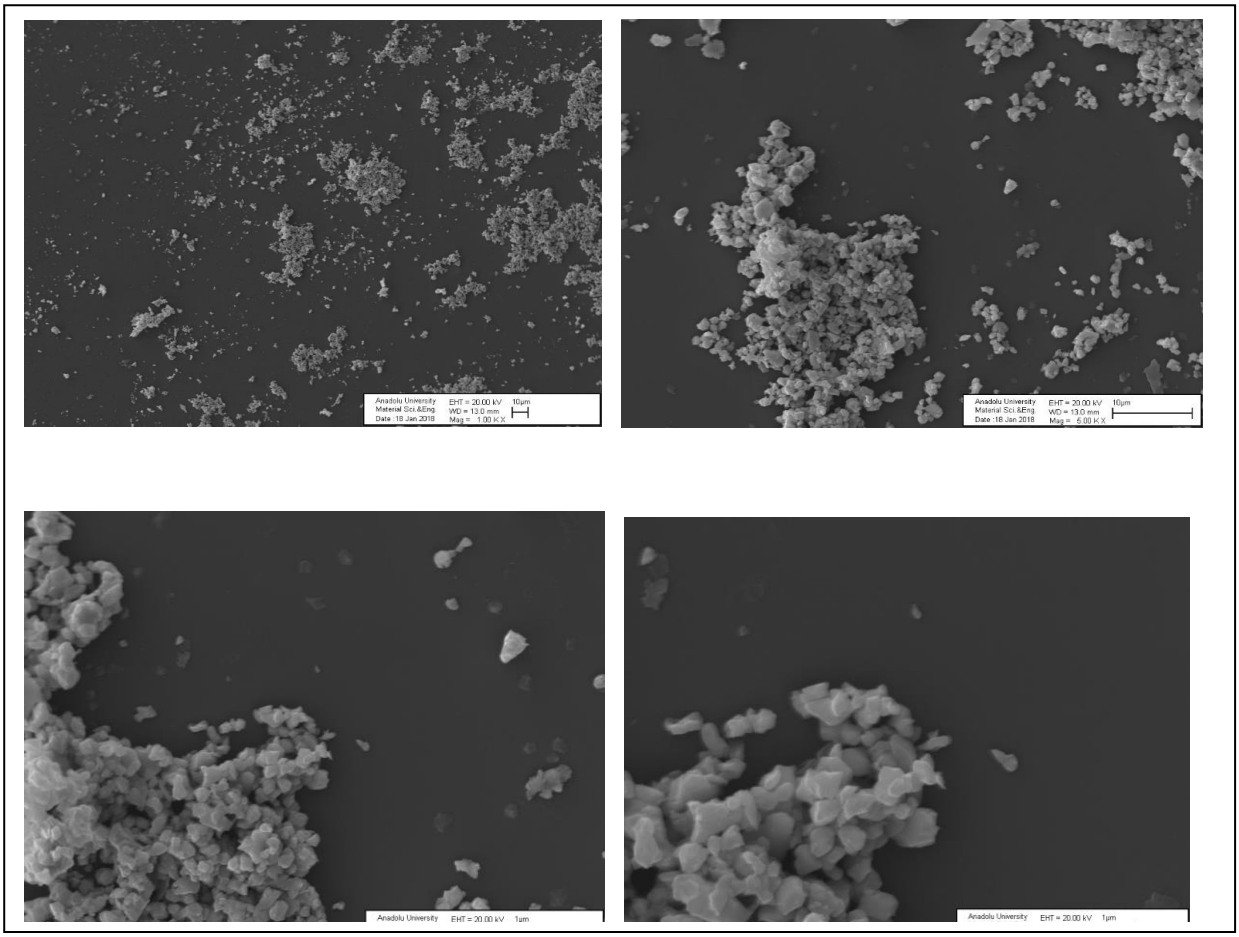

Figure 3. SEM images of GINPs

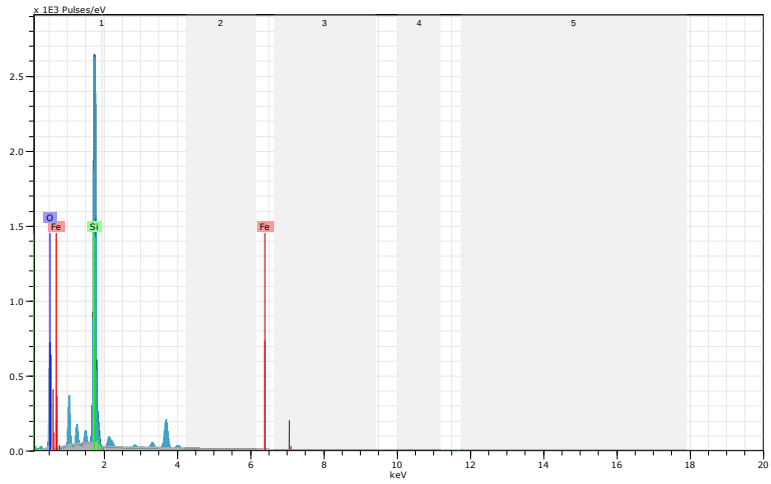

Figure 4. EDX spectrum of GINPs

SEM images demonstrated that size distribution of the cubic GnIPs ranged smaller than 100 nm. Also, it was clearly obtained that GnIPs come together and agglomerated. According to EDX result, the main elements were O, Si and Fe in EDX spectrum. Also, C, Na, Mg, Al, P, Cl, and K elements found in GnIP solution. EDX elemental results of green iron nanoparticles were summarized in Table 2. The result of EDX analysis indicated that GnIPs were produced as FeO form. Other SiO2 compound pointed that glass piece used for preparation of SEM-EDX analysis. In the literature, Sunitha et. al used TEM to determine the morphology and

shape of biosynthesized iron nanoparticles. They reported that the low magnification TEM micrographs revealed that the particles are spherical in shape and uniformly distributed (monodispersed) without significant agglomeration. They found that the iron nanoparticles synthesized using Indian gooseberry extracts were spherical with sizes ranging from 40 to 63 nm while TEM image of chemically synthesized iron nanoparticles have diameters of around 23 nm [30].

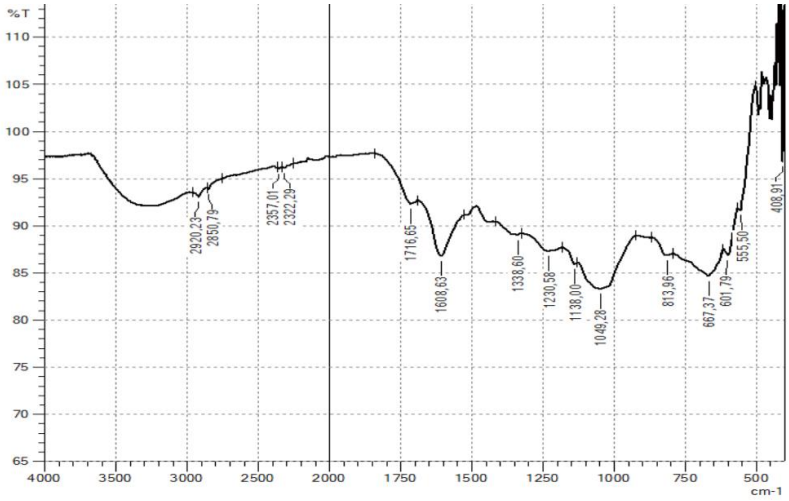

Figure 5. FTIR spectrum of GINPs

When analyzing final GInPs product, Fe-O peak observed between 600 and 1600 $cm^{-1,}$ Fe-CI peak observed at 1597 cm^{-1} and O-H peak observed 3000- 3500 cm^{-1} was disappeared in the spectrum at the end of GInPs production process. Kanagasubbulakshmi and Kadirvelu reported the strong peak at 624 cm^{-1} corresponds to the inorganic stretching indicates the iron NPs with -52 meV zeta potential proving highly stable due to the strong negative surface charge [27]. Also, Naseem and Farrukh reported that the absorption band between 3400 and 3200 cm^{-1} represented –OH group stretching and another peak at 2700 cm^{-1} referring C–H stretching. The peak at 612.63 cm^{-1} was reported due to Fe vibrations [28]. The inhibition zone determined in the disc diffusion test were summarized in the Table 1. The antibacterial inhibition zone images were given in the Figure

Table 5. Antibacterial Disc diffusion test result of GINPS

Bacteria strain		Initial bacterial concentration (CFU/mL)	Inhibition zone diameter (mm)
Gram negative	*Klebsiella pneumoniae*	$2.1x10^6$	24±2
	Escherichia coli	$2.2x10^6$	28±2
Gram positive	*Bacillus cereus*	$2.0x10^6$	31±3
	Staphylococcus aureus	$2.1x10^6$	21±1
	Staphylococcus faecalis	$2.3x10^6$	22±1

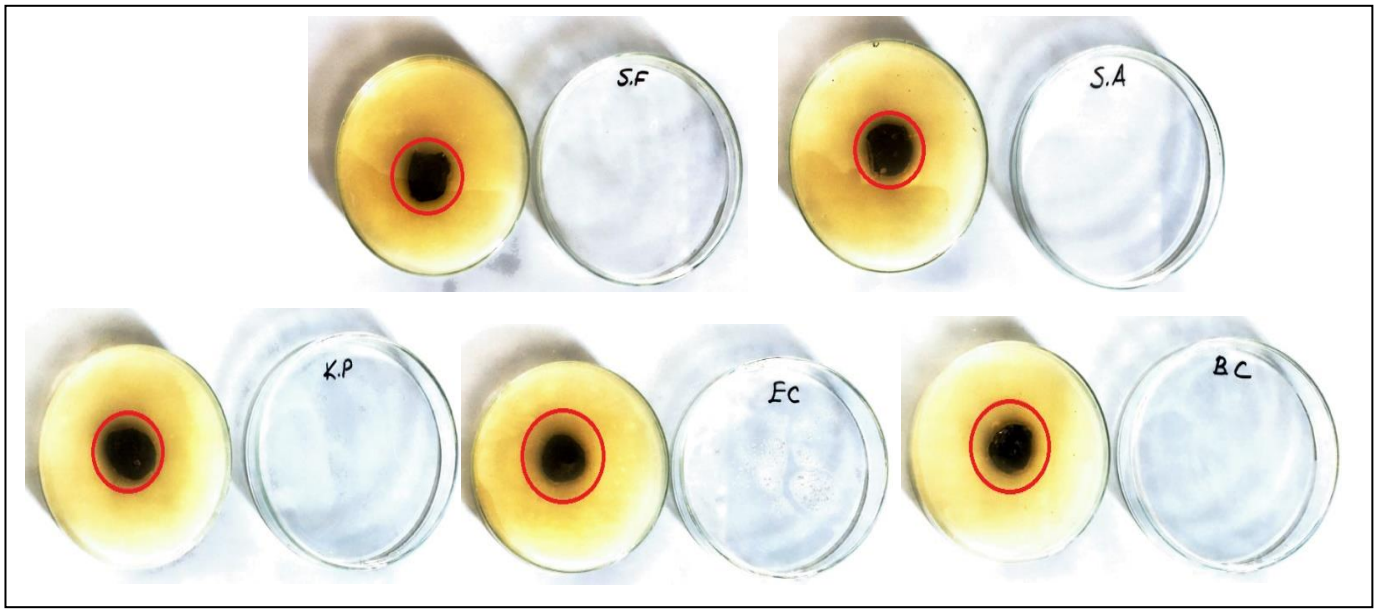

Figure 6. The antibacterial inhibition zone images of GINPS

Figure 6 showing the inhibition zone diameters of GINPS, large zone diameters were observed against all bacteria strain referring high antibacterial effect because the zone diameters were bigger than 20 mm Kanagasubbulakshmi and Kadirvelu stated that the antimicrobial property of synthesized INPs was evaluated against Gram negative *Escherchia coli*, Gram positive *Staphylococcus aureus* when the zone of inhibition was found to be 10 mm for *Escherchia coli*, and 8 mm for *Staphylococcus aureus* [28]. Mahdy et. al investigated that *St. aureus*, *E. coli* were grown in the presence of different ZVIN nanoparticles concentrations for 24 hours. MTT assays were performed and the results provide evidence that ZVIN nanoparticles. FeO nanoparticles MIC of *E. coli* and *St. aureus* at concentrations 30 µg/ml, whereas these bacteria were completely inhibited at concentrations 60 µg/ml [29]. Sunitha et. al investigated that the microbially synthesized iron nanoparticles prepared by the fungal strain showed antimicrobial activity against only Bacillus, E. coli, Staphylococcus sps. identified by zone formation. The green synthesized iron nano particles prepared by *Phyllanthus emblica* showed activity against *Klebsiella sps.* The chemically synthesized iron nanoparticles prepared by co-precipitation method showed antimicrobial activity against only Streptococcus sps. They explained the antibacterial activity of microbial synthesized iron nanoparticles against Bacillus, E.coli and Staphylococcus sps. They showed taht antimicrobial activity was seen in microbially synthesized iron nanoparticles is due to the fact that ROS such as superoxide radicals (O2−), hydroxyl radicals (-OH), hydrogen peroxide (H_2O_2), and singlet oxygen($^1O_2$), cause damage to proteins and DNA in bacteria by generating oxidative stress[30].

CONCLUSION

In this study, antibacterial effect of GINPs which were synthesized by the environmentally friendly method using Camellia sinensis extract were carried out. The average particle size of GINPs is in the range of 10-100 nm. The prepared nanoparticles have amorph structure and uniform particle size distribution and mostly zero-oxidation state. The prepared GINPs showed high antimicrobial activity against gram-negative and gram-positive bacteria.

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➢ *ORAL PRESENTATION*

Computational selection of novel 4-thiazolidinone derivatives containing sulfonamide structure as inhibitors against MPro and RdRp of SARS-CoV-2: A molecular docking study.

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Abstract
COVID-19 is a disease that has spread all over the world since December 2019 and has fatal consequences. The main protease (Mpro) and RNA-dependent RNA polymerase (RdRp) that among the identified proteins/enzymes of the SARS-CoV-2 by structural biological approaches, have been best drug discovery targets for computer-aided drug discovery. In this study, mechanism of binding to SARS-CoV-2 main proteaz (MPro) and SARS-CoV-2 RNA dependent RNA polymerase (RdRp) enzymes for new 5-arylidene-1,3-thiazolidin-4-one derivatives containing sulfonamide structure was evaluated using computer-assisted molecular modelling techniques. As results of, compounds **H4** and **H17** have exhibited the best binding poses with -7.9 kcal/mol and -8.1 kcal/mol binding energy against Mpro and RdRp, respectively.
Keywords: SARS-CoV-2 RdRp, SARS-CoV-2 MPro, 1,3-thiazolidin-4-one, molecular docking..

INTRODUCTION

Human coronavirus (COVID-19) caused by SARS-CoV-2 that is new emerged, highly-infectious and lethal pandemic strain, was identified from China in December 2019. The outbreak was declared a pandemic on 11th March, 2020 by WHO and 58.900.547 confirmed cases of COVID-19, including 1.393.305 deaths were reported until November 2020. Additionally the COVID-19 outbreak has imposed major challenges for global health, society and economy due to mandatory isolations and quarantines of millions of people. Today, besides to ongoing vaccine studies, there are no specific drugs are available for treatment and management of this disease and the need for the development of specific antiviral therapeutics toward SARS-CoV-2 has been continued (Parvez et al, 2020). Numerous clinical trials and drug discovery efforts for identifying potential therapies against COVID-19 require high cost and time. However, computer-aided drug discovery provides significant advantage for the prediction of potential molecules before synthesis and *in-vitro* testing. The structures of different proteins/enzymes of the SARS-CoV-2 were identified by structural biological approaches, and RNA-dependent RNA polymerase (RdRp), papain-like protease, and the main protease have been important drug targets for molecular docking studies. Among these targets, the main protease (MPro) also known as 3-chymotrypsin-like protease (3CLpro), and RNA-dependent RNA polymerase (RdRp) that has been targeted for various viral infections such as hepatitis C have been best drug discovery targets (Al-Masoudi et al, 2020; Elfiky, 2020). In previous studies by our group, 1,3-thiazolidin-4-one derivatives were determined as inhibitor of HCV RdRp enzyme Küçükgüzel et al, 2013; Çakır et al, 2015). Morever, HIV protease inhibitor tipranavir, which has a sulfonamide structure, is still used in the treatment of HIV infections (De Clercq, 2001). In the present study, *in silico* inhibition potentials of new 5-arylidene-1,3-thiazolidin-4-one derivatives containing sulfonamide structure designed against SARS-CoV-2 RdRp (nsp12) and main protease (MPro) were investigated.

MATERIALS AND METHODS

Preparation of ligands

The designed compounds were drawn with the Spartan 04 software (SPARTAN 04, Wavefunction, Inc., Irvine, USA) and optimized for each compound by using the semi-empirical PM3 method. For each compound, the most stable conformation was utilized in docking calculation and The AutoDock Tools program was used to generate the docking input files (Morris et al, 2009).

Preparation of enzymes for docking studies

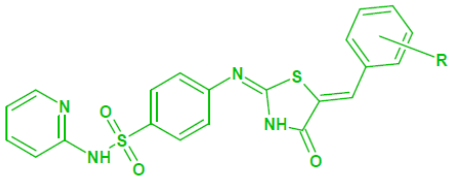
The structures of MPro (pdb code: 6zru) (Orlemans et al, 2020) and RdRp (pdb code: 6m71)(Gao etal, 2020) were obtained from the protein databank. Water molecules and co-crystallized inhibitor (boceprevir for MPro) were deleted. This structure was protonated using the The AutoDock Tools program and thereafter the obtained structure was energy-minimized.

Docking studies

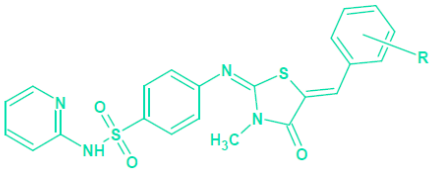
AutoDock Vina software was used for the designed compounds into the both enzymes MPro and RdRp structures docking calculations5. The results files were analyzed using Accelrys Discovery Studio Visualizer 4.0 program. For MPro enzyme, the size of binding pocket was detected 40x40x40 points in x (-19.103), y (-15.385), and z (16.652) dimensions was built; appropriate to the position of boceprevir in the 6ZRU structure. For RdRp enzyme, the size of binding pocket was detected 30x30x30 points in x (114.52), y (114.11), and z (122.91) dimensions was built; appropriate to the positions of Asp760 and Asp761 residues in the 6M71 structure (Ahmad et al., 2020). The Vina parameters "exhaustiveness" were set to the value of 10, besides a grid spacing of 0.375 Å were employed for the calculation of the energetic map of both enzymes. During study of validations as well as our docking of ligands was used flexible ligand in rigid protein.

RESULTS and DISCUSSION

In this study, *in silico* inhibition potentials of designed compounds **H1-20** and **M1-20** against SARS-CoV-2 RdRp (nsp12) and MPro (nsp5) were investigated and the results obtained by using Autodock Vina are presented in the Table 1. Sulfamoyl group of all designed compounds showed hydrogen bond interaction with active site of both enzymes. In addition to, hydrogen bond interaction was detected 1,3-thiazolidin-4-on ring of compounds **H1-20** with His41 and Cys145 residues of MPro enzyme as well as Asn623 and Asn691 residues of RdRp enzyme. When the results of compounds **M1-20** are examined, while the interactions of the sulfamoyl group with both enzymes active sites continues, the hydrogen bond interactions of the thiazolidinone ring with related aminoacids have decreased and even these interactions have been disappeared for RdRp. Also presence of substituted 5-arylidene structure has been contributed to the affinity against the active site with hydrophobic and hydrogen bond interactions.



Compound **H1-20**



Compound **M1-20**

Table 6. Binding energy of designed compounds **H1-20** and **M1-20** in active site of Mpro and RdRp enzymes for SARS-CoV-2.

Compound	R	MPro ΔG (kcal/mol)	RdRp ΔG (kcal/mol)	Compound	R	MPro ΔG (kcal/mol)	RdRp ΔG (kcal/mol)
H1	4-OCH_3	-7.3	-7.0	M1	4-OCH_3	-7.2	-7.3
H2	4-$N(CH_3)_2$	-7.6	-7.2	M2	4-$N(CH_3)_2$	-7.0	-7.7
H3	4-F	-7.4	-7.5	M3	4-F	-7.3	-7.1
H4	4-Cl	-7.9	-7.0	M4	4-Cl	-7.3	-7.2
H5	4-OH	-7.7	-7.2	M5	4-OH	-7.1	-7.5
H6	2-OCH_3	-7.3	-7.0	M6	2-OCH_3	-7.1	-7.2
H7	2-F	-7.8	-7.1	M7	2-F	-7.6	-7.5
H8	2-Cl	-7.1	-7.0	M8	2-Cl	-7.0	-7.5
H9	3-OCH_3	-6.9	-7.3	M9	3-OCH_3	-7.1	-7.3
H10	3-F	-7.7	-7.6	M10	3-F	-7.1	-7.4
H11	2,6-F_2	-7.5	-8.0	M11	2,6-F_2	-7.5	-7.7
H12	2,6-Cl_2	-7.3	-7.7	M12	2,6-Cl_2	-7.3	-7.0
H13	2,4-F_2	-7.4	-7.8	M13	2,4-F_2	-7.4	-7.8
H14	2,4-Cl_2	-7.7	-7.8	M14	2,4-Cl_2	-7.7	-7.5
H15	2-Cl-6-F	-6.8	-7.9	M15	2-Cl-6-F	-6.8	-7.6
H16	4-CF_3	-7.4	-8.0	M16	4-CF_3	-8.0	-7.6
H17	4-OCF_3	-7.5	-8.1	M17	4-OCF_3	-7.5	-7.7
H18	4-CH_3	-7.2	-7.8	M18	4-CH_3	-7.2	-7.4
H19	2-CH_3	-7.1	-7.7	M19	2-CH_3	-7.1	-7.8
H20	H	-6.9	-7.7	M20	H	-6.9	-7.2

When interactions with the binding site are examined, molecular docking studies showed that most active compounds **H4** and **H17** against MPro and RdRp, respectively. The interactions of both compounds with the binding site of the related enzyme are given in the Figure 1.

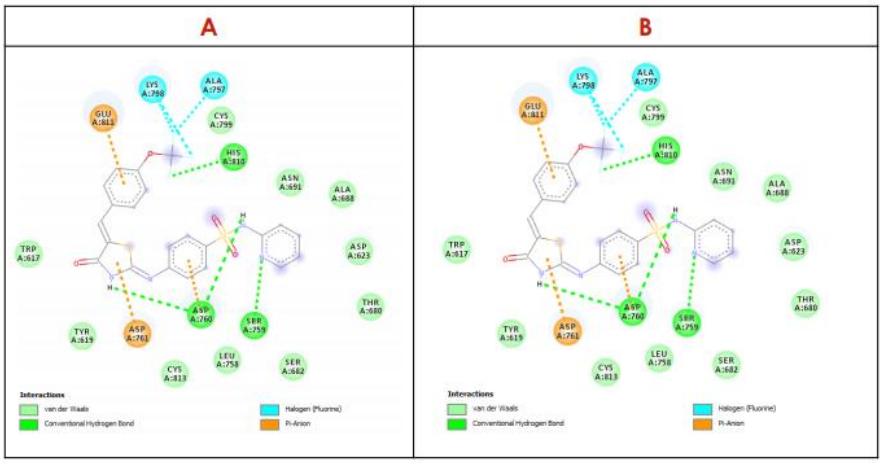


Figure 1. A) Interactions of compound H4 with SARS-CoV-2 MPro active site. B) Interactions of compound H17 with SARS-CoV-2 RdRp active site.

CONCLUSION

In the present study, *in silico* inhibition potentials and mechanism of binding of new 5-arylidene-1,3-thiazolidin-4-one derivatives containing sulfonamide structure against SARS-CoV-2 main proteaz (MPro) and RNA dependent RNA polymerase (RdRp) enzymes were evaluated. Molecular docking studies showed that sulfamoyl group and 1,3-tiazolidin-4-one ring are required for hydrogen bond interaction with the active site of related targets. However methyl substitution at position 3 of the thiazolidinone ring has been limited the H-bond interactions of the ring with the active site, especially for RdRp. In our study, while thiazolidinone derivatives designed based only on sulfapyridine have been docked for MPro and RdRp, and the data obtained have been showed that new derivatives from other sulfonamides could promise hope for COVID-19. Finally, synthesis studies of the designed compounds based on sulfapyridine have been started.

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➢ *ORAL PRESENTATION*

Tedavi amaçlı kullanılan *Ganoderma lucidum* (kırmızı reishi mantarı) etkinliğinin karbon tetraklorür toksikasyonunda siklooksijenaz-2 (COX-2) immunoreaktivitesine etkisi

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Özet

Karbon tetraklorür (CCl_4) genel toksik etkiye sahip bir bileşiktir. Deri, solunum ve gastrointestinal sistem tarafından emilir. Çalışmamızda yetişkin dönemdeki sıçanlara karbon tetraklorür ve ganoderma lucidum uygulaması yapılarak siklooksijenaz-2 (COX-2)'nin immunohistokimyasal lokalizasyonu incelendi. Çalışmada 8-10 haftalık 32 adet yetişkin Spraque-Dawley ırkı erkek sıçan kullanıldı. Sıçanlar; konrol-sham, CCl_4, Ganoderma lucidum (GL), CCl_4+GL olmak üzere 4 gruba ayrıldı. Deneysel uygulamalar sonucunda kontrol ve GL grubunda karaciğer dokusu normal, CCl_4'ün ise çeşitli histoptolojik lezyonlar oluşturduğu gözlendi. GL verilen grupta ise lezyon şiddet ve yoğunluğunda azalmalar tespit edildi. Karaciğer dokusunda COX-2 lokalizasyonu Streptavidin-Biotin-Peroksidaz Kompleks tekniği ile immunohistokimyasal yöntemle tespit edildi. Tüm gruplarda COX-2 immunoreaktivitesi benzer yoğunlukta tespit edilirken, CCl_4 gubunda ise COX-2 immunoreaktivitesi sentrilobüler ve perilobüler bölgede diğer gruplara oranla daha yoğun olarak gözlendi. COX-2 immunoreaktivitesi; sentral venadan kierman aralığına kadar olan bölgede hepatosit sitoplazmasında daha yaygın olarak saptanırken, hepatosit nükleusunda sporadik olarak tespit edildi. Biyokimyasal parametre olarak ise Total antioksidan seviye (TAS), Total oksidan seviye (TOS), Aspartat aminotransferaz (AST) ve Alanin aminotransferaz (ALT) düzeyleri değerlendirildi. Kontrol ve GL grupları arasında Total antioksidan seviye (TAS), Total oksidan seviye (TOS), Aspartat aminotransferaz (AST) ve Alanin aminotransferaz (ALT) düzeyleri bakımından istatistiki anlamda bir fark görülmedi. CCl_4 ve CCI4+GL gruplarından elde edilen biyokimyasal veriler arasında TAS, TOS, AST ve ALT düzeyleri bakımından istatistiki bir fark bulunmamaktadır. Ancak TAS, TOS ve AST düzeyleri bakımından bu iki grup ile kontrol ile Ganoderma lucidum grupları arasında istatistiki önemde bir fark tespit edildi ($p<0.01$). ALT düzeyi değerlendirildiğinde; CCl_4 grubu ile kontrol ve Ganoderma lucidum arasında istatistiki bir fark vardır ($p<0.05$). Ancak diğer biyokimyasal belirteçlerden farklı olarak CCl_4+GL grubu ile kontrol ve Ganoderma lucidum grupları arasında istatistiki bir fark gözlenmemiştir ($p>0.05$).

Anahtar Kelimeler: Ganoderma lucidum, karbon tetraklorür, siklooksijenaz-2, immunoreaktivite, total oksidan/antioksidan kapasite

Effect of Ganoderma lucidum (red reishi mushroom) used for therapeutic purposes on cyclooxygenase-2 (COX-2) immunoreactivity in carbon tetrachloride toxicity

Abstract

Carbon tetrachloride (CCl_4) is a compound with a general toxic effect. Respiration involves absorption by the skin and gastrointestinal tract. In the present study, immunohistochemical localisation of cyclooxygenase-2 (COX-2) was investigated in adult rats that were administered carbon tetrachloride and ganoderma lucidum. In the study, 32 adult Spraque-Dawley male rats aged 8-10 weeks were used. The rats were divided into 4 groups; control-sham, CCl_4, Ganoderma lucidum (GL) and CCl_4+GL. As a result of the experimental applications, it was observed that liver tissue was normal in the control and GL groups, while CCl_4 caused various histoptological lesions. Localisation of COX-2 in liver tissue was determined with immunohistochemical method using the Streptavidin-Biotin-Peroxidase Complex technique. In all groups, COX-2 immunoreactivity was found to have a similar intensity, while in the CCl_4 group COX-2 immunoreactivity was more intensive in the centrilobular and perilobular regions than in other groups. COX-2 immunoreactivity; while it was more common in the hepatocyte cytoplasm in the region from the sentral

vena to the kierman range, it was detected sporadically in the hepatocyte nucleus. Total antioxidant level (TAS), Total oxidant level (TOS), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels were evaluated as biochemical parameters. There was no statistically significant difference between the control and GL groups in terms of Total antioxidant level (TAL), Total oxidant level (TOL), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels. There was no statistical difference between the biochemical data obtained from CCI_4 and CCI_4+GL groups in terms of TAL, TOL, AST and ALT levels. However, a statistically significant difference was observed between these two groups and the control and Ganoderma lucidum groups in terms of TAL, TOL and AST levels ($p<0.01$). When ALT levels were assessed, there was a statistical difference between the CCI_4 group and the control and Ganoderma lucidum groups ($p<0.05$). However, unlike other biochemical markers, no statistical difference was observed between the CCI_4+GL group and the control and Ganoderma lucidum groups ($p>0.05$).
Keywords: Ganoderma lucidum, carbon tetrachloride, cyclooxygenase-2, immunoreactivity, total oxidant/antioxidant capacity

GİRİŞ

Ganoderma lucidum ve ilgili türleri, en az 4 bin yıldır tedavi edici amaçlar için kullanılmaktadır (Zhao ve Zhang, 1994). Bu mantar Japonya'da Reishi veya Mannetake (10 bin yıl mantarı) olarak adlandırılır. Çin ve Kore'de ise, farklı olarak Ling Chi, Ling Chih ve Ling Zhi (Ölümsüzlük Mantarı) olarak adlandırılmaktadır.

Binlerce yıldır geleneksel Çin halk tababetinde kullanılan bu mantarın basidiokarpının (fruiting body) (Nasreen ve ark., 2005; Liu ve ark., 2009) günümüzde de birçok hastalığa iyi geldiği bilimsel yollarla gösterilmiştir. Antialerjik, anti-inflamatuar (Kim ve ark., 2006), antioksidan (Yang ve ark., 2012), antihistaminik (Joseph ve ark., 2011), antikoagülant (Hsu ve ark., 2011), antimikrobiyal (Quereshi ve ark., 2010) aktivitelerinin yanısıra, karaciğer hastalıkları (Chien ve ark., 2004; Lin ve Zhang, 2004; Liu ve Zhang, 2005), nefrit (Fujita ve ark., 2005), artrit (Li ve ark., 2007), gastrik ülser (Liu ve ark., 2004), hiperlipemi (Kohguchi ve ark., 2004;Yang ve ark., 2006; Jung ve ark., 2006; Oluba ve ark., 2010), kanser (Tang ve ark., 2006, Cao ve Lin, 2003; Chen ve ark., 2004; Stanley ve ark., 2005; Kao ve ark., 2011; Montemayor ve ark., 2011), kronik koroner kalp hastalığı (Vukojevic ve ark., 2006), astım (Li ve ark., 2011), hipertansiyon (Zheng ve ark., 2006), bronşit (Kim ve ark., 2004), lökopeni (Gao ve ark., 2012), diyabet (Sheena ve ark., 2005), anoreksiya (Wagner ve ark., 2003; Sliva ve ark., 2003), skleroderma (Sliva, 2003) gibi hastalıklar üzerine pozitif etkileri mevcuttur. Ek olarak radyasyon zararlarına karşı koruyan (Fei ve ark., 2006), sedatif (Avtonomova ve ark., 2006) etki gösteren, yorgunluğu gideren (Zjaion, 2004; Lai ve ark., 2010; Ma ve ark., 2011), aneljezik (Han, 2010) etki gösteren ve immunolojik bozukluklardan kaynaklanan hastalıkların tedavisinde kullanılan popüler bitkisel bir ajandır (Karadeniz, 2012; Liu ve ark., 2002; Wicks ve ark., 2007).

MATERYAL VE METOD

Deneysel dizayn:

Çalışma için gerekli olan etik kurul izni Gaziantep Üniversitesi Hayvan Deneyleri Yerel Etik Kurulundan alınmıştır (GAÜN-DAM, Karar no: 2018/22). Bu çalışmada materyal olarak Gaziantep Üniversitesi Deney Hayvanları Araştırma Merkezinden alınan 250-300 gr. civarındaki 32 adet yetişkin (8-10 haftalık) Spraque-Dawley ırkı erkek sıçan kullanıldı. Sıçanların bakımları ve madde uygulamaları yine Gaziantep Üniversitesi Deney Hayvanları Araştırma Merkezindeki araştırma ünitelerinde yapıldı. Sıçanlar, ortam sıcaklığı 21 °C olan 12 saat aydınlık ve 12 saat karanlık ortamda tutulacak, standart sıçan yemi (%21 ham protein içeren) ve musluk suyu ile beslendi. Denekler, tüm gruplarda 8 er olmak üzere toplam 32 adet sıçandan oluşturuldu. Sıçanlar; konrol-sham, karbon tetraklorür (CCI_4), Ganoderma lucidum (GL), Karbon tetraklorür (CCI_4)+ Ganoderma lucidum olmak üzere 4 gruba ayrıldı. Kontrol grubuna 2 ml/kg dozunda serum fizyolojik gavaj yoluyla 14 gün, GL grubuna 1000 mg/kg dozunda Ganoderma lucidum ekstratı gavaj yoluyla 14 gün, CCI_4 grubuna 10 ml/kg dozunda karbon tetraklorür tek doz intraperitoneal olarak sadece ilk gün, CCI_4+GL gurubuna ilk 3 gün gavaj yoluyla Ganoderma lucidum ekstratı verildikten sonra 10 ml/kg dozunda CCI_4 tek doz uygulanır. Daha sonra 14 gün boyunca sadece gavaj yolla Ganoderma lucidum ekstratı uygulandı. Deney sonunda sıçanlar intra muskülar (i.m) yolla yapılan ketamin hidroklorür/xylasin (80/10 mg/kg) anestezisi altında servikal dislokasyonla öldürüldükten sonra disekte edildi. Çalışma başlangıcında ve sonunda tüm hayvanların tartımları yapıldı. Çalışmanın başlangıcında, hayvanlar ortalama ağırlıkları birbirine yakın olmak koşulu ile rasgele 4 gruba ayrıldı.

Deneysel uygulamalar sonunda çalışılacak bilimsel disiplinler için (histopatoloji, immunohistokimya, biyokimya) tüm gruplardan (Grup I, II, III, IV) doku alım planı:

Histopatoloji ve İmmunohistokimya analizi için: Sıçanlar anestezi altında iken servikal dislokasyonla öldürülerek bir kısım karaciğer dokuları alındı. Bu dokulardan elde edilen preparatlarda histopatolojik değişimler olup olmadığı gözlemlendi. İmmunohistokimya için ise alınan karaciğer dokularından elde edilecek histolojik kesitlere immunohistokimyasal metod uygulanarak COX-2 immunoreaktivitesi belirlendi.

Biyokimyasal analiz için: Sıçanlar anestezi altında iken intrakardiyak olarak kanları alındı. Daha sonra servikal dislokasyonla öldürülerek disekte edildi ve bir kısım karaciğer dokusu alınarak, total oksidan ve total antioksidan enzim düzeyleri saptandı.

Histolojik Analiz:

Deney süresi sonunda, genel anestezi altında servikal dislokasyonla öldürülen ve disekte edilen sıçanların karaciğer dokusu %10 luk tamponlu formol solüsyonu içerisinde tespit edildi. Tespit sonrası rutin doku takibi (dereceli alkoller, metil benzoat ve benzol takibi) ardından alınan dokular parafine gömülerek bloklardan önceden krom alum jelatin (CAG) ile kaplanmış lamlara mikrotom ile 5 µm'lik seri kesitler alındı. Alınan kesitlere histolojik boyama yöntemlerinden hematoksilen-eosin uygulanarak histopatolojik değişimler ışık mikroskobik düzeyde incelendi (Luna, 1968).

İmmunohistokimyasal Analiz:

Sıçanlardan alınan karaciğer dokuları COX-2 immunoreaktivitesi için %10'luk tamponlanmış formaldehit çözeltisinde tespit edildi ve daha sonra dereceli alkoller, metil benzoat ve benzollerden geçirildikten sonra parafinde bloklandı. Parafin bloklardan alınan 4-5 µm'lik kesitlere oda sıcaklığında 1 saat süreyle nemli ortamda anti-COX2 (Cyclooxygenase 2 antibody ab15191) primer antikoru 1/100 oranında uygulandı. Negatif kontrol grubu için doku kesitlerine sadece PBS (phosphate buffer solution) damlatıldı. Primer antikor inkübasyonundan sonra indirekt yöntemlerden Streptavidin-biotin peroksidaz tekniği kullanıldı (Shu ve ark., 1988; Nur ve ark., 2015). 3-Amino-9-Ethylcarbazole (AEC) eklenerek kromojen uygulaması yapıldı. AEC solüsyonu eklendikten sonra ışık mikroskobunda kesitler kontrol edilerek immunoreaktivite geliştiğinde reaksiyon distile su ile durdurularak, mayer hematoksilenle zıt boyama yapıldı. İşlemler sonunda kesitler kurulanarak su bazlı yapıştırıcı damlatılıp lamelle kapatıldı. Preparatlarda rastgele alanlar seçilerek Zeiss Primo Star marka entegre kameralı ışık mikroskobunda incelendikten sonra fotoğrafları çekildi ve değerlendirme semi-kantitatif olarak immunoreaktif bir skora göre yoğunluk incelendi. (Nur ve ark., 2015; Seidal ve ark., 2001; Zhu, 1989). Hücrelerdeki COX-2 immunoreaktivitesi renklerin koyuluk derecesine göre, birbiriyle mukayese edilerek belirlendi.

Biyokimyasal Analiz:

Total antioksidan Seviye-TAS (Erel, 2004) ve Total Oksidan Seviye-TOS (Erel, 2005) ölçümü, Thermo Scientific Multiskan GO model spektrofotometre aracılığıyla ticari kitler kullanılarak ölçüldü. Aspartat aminotransferaz (AST) ve Alanin aminotransferaz (ALT) aktivite analizleri otoanalizör (HumaStar 600, Germany) kullanılarak ölçüldü.

Verilerin Analizi:

Çalışmadan elde edilen verilerin istatistiksel işlemleri SPSS paket programında (IBM SPSS Statistics 22) yapıldı. Verilerin normal dağılıma uygun olup olmadığını ortaya koymak amacıyla Kolmogorow-Smirnov normallik testinden yararlanıldı. Normal dağılım gösteren gruplar için; parametrik bir test olan tek yönlü varyans analizi (ANOVA) ve eğer deney grupları ortalamaları arasında farklılık varsa gözlenen bu farklılığın hangi grup ya da gruplardan kaynaklandığının saptanılması için grup ortalamaları üzerinde ‘‘Anova-Duncan’’ testi uygulandı ve $p<0.05$ değeri istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Biyokimyasal Bulgular

Çalışmadan elde edilen biyokimyasal veriler değerlendirildiğinde, kontrol ve Ganoderma lucidum gruplarındaki değerler normal ve birbirlerine yakındır. Kontrol ve Ganoderma lucidum grupları arasında TAS, TOS, AST ve ALT değerleri bakımından istatistiki anlamda bir fark gözlenmedi. CCl_4 ve CCl_4+GL gruplarından elde edilen biyokimyasal veriler arasında TAS, TOS, AST ve ALT düzeyleri bakımından istatistiki bir fark bulunmamaktadır. Ancak TAS, TOS ve AST düzeyleri bakımından bu iki grup ile kontrol ile Ganoderma lucidum grupları arasında istatistiki önemde bir fark tespit edildi ($p<0.01$). ALT düzeyi değerlendirildiğinde CCl_4 grubu ile kontrol ve Ganoderma lucidum arasında istatistiki bir fark vardır ($p<0.05$). Ancak diğer biyokimyasal belirteçlerden farklı olarak CCl_4+GL grubu ile kontrol ve Ganoderma lucidum grupları arasında istatistiki bir fark gözlenmemiştir ($p>0.05$). Çalışmadan elde edilen gruplara ait veriler Tablo 1 de gösterilmiştir.

Histolojik Bulgular

Karaciğer dokuları rutin tespit ve doku takibi işlemlerinden sonra paraffin blokları gömüldü. Bu bloklardan mikrotom yoluyla alınan 5 µm'lik seri kesitlerin hematoksilen-eosin boya ile boyanmasından sonra ışık mikroskobunda incelendi. Gruplardan elde edilen kesitlerde; kontrol ve Gonaderma lucidum uygulanan gruplarda merkezi vena ve kierman aralığının normal görünümde olduğu, remark kordonlarının düzenli olduğu, parankimal yapının normal olduğu ve hepatositler arasında sinuzoidler tespit edilmiştir. Portal bölgede ise hepatik arter, portal ven ve safra kanalı üçlüsünü barındıran bağ dokusu alanı normal yapıda bulunmaktadır (Şekil 3a, b, c). CCl_4 uygulanan grupta ise vena sentralis ve portal bölgede bulunan vende konjesyona rastlandı. Bu grup kesitlerinde sentral ven ve portal bölge arasında multifokal nekroz alanları ve hepatosellüler dejenerasyon gözlendi. Ayrıca hücre infiltrasyonu, hepatosit sitoplazmasında vakuol, düzensiz remark kordonları, sinusoidal dilatasyon ve konjesyona rastlandı (Şekil 3d, e). CCl_4 ve Ganoderma lucidum'un birlikte uygulandığı grupta ise fokal nekroz alanları ve hepatik dejenerasyon, konjesyon, sinusoidal dilatasyon ve hücre infiltrasyonuna rastlandı (Şekil 3f, g, h). Bu grupta meydana gelen lezyonların sıklığı CCl_4'ün tek başına verildiği gruba yakın olarak belirlenmiştir. Bu nedenle Ganoderma lucidum, CCl_4'ün meydana getirdiği karaciğerdeki dejenerasyonu tedavi açısından tek başına yeterli değildir (Tablo 2). Karaciğer dokusunda oluşan histopatolojik lezyonlara ait doku değişim derecelendirmeleri Tablo 2'de dunulmuş olup, frekans derecelendirmeleri Bernet ve ark. (1999)'dan (Bernet ve ark, 1999) uyarlanmıştır.

Tablo 1. Gruplara ait TAS, TOS, AST ve ALT değerleri ve istatistiki önem.

PARAMETRELER	**GRUPLAR**				p<
	Kontrol (n:8) Ort±SD	**Ganoderma lucidum (GL)** (n:8) Ort±SD	**CCl_4** (n:8) Ort±SD	**CCl_4+GL** (n:8) Ort±SD	
TAS (mmol Trolax eqivalen/L**)**	1,91 ± 0,13^{a}	1,85 ± 0,12^{a}	1,43 ± 0,13^{b}	1,56 ± 0,10^{b}	*
TOS (µmol H_2O_2 equiv./L**)**	7,33 ± 0,57^{b}	7,40 ± 0,55^{b}	8,96 ± 0,46^{a}	8,34 ± 0,48^{a}	*
AST (U/L**)**	158,56 ± 9,61^{b}	165,79 ± 10,59^{b}	203,38 ± 10,46^{a}	194,07 ± 17,46^{a}	*
ALT (U/L**)**	65,27 ± 11,89^{b}	67,10 ± 7,18^{b}	79,37 ± 4,94^{a}	74,14 ± 6,57^{a,b}	**

*: p<0.01=İstatistik olarak anlamlı fark, **: p<0.05=İstatistik olarak anlamlı fark, a,b: Aynı satırda farklı harf taşıyan grup ortalamalrı arası fark önemlidir. n: gruptaki denek sayısı, Ort±SD: Ortalama±Standart sapma.

Tablo 2. Karaciğer dokusunda histopatolojik lezyonlara ait doku değişim derecelendirmeleri.

Karaciğer lezyonları	Gruplar			
	Kontrol grubu	Ganoderma lucidum (GL)	(CCl_4)+GL	Karbon tetraklorür (CCl_4)
Hepatositlerde dejenerasyon	-	-	++	+++
İnfiltrasyon	-	-	++	+++
Remark kordonlarında düzensizlik	-	+	++	+++
Vasküler dejenerasyon	-	-	++	++
Sinüzoidal dilatasyon	-	-	++	++
Sentral ve portal konjesyon	-	-	++	++
Nekroz	-	-	++	+++
Sinuzoidal konjesyon	-	-	++	++
Vakuolizasyon	-	+	++	++

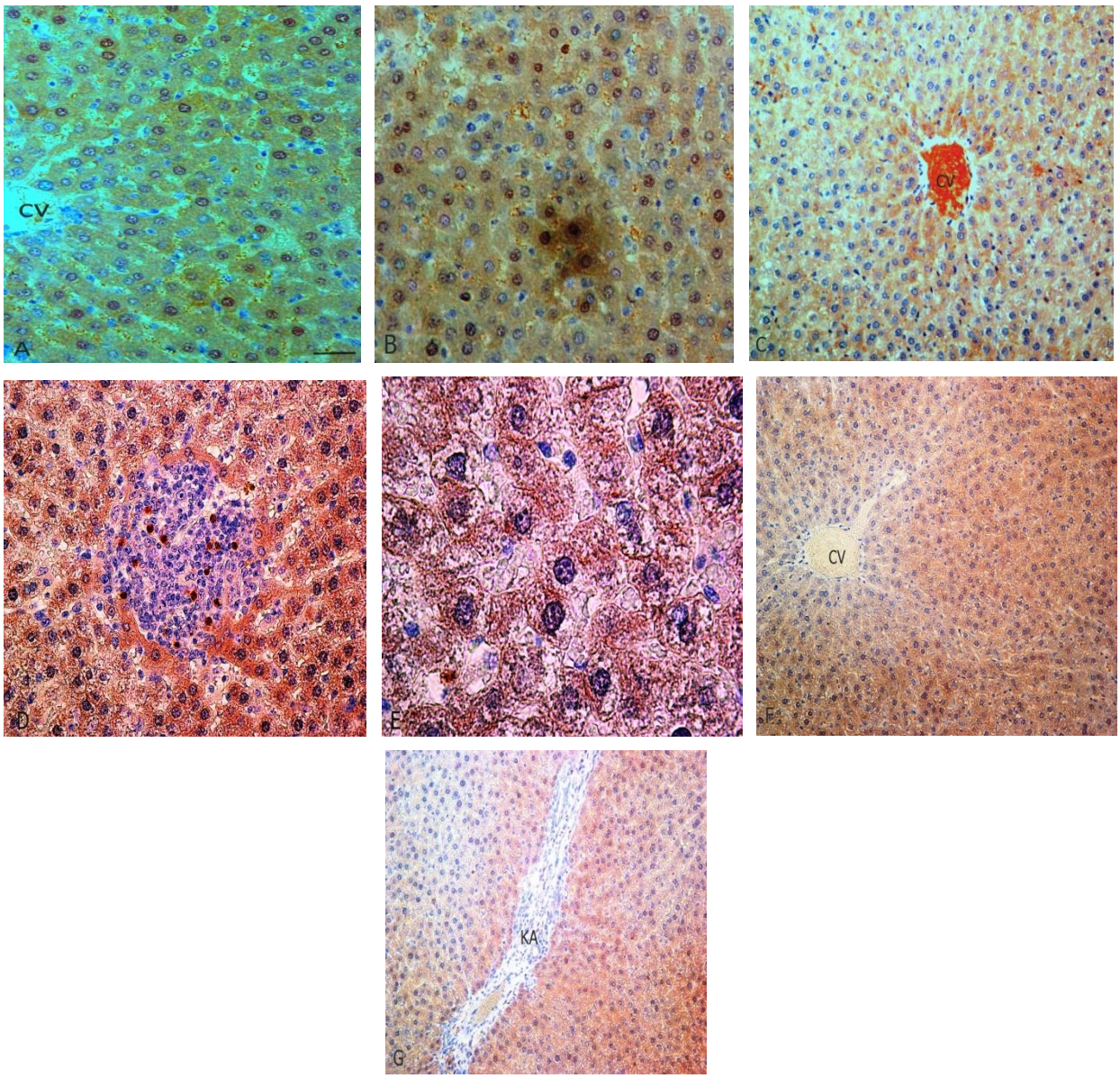
-: anormallik yok, +: anormallik frekansı düşük, ++: anormallik frekansı orta, +++: anormallik frekansı yüksek.

İmmunohistokimyasal Bulgular

COX-2 immunoreaktivitesi, kontrol, Ganoderma lucidum, CCl_4 ve CCl_4+Ganoderma lucidum gruplarından elde edilen preparatlarda değerlendirildi. Tüm gruplarda COX-2 immunoreaktivitesi vena sentralis bölgesinden kierman aralığına kadar olan bölgede hepatosit sitoplazmasında orta yoğunlukta yaygın olarak, hepatosit nükleusunda ise sporadik olarak gözlendi (Tablo 3, Şekil 1). COX-2 immunoreaktivitesi, CCl_4 grubunda gerek sentrilobüler gereksede perilobüler bölgede diğer gruplara oranla hepatosit sitoplazmasında yaygın olarak daha yoğun gözlendi.

Tablo 3. Kontrol, ve uygulama gruplarında COX-2 immunoreaktivite yoğunluğu. (+++) çok yoğun, (++) orta derecede yoğun, (+) az yoğun, (-) reaksiyon yok.

Siklooksijenaz	İmmun reaksiyon gösteren yapı	Kontrol grubu	*Ganoderma lucidum* grubu	CCl_4 grubu	*Ganoderma lucidum*+CCl_4 grubu
COX-2	Sentrilobüler bölge	+	+	+++	+
	Hepatosit sitoplazması	+	+	+++	++
	Hepatosit nükleusu	+	+	+	+
	Perilobüler bölge	+	+	+++	++



Şekil 1. Sıçan karaciğer dokusunda COX-2 immunoreaktivitesi. **A, B.** Kontrol grubu, bazı bölgelerde hepatosit sitoplazması ve nükleusunda orta yoğunlukta immunoreaktivite. **C.** Ganoderma lucidum grubu, vena sentralis çevresindeki hepatosit sitoplazmasında orta yoğunlukta immunorektivite. **D, E.** Karbon tetraklorür grubu, sentral vena ve kierman aralığı arasında hepatosit nükleuslarında orta yoğunlukta, hepatosit sitoplazmasında yoğun immunoreaktivite. **F, G.** Karbon tetraklorür+Ganoderma lucidum grubu, hepatosit sitoplazmasında vena sentralis civarında hafif, geri kalan kierman aralığına kadarki alanda orta yoğunlukta immun reaksiyon. CV: vena sentralis, KA: kierman aralığı, Bar: 50 µm.

TARTIŞMA VE SONUÇ

Karbon tetraklorür (CCl_4), karbondisülfürün klorlandırılmasıyla veya aynı bileşiğin kükürt monoklorür ile tepkimeye sokulmasıyla elde edilir. Bu madde solunum, deri ve gastrointestinal sistem ile emilir. Düşük dozlardaki CCl_4 karaciğer hücrelerinde yağlanmaya ve balon dejenerasyonuna, yüksek dozlarda ise karaciğer hücrelerinin nekrozuna neden olduğu bildirilmektedir (Şanlı, 1988; Vural, 1984). Karbon tetraklorür gibi bazı kimyasal maddeler olarak biyolojik aktiviteye sahip değildir ve bunların toksik etki gösterebilmeleri için önce hedef hücreleri etkileyen reaktif toksik metabolitlere dönüşmeleri gerekir. Bu dönüşüm genellikle karaciğer ve diğer organların hücrelerindeki düz endoplazmik retikülumda bulunan sitokrom P-450 tarafından geçekleştirilir. Metabolitleri hücre hasarına membranlardaki protein ve lipidlere doğrudan kovalent bağlarla bağlanarak yol açsa da hücre hasarına neden olan en önemli mekanizma serbest radikaller üzerinden olur. Karbon tetraklorür daha çok karaciğerde olmak üzere toksik bir serbest radikal olan karbon triklorüre (CCl_3) dönüşür ve bu madde öncelikle membran fosfolipidlerinin peroksidasyonu üzerinden hücre hasarına neden olur. CCl_4'e maruz kalınmasını izleyen 30 dakika içerisinde endoplazmik retikulum membranı parçalanır, karaciğerde enzim proteinlerinin ve plazma proteinlerinin sentezi azalır. 2 saat içerisinde ise endoplazmik retikulumlarda şişme izlenir. Hepatositlerin, trigliseridlerle kompleks oluşturup lipoprotein salgılanmasını kolaylaştıracak olan apoproteinleri sentezleyememesi nedeniyle bu hücrelerden çıkan lipid miktarı azalır. CCl_4 zehirlenmesinin sonucunda bu nedenle yağlı bir karaciğer oluşur. Bunu mitokondri hasarı izler ve ATP depolarının azalması kusurlu iyon transportuna ve hücrenin giderek şişmesine yol açar. Endoplazmik retikulumda lipid peroksidasyonuyla üretilen yağ aldehidleri plazma membranına daha fazla hasar verir. Sonuç olarak membranı zedelenmiş hücreye kalsiyum girişi olur ve hücre ölümü gerçekleşebilir (Canbay ve ark., 2004). G. lucidum polisakaritleri, karbon tetraklorür (CCl_4) kaynaklı hepatosit hasarı üzerinde hepatoprotektif etkilere sahiptir (Liu ve ark., 2015). İn vitro yapılan bir çalışmada, insan hepatik HepG2 hücrelerinde ganoderma triterpenoidlerin hepatoprotektif aktivitesi gösterilmiştir (Peng ve ark., 2014; Wuve ark., 2016). Siklooksijenaz (COX) enzimi, prostaglandin (PG) yapımı için anahtar rolü olan enzimdir. Bu moleküller hücresel fizyolojik süreçlerin düzenlenmesinde yardımcı olan lokal hormonlardır. Yarılanma ömürleri kısadır, etkilerini sentezledikleri hücrede ve komşu hücrelerde gösterirler. Bu moleküller hücre proliferasyonunu stimüle ederler, özellikle meme epitel hücrelerinin mitotik aktivitesini arttırırlar. Bununla birlikte Prostaglandin E2 (PGE2), immunregulatör lenfokinlerin üretimini, T ve B hücre proliferasyonunu ve doğal öldürücü (NK) hücrelerinin sitotaksik aktivitelerini inhibe eder. Böylece immun süpresif etkisi vardır. PG düzeylerinin yükselmesi sellüler siklik AMP (cAMP) artışına neden olur. Buda apoptozisin azalması ve hücre yaşam süresinin uzamasına yol açar. Aynı zamanda COX-2 artışı doğal substratı olan araşidonik asit düzeyinin de azalmasına yol açar ki buda apaptozisin azalmasıyla sonuçlanmaktadır (Singh-Ragner ve ark., 2008; Larkins ve ark., 2006). COX-2 enzimi yangıda veya iltihapta çok önemli role sahiptir (Smith ve ark., 2000; Vane ve ark., 1998; Smith ve ark., 1996; Tütüncü, 2009; Turini ve Dubois, 2002; Caterina ve Julius, 2001). COX-2, çeşitli sitokinler, büyüme faktörleri veya mitojenler tarafından uyarılarak yangısal süreçte görev alan makrofajlar, monositler, sinoviyal hücreler, kondrositler, fibroblastlar ve endotel hücrelerde eksprese edilmekte ve karaciğerde metabolize olmaktadır (Heitmeier ve ark., 2004; Inoue ve ark., 2000; Lembeck, 1987).

Yaptığımız bu çalışmada ise diğer araştırıcıları yaptıkları çalışmaların bulgularıyla parallel olarak, CCI_4 toksikasyonu oluşturulan ratlarda, artan TOS, AST ve ALT değerlerine karşın TAS düzeylerinde azalma belirlenmiştir. Ganoderma lucidum'un biyokimyasal belirteçler üzerinde CCI_4 ün etkilerini azaltarak TAS düzeylerinde artış, buna karşın TOS, AST ve ALT değerlerinde azalma tespit edilmiştir. Ancak Ganoderma lucidum'un, karbon tetraklorür ile birlikte verildiği gruptan elde edilen verilerin değeriendirilmesiyle, ALT düzeyi dışındaki TAS, TOS, AST değerlerindeki değişiklikler yönünden CCI_4 grubuyla arasında istatistiki anlamda bir farkın olmaması, Ganoderma lucidum'un tam iyileştirici etkisinin tek başına yeterli olmadığını düşündürmektedir. Gruplar histolojik olarak incelendiğinde kontrol ve GL gruplarının karaciğer dokusu normal yapıda incelenirken, CCI_4 grubunda hücre infiltrasyonu, sentral ve portal venada konjesyon, multifokal nekroz alanları, hepatosellüler dejenerasyon ve vakuol oluşumu gözlenirken CCI_4 ve GL nin birlikte uygulandığı grupta yukarıda sayılan lezyonlar aynı şekilde mevcut iken, lezyon şiddetinde azalmalar tespit edilmiştir. COX-2 immunoreaktivitesi, tüm gruplarda hepatositlerin sitoplazmasında daha yaygın olarak gerçekleşirken hepatosit nükleuslarında sporadik olarak reaktivite gözlenmiştir. COX-2 yoğunluğu CCI_4 grubunda meydana gelen yangıya bağlı olarak diğer gruplardan daha yoğun olarak gözlemlenmiş, diğer gruplardaki yoğunluk ise birbirine yakın olarak orta düzeyde tespit edilmiştir. Özellikle CCI_4 ve Ganoderma lucidum'un birlikte verildiği grupta COX-2 immunoreaktivitesinin CCI_4 grubuna kıyasla azalması inflamatuar yanıtın baskılandığı anlamına geldiğini düşünüyoruz.

Özellikle ganoderma lucidum'un yapısında bulunan polisakkarit ve triterpenler, organizmada oluşan toksik maddelerin zararlı etkilerine karşı antioksidatif etkinin yanında bağışıklık sistemini güçlendirici ve

öğelerini uyarıcı özellik göstererek karaciğeri korumaktadırlar. Yaptığımız bu çalışmada, toksik maddelerle yapılan diğer çalışmalara benzer şekilde karbon tetraklorür'ün ratlarda oksidatif hasara bağlı biyokimyasal ve histolojik olarak önemli değişiklikler meydana getirebileceği, buna karşı Ganoderma lucidum'un ise lezyon şiddetinde azaltıcı özellik göstererek tedaviye yardımcı özelliğe sahip olduğu sonucuna varıldı. Ganoderma lucidum'un hepatoprotektif etkisi, serbest radikal süpürücü özelliği ile oksidatif stresi azaltması ve karaciğerdeki enflamatuar yanıtı inhibe etmesi ile bağıntılı olabilir. Çalışmamızın sonuçları değerlendirildiğinde ganoderma lucidumun, karbon tetraklorür'ün metabolik aktivasyonunu inhibe edip ve enflamatuar yanıtı ve hepatositlerde gelişen apoptotik reaksiyonu azaltmak için karbon tetraklorür kaynaklı oksidan kapasitenin artışını önleyerek ratlarda karbon tetraklorür kaynaklı akut karaciğer hasarına karşı koruyucu bir karakter gösterdiğini düşünüyoruz.

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➢ *ORAL PRESENTATION*

Güneydoğu Karadeniz'de (Rize kıyıları, Türkiye) deneysel krişli trol ile örneklenen balık (Pisces) türleri

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Özet

Bu çalışma, Güneydoğu Karadeniz sahillerinde (Rize civarında) bulunan balık türlerini belirlemek amacıyla yapılmıştır. Aralık 2012 ve Kasım 2013 tarihleri arasında aylık olarak gerçekleştirilen ve torba ağ gözü açıklığı 15mm sahip deneysel krişli trol ile toplamda 146 örnekleme yapılmıştır. Çalışmada iki sınıfa ait (Actinopterygii ve Chondrichthyes) 9 takımdan (Atheriniformes, Clupeiformes, Syngnathiformes, Ophidiiformes, Perciformes, Pleuronectiformes, Scorpaeniformes, Rajiformes ve Gadiformes) 22 familyaya ait (Atherinidae, Engraulidae, Gadidae, Latidae, Ophiidae, Blenniidae, Callionymus, Carangidae, Centracanthidae, Gobidae, Labridae, Mullidae, Sparidae, Trachinidae, Uranoscopidae, Bothidae, Pleuronectidae, Scophthalmidae, Soleidae, Scorpaenidae, Syngnathidae ve Rajidae) 25 balık türü belirlenmiştir.

Anahtar Kelimeler: Karadeniz, Biyoçeşitlilik, Balık

Fish (Pisces) species sampled with experimental beam trawl in the Southeast Black Sea (Rize coasts, Turkey)

Abstract

This study was conducted to determine the fish species found in the Southeastern Black Sea coast (around Rize). A total of 146 sampling was conducted monthly between December 2012 and November 2013 with an experimental beam trawl with a 15 mm cod-end mesh size. Belong to two classes in the study ((Actinopterygii and Chondrichthyes) from 9 ordo(Atheriniformes, Clupeiformes, Syngnathiformes, Ophidiiformes, Perciformes, Pleuronectiformes, Scorpaeniformes, Rajiformes and Gadiformes) belonging to 22 families (Atherinidae, Engraulidae, Gadidae, Latidae, Ophiidae, Blenniidae, Callionymus, Carangidae, Centracanthidae, Gobidae, Labridae, Mullidae, Sparidae, Trachinidae, Uranoscopidae, Bothidae, Pleuronectidae, Scophthalmidae, Soleidae, Scorpaenidae, Syngnathidae and Rajidae) 25 fish species were determined.

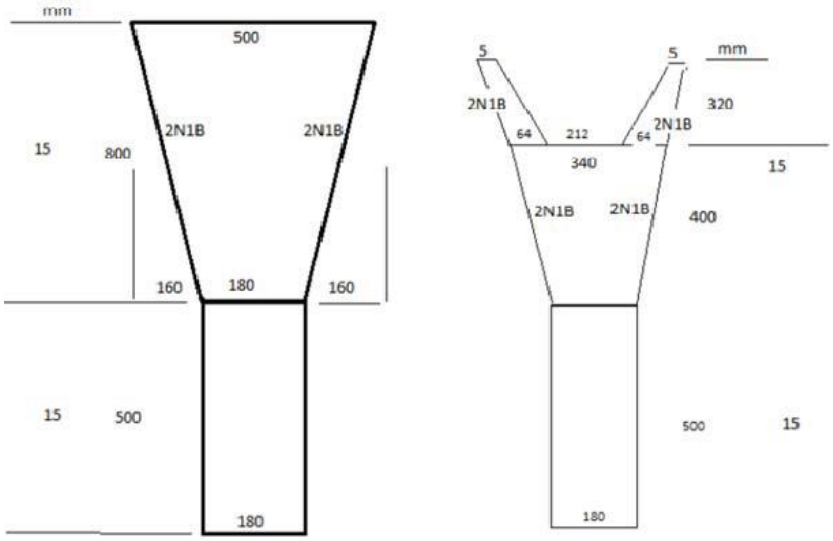
Keywords: Black Sea, Biodiversity, Fish

GİRİŞ

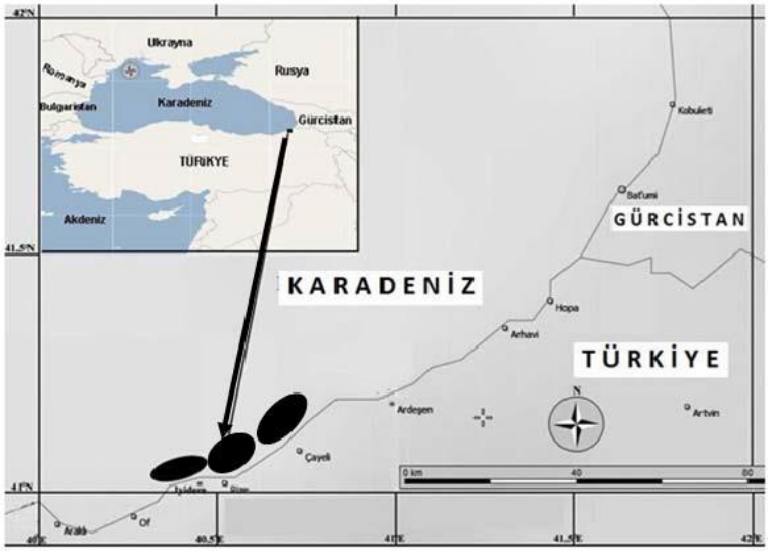
Biyoçeşitliliğin korunması için canlı türlerinin belirlenmesi ve yaşam yerlerinin bilinmesi gerekmektedir. Bozulmadan önceki durumunu bilmeden o ekosistem sağlığının bozulmaya başladığını tespit edebilmek oldukça güç olmaktadır. Birçok balık türünün genç bireylerinin, beslenme ve büyüme alanlarını yakın sahil meraları oluşturmaktadır. Sahil kenarlarında yapılaşmaların ve zirai faaliyetlerin etkileri öncelikle kıyısal alanları ve dolayısıyla bu alanlarda yaşayan canlıları etkilemektedir. Biyoçeşitliliğin korunabilmesi için öncelikle söz konusu bölge içindeki biyoçeşitliliğin bilinmesi gerekmektedir (Lazzarı ark., 1999; Nash ve Santos, 1998). Çalışma sahası olarak belirlenen Doğu Karadeniz Bölgesi, üzerinde durulması gereken bir ekosistemdir ve balık faunası hakkında az sayıda çalışma bulunmaktadır. Bu çalışmayla, Rize kıyıları açıklarında bir yıl boyunca aylık örnekleme sonucu, deneysel krişli trol çekimlerinden elde edilen balık faunası tespit edilmeye çalışılmıştır.

MATERYAL VE METOD

Bu çalışma, Rize kıyılarından 3 istasyon (İyidere, Merkez ve Çayeli), 4 farklı derinlik grubundan (1-5 m, 5-10 m, 10-20 m ve 20-30+ m), deneysel krişli trol kullanılarak (Şekil 1) Aralık 2012 ve Kasım 2013 tarihleri arasında aylık olarak örnekleme yapılarak yürütülmüştür (Şekil 2). Toplam 146 örnekleme Recep Tayyip Erdoğan Üniversitesi, Su Ürünleri Fakültesine ait SUAR isimli araştırma teknesiyle yapılmıştır.



Şekil 1. Deneysel krişli trol ağının kesim planı (orijinal).



Şekil 2. Örnekleme alanı

Çalışmada çamurlu, kumlu, çamurlu-kumlu, kayalık ve deniz otu (yosun ya da alg) habitatları başta olmak üzere ticari dip trol balıkçılığına kapalı Rize sularında epibentik makrofaunanın örneklenmesi yapılmıştır. Bu çalışmada örneklemeler için 2 m kiriş uzunluğunda ve torba ağ gözü açıklığı 15 mm olan deneysel amaçlı krişli trol kullanılmıştır. İyidere istasyonu Of-İyidere arasındaki ilçe sınırından başlayarak İyidere-Derepazarı ilçe sınırına kadar olan alanı kapsamaktadır. Merkez istasyonu Derepazarı ilçe sınırı ile Askoroz deresinin (Salarha havzasından doğan dere) denize döküldüğü yere kadar olan alanı kapsamaktadır. Çayeli istasyonu Büyük Köy kıyılarından başlayıp Liman Köy mahallesi mevkii boyunca olan alanı kapsamaktadır. Çalışmadaki her çekim sonucunda tekneye alınan ağdaki örnekler güverteye boşaltılmıştır. Çekimler sonucunda elde edilen örnek aynı gün içerisinde RTEÜ, Su Ürünleri Fakültesi, Balıkçılık laboratuarına getirilerek balık türleri tür düzeyinde tanımlanmış ve FishBase referans alınarak doğrulanmıştır (Froese & Pauly, 2013).

BULGULAR

Araştırma süresince İyidere, Merkez ve Çayeli istasyonlarından 2 m genişliğinde kirişli trol kullanılarak toplamda 146 çekim neticesinde iki sınıfa ait (Actinopterygii ve Chondrichthyes) 9 takımdan (Atheriniformes, Clupeiformes, Syngnathiformes, Ophidiiformes, Perciformes, Pleuronectiformes, Scorpaeniformes, Rajiformes ve Gadiformes) 22 familyaya ait (Atherinidae, Engraulidae, Gadidae, Latidae, Ophiidae, Blenniidae, Callionymus, Carangidae, Centracanthidae, Gobidae, Labridae, Mullidae, Sparidae, Trachinidae, Uranoscopidae, Bothidae, Pleuronectidae, Scophthalmidae, Soleidae, Scorpaenidae, Syngnathidae ve Rajidae) 25 balık (*Atherina boyeri* Risso, 1810, *Engraulis encrasicolus* (Linnaeus, 1758), *Merlangus merlangus euxinus* (Noramnn, 1840), *Gaidropsarus mediterraneus* (Linnaeus, 1758), *Ophidion barbatum* (Linnaeus, 1758), *Blennius sp*., *Callionymus sp*., *Trachurus mediterraneus* Steindachner, 1868, *Spicara smaris* (Linnaeus, 1758), *Aphia minuta* Risso, 1810, *Gobius niger* Linnaeus, 1758, *Pomatoschistus marmoratus* (Risso, 1890), *Symphodus tinca* (Linnaeus, 1758), *Mullus barbatus* Linnaeus, 1758, *Diplodus annularis* (Linnaeus, 1758), *Trachinus draco* Linnaeus, 1758, *Uranoscopus scaber* Linnaeus, 1758, *Arnoglossus kessleri* (Schmidt, 1915), *Platichthys flesus* Linnaeus, 1758, *Scophthalmus maximus* (Linnaeus, 1758), *Pegusa nasuta* (Pallas, 1814), *Raja clavata* Linnaeus, 1758, *Hipocampus sp* (Linnaeus, 1758), *Syngnathus typhle* Linnaeus, 1758 ve *Scorpaena porcus* Linnaeus, 1758) türü örneklenmiştir. Türlerin sistematiği aşağıdaki gibidir.

Örneklenen yengeç türleri ve sistematiği:

Phylum: Chordata Bateson, 1885

1. Class: Actinopterygii Klein,1885

1. Order: Atheriniformes

1. Family: Atherinidae

1. Tür: *Atherina boyeri* Risso, 1810

2. Order: Clupeiformes

1. Family: Engraulidae

1. Tür: *Engraulis encrasicolus* (Linnaeus, 1758)

3. Order: Gadiformes

1. Family: Gadidae

1. Tür: *Merlangus merlangus euxinus* (Noramnn, 1840)

2. Family: Latidae

1. Tür: *Gaidropsarus mediterraneus* (Linnaeus, 1758)

4. Order: Ophidiiformes

1. Family: Ophiidae

1. Tür: *Ophidion barbatum* (Linnaeus, 1758)

5. Order: Perciformes

1. Family: Blenniidae

1. Tür: *Bllenius sp.*

2. Family: Callionymus

1. Tür: *Callionymus sp.*

3. Family: Carangidae

1. Tür: *Trachurus mediterraneus* Steindachner, 1868

4. Family: Centracanthidae

1. Tür: *Spicara smaris* (Linnaeus, 1758)

5. Family: Gobidae

1. Tür: *Aphia minuta* Risso, 1810

2. Tür: *Gobius niger* Linnaeus, 1758

3. Tür: *Pomatoschistus marmoratus* (Risso, 1890)

6. Family: Labridae

1. Tür: *Symphodus tinca* (Linnaeus, 1758)

7. Family: Mullidae

1. Tür: *Mullus barbatus* Linnaeus, 1758

8. Family: Sparidae

1. Tür: *Diplodus annularis* (Linnaeus, 1758)

9. Family: Trachinidae

1. Tür: *Trachinus draco* Linnaeus, 1758

10. Family: Uranoscopidae

1. Tür: *Uranoscopus scaber* Linnaeus, 1758

6. Order: Pleuronectiformes

1. Family: Bothidae

1. Tür: *Arnoglossus kessleri* (Schmidt, 1915)

2. Family: Pleuronectidae

1. Tür: *Platichthys flesus* Linnaeus, 1758

3. Family: Scophthalmidae

1. Tür: *Psetta maxima* (Linnaeus, 1758)

4. Family: Soleidae

1. Tür: *Pegusa nasuta* (Pallas, 1814)

7. Order: Scorpaeniformes

1. Family: Scorpaenidae

1. Tür: *Scorpaena porcus* Linnaeus, 1758

8. Order: Syngnathiformes

1. Family: Syngnathidae

1. Tür: *Hipocampus sp* (Linnaeus, 1758)

2. Tür: *Syngnathus typhle* Linnaeus, 1758

2. Class: Chondrichthyes

1. Order: Rajiformes

1. Family: Rajidae

1. Tür: *Raja clavata* Linnaeus, 1758

TARTIŞMA

Bu çalışmada Rize sahillerinde yapılan av operasyonları sonucunda elde edilen balık faunası değerlendirilmiştir. Av operasyonları sonucunda Crustacea (kabuklu), Mollusca (yumuşakça) ve Pisces (balık) türleri örneklenmiştir. Beam trol ağından çıkan balık türleri sistematik açıdan incelenmiştir. Toplam 9 takıma ait 22 familyadan 25 tür saptanmış olup, bunlardan 1 türü kıkırdaklı ve 24 türü de kemikli balıklara aittir. Çalışmada yakalanan balıkların büyük çoğunluğu demersal türler olmakla beraber, *T. mediterraneus*, *E. ancrasicolus* ve *A. boyeri* gibi pelajik türlerde az miktarda elde edilmiştir. Karadeniz'deki balık faunası ilgili ilk bilimsel çalışma Slastenenko, 1955-1956 tarafından yapılmıştır. Karadeniz de

balık yumurta ve larvalarının dağılımını inceleyen Dehnik (1973) ise 50 familyaya ait 119 balık türünün yayılım gösterdiğini bildirmiştir. Keskin (2010) ise Karadeniz'in Türkiye kıyılarında toplam 161 balık türünün barındığını rapor etmiştir. Bu çalışmada belirlenen daha düşük familya ve tür sayısının olası nedeni bu çalışmanın sadece Rize kıyılarında yapılmış olması olabilir. Yapılan diğer çalışmalarda, Karadeniz Trabzon kıyılarında dip trolü kullanılarak yapılan çalışmada Ak ark. (2008) 2 sınıf, 8 takım ve 25 familyaya ait 28 tür belirlemiştir. Bunlardan 3 tanesi kıkırdaklı ve 25 tanesi de kemikli balık olarak tespit edilmiştir. Doğu Karadenizde dip trolü kullanılarak yapılan bir başka çalışmada ise 10 familyaya ait 10 tür tespit edilmiştir. Bu türlerin, 6 'sı kemikli balık, l'i kıkırdaklı balık, l'i kabuklular ve 2 'si yumuşakçalar grubundandır (Koç, 2005). Diğer bir çalışmada Öğreden ve Yağlıoğlu (2017) Güneybatı Karadeniz Düzce kıyılarında dip trolü ile yaptıkları örnekleme sonucunda 27 familyaya ait 36 tür belirlemiştir. Başka bir çalışmada Başkaya (2012) Batı Karadeniz kıyılarında 34 trol operasyonu sonucunda 25 kemikli balık, 2 kıkırdaklı balık, 4 kabuklu (crustacea), 2 derisidikenli (echinodermata) ve 1 yumuşakça (mollusca) türü rapor etmiştir. Batı Karadeniz kıyılarında yapılan çalışmada 21 dip trol operasyonu sonucunda 22 balık türü, 2 eklembacaklı türü, 1 gastropod ve 1 çift kabuklu olmak üzere toplam 26 tür tespit edilmiştir (Ceylan ark., 2013). Batı Karadeniz kıyılarında Yıldız ve Karakulak (2018) çalışmalarında toplam 66 dip trolü çekimi yapmış ve 32 balık türü rapor etmişlerdir. Bu çalışmalarda saptanan familya ve tür sayısı ise bizim çalışmamız ile benzerlik göstermektedir. Çalışmalar arasındaki bu benzerlik ve farklılıkların nedeni ise kullanılan av aracının (göz açıklığı, ağ uzunluğu gibi).farklı özelliklere sahip olmasından kaynaklanıyor olabilir. Ayrıca avcılık operasyonları çekim sayı ve süreleri ve çekim hızından kaynaklanan farklılıklardan oluşmuş olabilir.

SONUÇ

Sonuç olarak deniz ekosistemleri ve biyoçeşitliliğin dağılımı belirlenererek meydana gelen değişikliklerin etkileri erken tespit edilmeldir. Sucul ekosistemin sürdürülebilirliği, ortamda yaşayan türlerin çeşitliliğine bağlıdır.

TEŞEKKÜR

Bu çalışma, Recep Tayyip Erdoğan Üniversitesi Bilimsel Araştırma Projeleri (BAP) birimi tarafından 2013.103.03.1 Numaralı proje ile desteklenmiştir. Desteklerinden dolayı ilgili birimlere teşekkür ederiz.

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➢ *ORAL PRESENTATION*

Germ Hücreleri ve İnfertilite Tedavisi

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Özet
Germ hücrelerinin izolasyonu ve laboratuar koşullarında çoğalabilme yetenekleri birçok hastalığın tanı ve tedavisinde etkili olabilir. Bu hastalıklardan biri olan infertilite tedavisinde in vitro proliferasyon yöntemi ile çeşitli yerlerden kök hücre kaynaklarına ulaşmak, onları germ hücrelerine ayırmak veya direkt olarak PGC veya SSC izole etmek mümkündür. Kısırlık için başvuran erkeklerin yaklaşık% 5-20'sinde ejakülat örneğinde canlı sperm bulunmaz. Sadece erken evre spermatogenezi (olgunlaşma duraklaması) veya sadece Sertoli destek hücreleri (sadece Sertoli hücre) olan hastalarda spermatogenezi indüklemek ve spermatozoa elde etmek için yapılan çalışmalar histopatolojik değerlendirmelerde klinik alanda başarılı olmamıştır. Güncel literatüre dayalı olarak elde edilen bulguların paylaşılması amaçlanmaktadır.
Anahtar kelimler: İnfertilite, kök hücre, germ hücresi, spermatogonial kök hücre

Giriş

İnfertilite, 12 ay üst üste doğum kontrolü yapılmadan düzenli cinsel ilişkiye girilmesine rağmen gebe kalamama olarak tanımlanmaktadır. Bu sorun genç çiftler arasında bile, hızla yaygın bir sorun haline gelmektedir (Mehra, et al., 2018; Hansen, et al., 2016). Bu nedenle, 2009'da Uluslararası Üremeye Yardımcı Teknolojinin İzlenmesi Komisyonu (ICMART) ve Dünya Sağlık Örgütünün (WHO) ortaklaşa infertiliteyi sistemik bir hastalık olarak tanımlamıştır (Barratt, et al., 2017). Dünya çapında, doğurganlık çağındaki (186 milyona kadar) çiftlerin % 8-12'si kısırlık çekmektedir [4]. İnfertilite küresel olarak azalan çocuk sayısının ve birincil ve ikincil kısırlık olarak kategorize edilen kısırlığın temel nedenlerinden biri olarak kabul edilir. Hamileliğin gerçekleşmesi için yumurtlama ve döllenme sürecindeki her adımın doğru şekilde gerçekleşmesi gerekir. Erkek faktörleri, kısırlık vakalarının üçte birinin nedenidir, esas olarak spermdeki morfolojik ve fonksiyonel bozukluklar, erken ergenlik, kalıtsal hastalıklar ve testis tıkanıklığı gibi yapısal sorunlar dahil; sperm disfonksiyonuna yol açan genital hasar veya yaralanma, çevresel ve psikolojik faktörlerdir (D'Antonio, et al., 2017; Kartz, et al., 2017).

İnfertilite tedavisi, nedene ve hasta özelliklerine bağlı olarak farmakolojik tedaviden yardımcı üreme teknolojisine kadar değişir. Erkek fertilitesinin teşhisi için sperm analizi, hormonal muayene, genetik testler ve testis biyopsisi birincil muayene seçenekleridir. Erkek infertilitesinin tedavisinde, yaşam tarzı iyileştirmeleri, ilaçlar, ameliyat ve sperm rejenerasyonu yer almaktadır. Son yıllarda, kök hücreler infertilite alanında önemli ilgi görmüştür (Mouka, et al., 2016). Kök hücreler, onarım, geliştirme ve rejenerasyon için çeşitli diğer hücrelere bölünebilen çok potansiyelli orijinal hücrelerdir. Deneysel modellerle ilgili çalışmalar, kısırlığın kök hücre tedavisi ile tedavi edilmesinin kabul görmeye başladığını göstermiştir (Somigliana, et al., 2016). Cinsel infertiliteyle ilişkili hastalıklar üzerine yapılan klinik öncesi çalışmalar, infertilitenin tedavisi için dikkate alınması gereken yeni yönler önermektedir (Vanni, et al., 2017). Deneysel modeller kullanan çalışmalar, infertilitenin tedavisinde kök hücre tedavisinin gücünü ortaya çıkarmış ve bu sonuçları doğrulamıştır (Brunauer, et al., 2017). Bu derlemede kısırlığın tedavisi için kök hücrelerin uygulanmasına ilişkin araştırmalar gözden geçirilmiştir.

HÜCRE TEDAVİSİNDE YENİLİKLER

Son yıllarda, erkek germ hücrelerinin in vitro pluripotent kök hücrelerden in vitro farklılaşmasında önemli ilerlemeler kaydedilmiştir (Smith, et al., 2016). Kök hücreler, embriyolarda, fetüslerde ve yetişkinlerde farklılaşmış hücreler üretmek için bulunan farklılaşmamış hücrelerdir. Tipik olarak iki kaynaktan gelirler: erken embriyonik hücreler ve yetişkin dokular. Dokuya özgü kök hücreler, farklılaşmış organlarda, yaşamın doğum sonrası ve yetişkinlik dönemlerinde bulunur ve organ hasarının onarımında önemli bir rol oynar. Ana kök hücre tipleri embriyonik kök hücreler (ESC'ler), mezenkimal kök hücreler (MSC'ler), spermatogonial kök hücreler (SSC'ler) ve indüklenmiş pluripotent kök hücrelerdir (iPSC'ler). Kök hücreler kendi kendine homing oluştururlar ve insan vücuduna enjekte edildiklerinde hasarlı organlarda ve bunlara karşılık gelen kısımlarda birikerek bu organlara ve parçalara özgü hücre tiplerine farklılaşırlar. Örneğin, SSC'lerin homing yeteneği, onları steril testislere nakledildikten sonra nişlerine yönlendirir. Nakledilen SSC'ler daha sonra Sertoli hücrelerine bağlanır ve kan-testis bariyerini (BTB) bazal membran üzerindeki nişlerine geçmek için yakından bağlar (Chen, et al., 2017).

SPERMATOGONİAL KÖK HÜCRELER

Spermatogenez, IL-1 ailesi gibi testosteron, endokrin ve parakrin sekresyon / otokrin faktörlerle düzenlenen bir süreçtir. Patolojik koşullar altında, spermatogenez üzerinde olumsuz bir etkiye sahip olan proinflamatuar sitokinlerin seviyesi artar. Bu nedenle, testiküler parakrin/otokrin faktörün ekspresyonu ve regülasyon mekanizması, erkek kısırlığının gelecekteki tedavi stratejisinde dikkate alınmalıdır (Rozwadowska, et al., 2007). Sağlıklı SSC'ler sperm rejenerasyonuna yol açabilir. 1971'de, sıçan spermatogenezi ve erkek fertilitesini sürdürmek için SSC'lerin kullanımı kabul edilmiştir (Arkoun, et al., 2015). SSC'ler, embriyonik gelişim sırasında primordiyal germ hücrelerinden (PGC'ler) türetildiler. SSC transplantasyonu, spermatogenez ve kök hücrenin kendini yenilemesine dayanan yeni ve ümit verici bir strateji olarak sunulmuştur. 1994 yılında Brinster ve ark. ilk olarak fertil donör erkek farelerden spermatogonial kök hücreler steril farelerin seminifer tübüllerine enjekte edildi. Sonuç olarak, alıcı fareler döllenme kabiliyetine sahip sperm üretti ve normal yavrular elde edildi (Mulder, et al., 2016). SSC'ler kendi kendini yeniler ve yaşam boyunca spermlere dönüşebilen çok sayıda adanmış progenitör hücre üretebilirler. Farelerde, DBA / 2J fare suşu, yoğun şekilde paketlenmiş bir hücre kütlesi oluşturabilir ve glial hücre çizgisinden türetilmiş nörotrofik faktörün (GDNF) müdahalesi altında çoğalmaya devam edebilir (Hirt, et al., 2018). Kültürdeki SSC'ler altı aydan fazla bir sürede çoğalmıştır. Alıcının testisine transplantasyonundan sonra sperm yeniden oluşturulabilir ve kısır alıcıya fertilite geri kazandırabilir. Nitekim donör erkek farelerin testislerinden izole edilen kök hücreler seminifer tübüllere enjekte edilmiş ve donör spermatogonial kök hücreler, testiste normal morfolojik özelliklerle spermatogenezi indükleyerek olgun sperm üretebilmiştir. İnsanlarda, SSC'ler erkek sperminin sürekli üretiminden sorumludur. Spermatogonial kök hücreler, spermatogenezin seminifer tübüllerini muhafaza eden bazal membranda bulunur. İnsan SSC'lerinin transplantasyonu, erkek kısırlığı için etkili bir tedavi olabilir. SSC'ler, ebeveyn genetik bilgilerini yavrulara aktarabilen tek kök hücredir. Neonatal SSC'ler prostat, uterus ve deri epitelini üretmek için doku rekombinasyon tekniklerinde kullanılır. Fare SSC'leri in vivo hematopoietik hücrelerin morfolojik ve fonksiyonel özelliklerini kazabilmektedir. Dahası, bu teknoloji domuzlar, keçiler ve maymunlar gibi diğer hayvan türlerine de uygulanabilir (Sun, et al., 2018). Bu teknoloji aynı zamanda teknik problemlerin çözümü bağlamında insan kısırlığını tedavi etmek için de kullanılabilir.

KÖK HÜCRELER VE ERKEK İNFERTİLİTESİ

Spermatogenez, SSC'nin kendini yenilemesinin ve haploid spermatozoaya farklılaşmanın karmaşık bir sürecidir. SSC'ler yetişkin testislerde bulunur ve yaşam boyunca spermatogenezi sürdürür. SSC'ler, ESC'lere benzer bir farklılaşma potansiyeline sahip olan pluripotent yetişkin germ hattı kök hücreleri (maGSC) olarak bilinen yetişkin kök hücrelerdir. In vitro, maGSC'ler kendiliğinden tüm embriyonik germ katmanlarının türevlerine farklılaşabilir ve bağışıklığı yetersiz farelerde teratomlar oluşturabilir. Otolog ve allojenik SSC'ler, alkilasyon kemoterapisi ile infertil hale getirilen yetişkin ve ergenlik öncesi rhesus maymunlarının testislerine transplante edildiğinde, spermatogenez yeniden başladığı ve fonksiyonel sperm üretildiği görüldü (Hermann, et al., 2009). Bu sonuçlar, SSC naklinin erken kemoterapinin neden olduğu erkek infertilitesi için başarılı bir tedavi olabileceğini göstermektedir. Bununla birlikte, SSC'ler erkek kısırlığının kök hücre temelli tedavisi için iyi bir aday gibi görünürken, memeli testislerinde düşük SSC konsantrasyonları ile ilişkili zorluklar ve izolasyon, tanımlama ve kültür protokolü klinik uygulamadan önce ele alınmalıdır. Benzer şekilde, insan ESC'lerinin çalışmaları, spermatogenezin ileri aşamalarına farklılaşma yeteneklerini, in vitro, yumurtaları dölleyemeyen yuvarlak sperm dahil gelişmiş memelilerde ortaya çıkardı (Makoolati, et al., 2016). İnsan ESC'lerinin izolasyonu etik açıdan tartışmalıdır. ESC'ler genetik olarak hastalarla ilgisiz olmalarına rağmen, bunların toplanması insan embriyonik dokusunun yok edilmesini içerir. Kökte büyük atılımlar hücre biyolojisi

ve hastaya özgü iPSC'lerin keşfi bu sorunların üstesinden gelebilir. Bazı çalışmalar yakın zamanda hem insan hem de murin iPSC hücrelerinin erkek germ hücrelerine farklılaşabildiğini bildirmiştir. Fare iPSC hücrelerinin fonksiyonel sperm oluşturduğu gösterilmiştir (Sanjo, et al., 2018). Fonksiyonel testler, iPSC'ler tarafından üretilen spermin, intra-sitoplazmik enjeksiyondan sonra yumurtaları dölleyebildiğini ve embriyo transferinden sonra verimli yavrular oluşturabildiğini göstermiştir. Ancak şimdiye kadar, insan iPSC hücrelerinden fonksiyonel erkek gametler elde edilmemiştir. Sato vd. yenidoğan farelerin testis doku parçalarının sadece germ hücreleri veya ilkel spermatogoni içerdiğini göstermiştir [63]. İn vitro sperm üretilebilir ve sağlıklı doğurgan yavrular oluşturabilirler. Kök hücrenin farklı aşamalarında in vitro gelişimini anlamak, kök hücrelerden germ hücrelerinin üretimini kolaylaştırmaya yardımcı olabilir. Bununla birlikte, kök hücrelerden in vitro germ hücrelerinin üretimi ümit vaat ederken, hala çözülmesi gereken birçok sorun bulunmaktadır.

SONUÇLAR

Güncel araştırmalar, kök hücre tedavisinin dejeneratif hastalıkları tedavi edebileceğini, kötü huylu kanserleri iyileştirebileceğini ve hasarlı dokuyu onarabileceğini göstermiştir. Bununla birlikte, kök hücre tedavisinin bazı yönleri keşfedilmemiştir; bu nedenle, kısırlık gibi hastalıkların tedavi edilmesine ilişkin uygulamalarda hâlâ kullanılmayan büyük bir potansiyel mevcuttur. Doğru yaklaşım bulunduğunda bilimin kısırlığı tedavi edeceğinden eminiz.

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➢ *ORAL PRESENTATION*

Polypeptide-functionalized Polymer Decorated with Gold Nanoparticles as Immobilization and Sensing Platform for Codeine Analysis

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Abstract
An aptamer based electrochemical biosensor was designed for the detection of codeine. Polypeptide-functionalized polymer (EBP) was coated on a glassy carbon electrode (GCE). Cysteamine modified gold nanoparticles (AuNP-Cys) were attached on the polymer coated electrode surface via glutaraldehyde through the amino functional groups of the polymer. Immobilization of aptamer was achieved via AuNP-Cys through Au–SH affinity. Surface modifications were studied via electrochemical techniques such as cyclic voltammetry, electrochemical impedance spectrometry, and differential pulse voltammetry. The designed sensor showed an excellent electrocatalytic response towards the sensing of codeine with a wide linear range.

Keywords: Biosensor, aptasensor, codeine.

INTRODUCTION

The use of illicit drugs is still a major issue worldwide in terms of social, economic and health problems. According to the latest United Nations Office for Drugs and Crime World Drug Report, approximately 271 million people, or 5.5 percent of the world population aged 15-64 used illicit drugs in 2017, and an estimated 585,000 people died worldwide due to drug use. 53.4 million people used opioids which was 56 percent increase compared to the estimate for 2016 (World Drug Report, 2019). Opioids are divided into three groups as natural opiates such as morphine, codeine, thebaine, their semi-synthetic analogues such as heroin, oxycodone, hydrocodone, and synthetic derivatives such as alfentanil, fentanyl, methadone (Laycock and Bantel, 2019).

Codeine (3-methyl morphine) is a natural opiate alkaloid which is obtained from poppy plants. It is one of the most commonly utilized alkaloids in the treatment of mild to moderate pain and cough. Codeine has pharmacological activity similar to morphine and heroin, but it has less sedation and respiratory depression than the latter (Asturias-Arribas et al. 2014, Simioni et al. 2017). Excessive and long-term use of codeine may also be addictive and even cause death (Mohamed et al. 2018). Therefore, development of rapid, sensitive, and adaptable platforms for codeine detection is crucial for forensic sciences. Recently, several methods for the detection of codeine have been reported, such as high-performance liquid chromatography (HPLC), (Manassra et al. 2010, Freiermuth and Plasse, 1997, El-Kommos and Emara, 1989) gas chromatography tandem mass spectrometry (GC-MS), (Meadway et al. 2002, Mulé and Casella, 1988) and chemiluminescence (Barnett et al. 1996, Greenway et al. 2000). Although these methods have high sensitivity and reliability, they have drawbacks such as high cost and tedious sample preparation with complex analytical procedures. In contrast, electrochemical methods offer advantages like low cost, convenience in use, and rapid analysis.

Aptamers are single-stranded oligonucleotides that can bind to target cells with high specificity as well as high selectivity and affinity to the target, hence, they can be used for analysis of a myriad of analytes. Aptamer-based methods or signal amplification methods using aptamers show great potential in improving the selectivity and sensitivity. During the recent decades, several biosensor studies such as optical, (Liu et al. 2006) chemiluminescence, (Li et al. 2007) and electrochemical (Jin et al. 2019, Feng et al. 2011) have been reported involving the use of aptamers as biorecognition elements. In addition, there are studies related to antibody-based biosensors in the literature (Yilmaz et al. 2017, Peterson et al. 2015, Balaban et al. 2019). Compared to antibodies, use of aptamers is relatively advantageous due to ease of fabrication with SELEX (selective

evolution of ligands by exponential enrichment) technique and the lack of need to depend on cells or animals. Moreover, aptamer-based biosensors (aptasensors) have various advantages over antibody-based biosensors, such as long shelf life, denaturation resistance, low cost, and stability (Zejli et al. 2018, Bai et al. 2013).

Poly-L-phenylalanine-bearing electroactive macromonomer (EDOT–BTDA–PPhe, or EBP) was synthesized and characterized in our previous studies (Yilmaz et al. 2016). In addition, electrochemical biosensors with efficient biomolecule immobilization performances were successfully developed for the detection of cocaine (Yilmaz et al. 2017) using this polymer. Presence of well-oriented polypeptides in polymer backbones can improve the interaction between the biorecognition element and the target analyte in biosensing applications by acting as a spacer arm.

Here, we designed a sensitive electrochemical codeine aptasensor based on EBP/AuNP-Cys modified glassy carbon electrode (GCE). This platform contains many advantages of using both EBP polymer and gold nanoparticle along with aptamers. First, the GCE was covered with polymer which ensured immobilization of AuNP-Cys. Immobilization of codeine aptamer was achieved by Au-S affinity between the amino groups of AuNP-Cys and the thiol groups of aptamer.

MATERIALS AND METHODS

Materials and reagents

(5'-SH$(CH_2)_6$CCCCCTGGGTCGGGAGGGAAGGGGGTTGGGGGTGCGG-3'), the codeine aptamer, was obtained from Takara Biotechnology Co., Ltd. (Dalian, China). Codeine, cocaine, morphine, caffeine, and methylphenidate HCl were purchased from Cerilliant (Cerilliant Corp., Round Rock, TX, USA). Glutaraldehyde (GA, 25%), potassium hexacyanoferrate (III) [$K_3Fe(CN)_6$] (as redox probe) and other chemical reagents were purchased from Sigma Chem. Co. (St. Louis, MO, USA). All other chemicals were analytical grade. Sodium phosphate buffer (0.05 M, pH 7.4) was used as the working buffer in all electrochemical experiments. Working buffer was adjusted to pH 7.4 to simulate the physiological pH.

Instrumentation

Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) measurements were performed with a PalmSens Potentiostat (Palm Instruments, Houten, Netherlands). Electrochemical impedance spectroscopy (EIS) was carried out with a CHI 6005 C electrochemical analyzer (CHI, Austin, Texas, USA).

The three-electrode system consisted of the glassy carbon (GCE as working electrode), platinum (Pt as counter electrode) and Ag/AgCl (as reference electrode, 3.0 M KCl, Metrohm, Switzerland) was used through the electrochemical measurements. All experiments were conducted at ambient conditions.

Synthesis of AuNP-Cys

5.0 mL Cys (1.0 mg/mL) was added to 2.0 mL stabilized AuNP solution. The solution was shaken at 1000 rpm, 25^{o}C for 2 h. After conjugation, it was centrifuged at 3000 rpm, 4^{o}C for 15 min. The solution was washed with sodium phosphate buffer (10 mM, pH 7.4) 2 times. Supernatant was discarded, and final volume was completed to 30 mL with sodium phosphate buffer (10 mM, pH 7.4). The stock AuNP-Cys solution was diluted 1000 times with ultra pure water.

Surface Modification

GCE was polished on wet emery paper with alumina slurry (3.0; 1.0; 0.3; 0.1; 0.05 µm sized) and then sonicated for 5 min in ethanol: distilled water (1:1) solution. 5.0 µL EBP solution (from 0.5 mg/mL stock solution in acetonitrile:tetrahydrofuran) was dropped onto the electrode surface and was allowed to dry at room temperature. Before each measurement, the electrode surface was washed with ultra pure water to remove any residue. 20 µL GA (1.0% in 50 mM, pH 7.4 sodium phosphate buffer) was added onto the polymer coated surface, followed by 20 µL AuNP-Cys and incubated at room temperature for 3 h. 10 µM aptamer solution (25 µM in 10 mM phosphate buffer, pH 7.4) was added onto the electrode surface and incubated for 2 h at room conditions. 10 µL codeine solutions with known concentrations were applied on the electrode surface and incubated for 1 hour at room conditions for the testing of sensing capabilities of aptasensor.

Electrochemical Measurements

Electrochemical response signals of bare, polymer coated, aptamer immobilized, and codeine treated electrodes were obtained via DPV with a potential range of −0.4 to +0.8 V, where a water soluble redox probe

was used (5.0 mM $Fe(CN)_6^{3-/4-}$, in 0.1 M KCl). A calibration graph was plotted. CVs were carried out in the potential range of -0.8 to 0.7 V (Scan rate = 50 mV/s) in the presence of 5.0 mM $Fe(CN)_6^{3-/4-}$ (in 0.1 M KCl).

EIS measurements were performed in sodium phosphate buffer (50 mM, pH 7.4) in the presence of 5.0 mM $Fe(CN)_6^{3-/4-}$, in 0.1 M KCl. The frequency range of EIS measurements were 0.03 and 10 kHz at 0.18V.

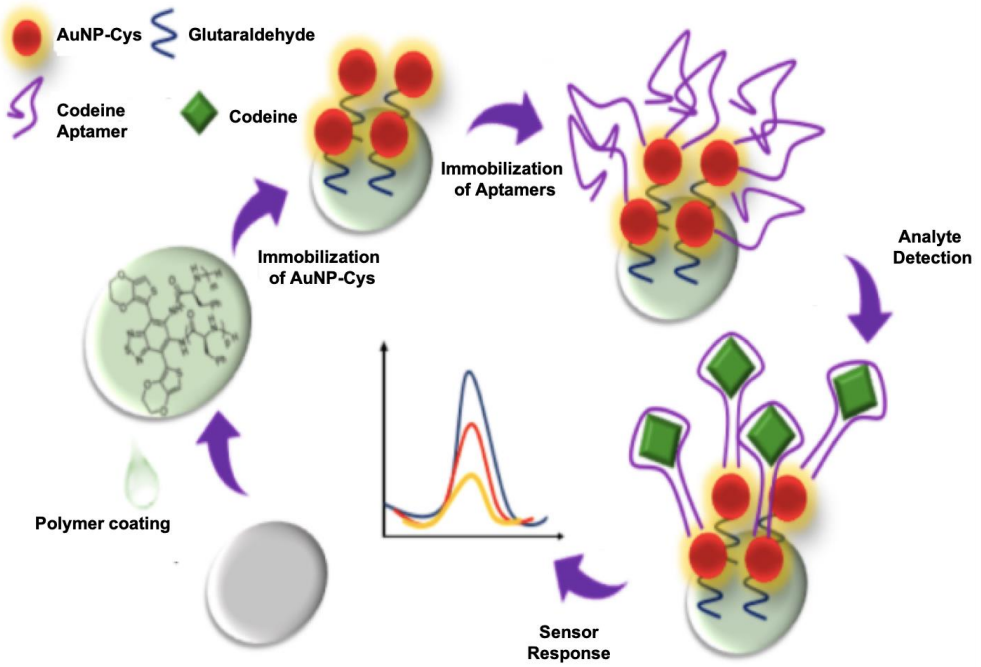
Sample Application

Effects of interference molecules were analyzed via DPV by applying cocaine, morphine, caffeine, and methylphenidate (100 pg/mL) separately on the modified electrode surface.

RESULTS and DISCUSSION

Fabrication of Codeine Aptasensor and Surface Characterization

For the construction of the aptasensor, polypeptide bearing polymer, EBP, was coated on GCE. Amino functional groups on the polypeptide-functionalized polymer backbone served to immobilize cysteamine modified gold nanoparticles (Cys-AuNP) via glutaraldehyde. Codeine aptamers were then conjugated on the modified surface by Au affinity of their thiol groups. The schematic representation of surface modification was demonstrated in Scheme 1.



Scheme 1. Design of codeine aptasensor & electrochemical analyses.

Surface modifications were studied by electrochemical methods. Current responses of bare electrode, polymer coated electrode, and aptamer immobilized electrode before and after conjugation with codeine aptasensor, followed by analyte addition in the presence of redox probe were examined.

Step by step surface modification and analyte binding monitored by CV are given in Figure 1. A gradual decrease was observed in anodic and cathodic current peaks. The anodic and cathodic peak currents were obtained as 50,17 µA and −49.81 µA (peak-to-peak separation, or pps = 0.078 V) for bare GCE, 37.79 µA and −39.58 µA (pps = 0.158 V) for GCE/EBP, 29.97 µA and −33.82 µA (pps = 0.26 V) for GCE/EBP/AuNP-Cys, 24.81 µA, and −29.71 µA (pps = 0.354 V) for GCE/EBP/AuNP-Cys/Aptamer, and 21.64 µA and −26.22 µA (pps = 0.422 V) for GCE/EBP/AuNP-Cys/Aptamer/Codeine surfaces. Therefore, upon every surface modification, electron transfer between electrolytes and electrodes was hindered. Eventually, the interactions and the subsequent binding reaction between aptamer and codeine hinders the electron transfer of the redox

probe, causing a decrease in the electrochemical signals compared to the initial signals. The drop in the values are directly related to the codeine concentrations.

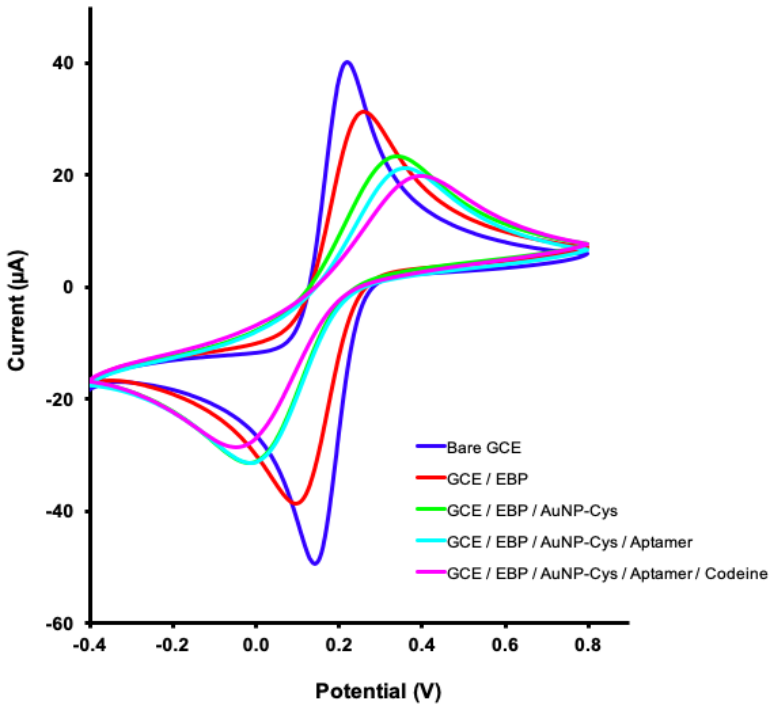


Figure 1 CV of bare GCE, GCE/EBP, GCE/EBP/AuNP-Cys, GCE/EBP/AuNP-Cys/Aptamer, and GCE/EBP/AuNP-Cys/Aptamer/Codeine surfaces. Measurements were carried out in 50 mM sodium phosphate buffer (pH 7.4) containing 5,0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl.

Surface modification was further confirmed by EIS as seen in Figure 2. Data were fitted according to the circuit design including the solution resistance (R_s), the double layer capacitance (C_{dl}), Warburg impedance (Z_w) due to the diffusion of the $[Fe(CN)_6]^{3-/4-}$ redox probe, and the charge transfer resistance (R_{ct}), shown in the inset of Figure 2. Rct values displayed an increase after every surface modification due to changes in electron transfer properties proving the success of modifications.

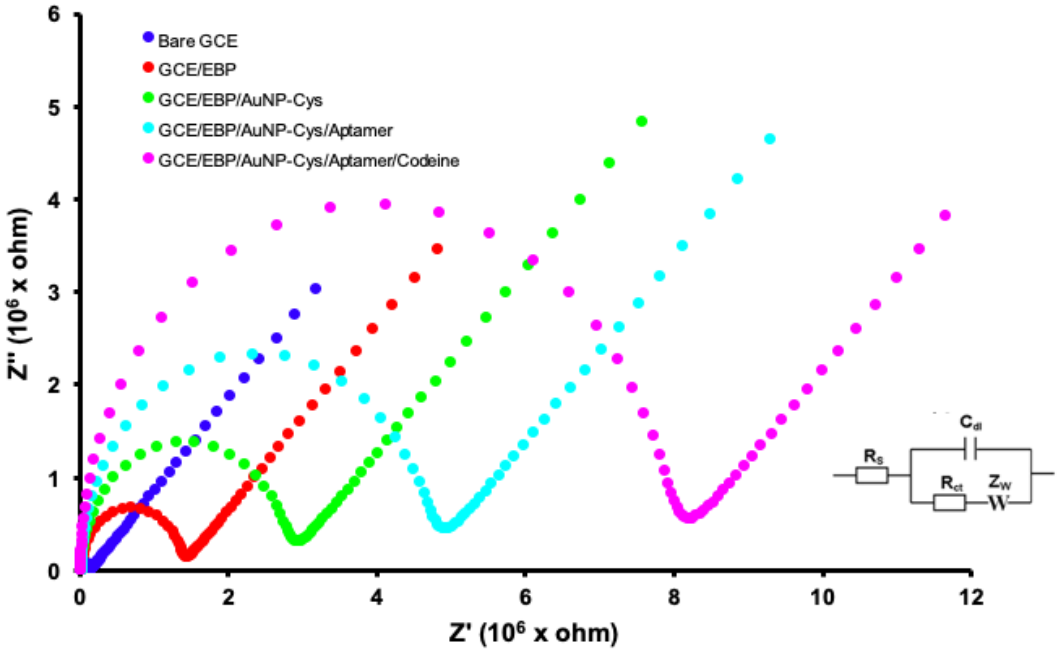


Figure 2 Nyquist diagrams of the modified electrode surfaces. (50 mM sodium phosphate buffer (pH 7.4) containing 5,0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl)

DPV technique was employed to investigate the analytical performance of the biosensor. DPV peak current values of the bare GCE, GCE/EBP, GCE/EBP/AuNP-Cys, GCE/EBP/AuNP-Cys/Aptamer, and GCE/EBP/AuNP-Cys/Aptamer/Codeine were 77.3 μA, 50.9 μA, 24.3 μA, 11.0 μA, and 6.7 μA, respectively (Figure 3). As seen in Figure 3, the current peaks decrease consecutively after each surface modification as the current passing through the electrode surface decreases due to the increased thickness of diffusion layers on the surface. Furthermore, the potentials obtained after immobilization of the aptamer to the surface are observed to shift in the positive direction. This may be due to the physical adsorption of the polymer and AuNP-Cys coated surface causing the formation of a non-homogeneous layer containing hydrophilic and hydrophobic groups. This may cause a change in the interaction between the water-soluble redox probe and the electroactive area. Depending on the chemical structure and layer thickness, these changes can be effective for oxidation of the redox mediator.

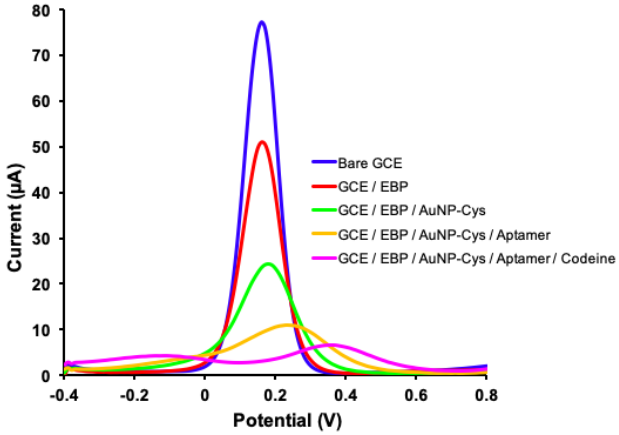


Figure 3 DPVs of bare GCE, GCE/EBP, GCE/EBP/AuNP-Cys, GCE/EBP/AuNP-Cys/Aptamer, and GCE/EBP/AuNP-Cys/Aptamer/Codeine surfaces.

A calibration curve was plotted using the change in the current values in differential pulse voltammograms as a function of analyte concentration, given with the linear equation y=0,37x+0,862 where R^2 equals 0,9998. Linear range was found as 0,1 pg/mL–50 ng/mL (3.3×10^{-4} – 33 nM), which is a wide linear range for the sensing system.

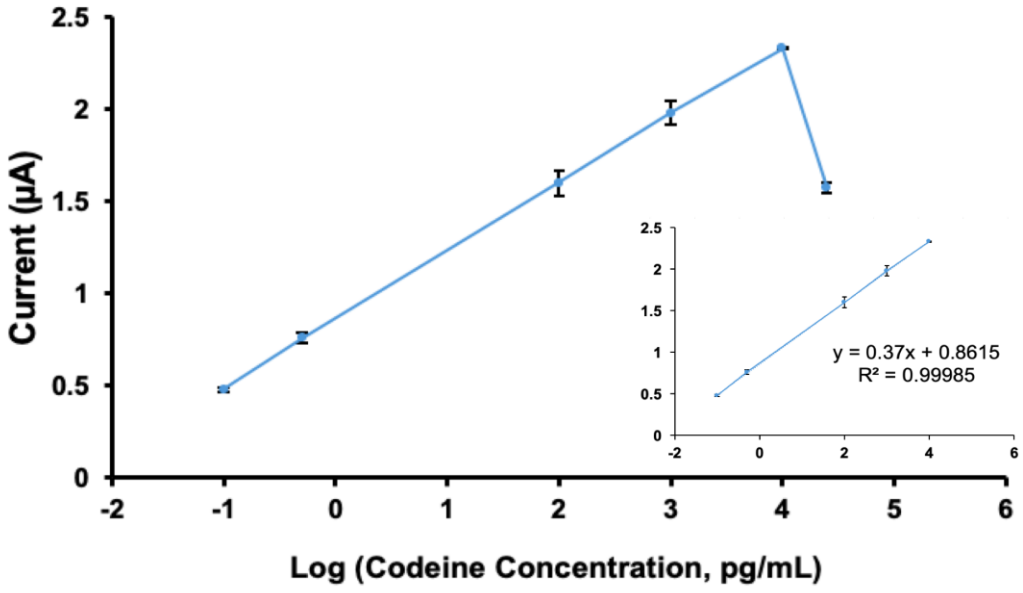


Figure 4 The calibration curve of the proposed sensor at different concentrations.

Analytical parameters such as LOD and repeatability for the proposed aptasensor were investigated. The repeatability was confirmed with 10 successive measurements. Values of standard deviation and coefficient of variation were calculated as 0.15 and 3.12%, respectively. LOD was found as 1.13 pg/mL (2.4×10^{-2} nM) for codeine analysis by using $3S_b/m$ formulation, where S_b is the standard deviation of 10 measurements for the lowest concentration value in the calibration curve, and m is the slope of the linear equation.

Table 1. Analytical features of the proposed sensor platform.

Linear range (nM)	3.3×10^{-4} – 33
Limit of detection (nM)	3.8×10^{-3}
Repeatability (∓S.D.)	0.15
Coefficient of variation (%)	3.12

S.D.: Standard Deviation

Comparison of analytical performances of GCE/EBP/AuNP-Cys/Aptamer with other reported codeine biosensors is provided in Table 2. Values show that the proposed biosensor demonstrated better analytical performance for codeine detection with a very low LOD and a wide linear range.

Table 2. Comparison of Analytical Features to Other Codeine Biosensors

Modified Electrode	Method	LOD (nM)	Linear Range (nM)	Ref
GCE/ND-DHP	SWV	54.5	299 – 1.08×10^4	(Simionia et al. 2017)
SPE/FSG	SWV	5.8	0.2 – 2×10^5	(Mohamed et al. 2018)
CMSPE	SWV & FIA	20	16.7 – 400	(Shih et al. 2002)
GCE / GR-NF	SWV	15	50 – 3.0×10^4	(Li et al. 2013)
BDDF	DPV	80	100 – 6.0×10^4	(Švorc et al. 2013)
Au-MSN	DPV	3.0×10^{-3}	0.01 – 100	(Huang et al. 2013)
SPCE/AChE-TTF	Chronoamperometric	2.0×10^4	NR	(Asturias-Arribas et al. 2013)
GCE/Nafion-MWCNTs	DPV	14	50 – 500	(Piech et al. 2015)
GCE/MWCNT/SmHCF	DPV	60	200 – 2.0×10^4	(Mashadizadeh et al. 2016)
GCE/EBP/AuNP-Cys/Aptamer	Amperometric	3.8×10^{-3}	3.3×10^{-4} – 33	This work

Abbreviations
Au-MSN: Au-Mesoporous Silica Nanoparticles, AChE: Acetylcholinesterase, BDDF: Boron-Doped Diamond Film, CMSPE: Nontronite Clay-Modified Screen Printed Electrode, FIA: Flow Injection Analysis, FSG: Adenine-Functionalized Spongy Graphene, GCE: Glassy Carbon Electrode, GR-NF: Graphene composited Nafion, MWCNT: Multiwalled Carbon Nanotubes, ND-DHP: Nanodiamonds – Dihexadecyl Phosphate, NR:

Not Reported, TTF: Tetrathiafulvalene, SmHCF: Samarium Hexacyanoferrate, SPE: Screen Printed Electrode, SPCE: Screen Printed Carbon Electrode, SWV: Square-Wave Voltammetry

Selectivity of the aptasensor designed for codeine detection was evaluated by determining its response to various interferences that can be found in biological matrices and could be present together with codeine, such as cocaine, morphine, caffeine, and methylphenidate. Concentration of codeine and all interferants was 100 ng/mL. Relative response signals of the interferants with respect to that of codeine were assessed. Results showed that the proposed biosensor was highly selective to codeine.

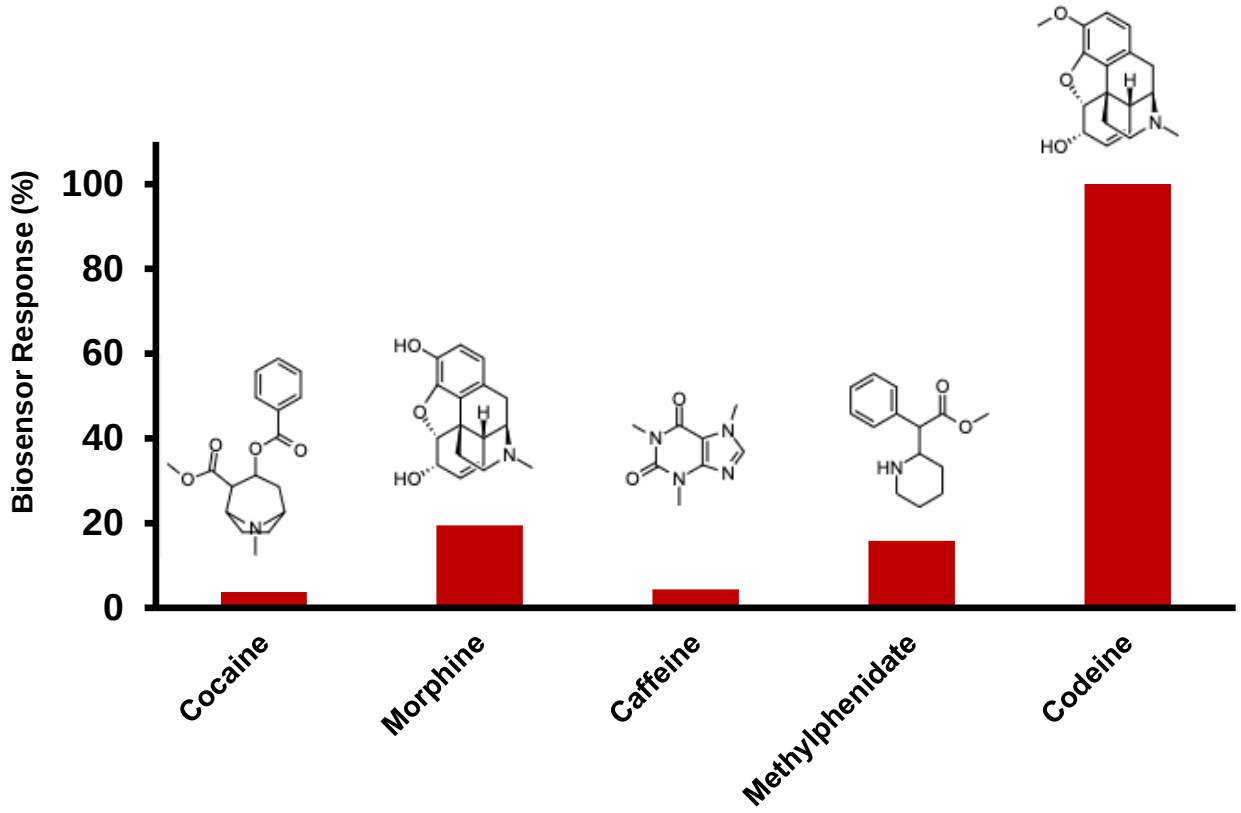


Figure 5 Influence of possible interferants

CONCLUSIONS

A new aptasensor platform was developed for the highly sensitive and selective detection of codeine, based on aptamer immobilized, Cys modified Au nanoparticles making use of the affinity of thiol groups towards gold surfaces. CV, EIS, and DPV was employed to analyze surface modifications and analytical performance of GCE/EBP/AuNP-Cys/Aptamer provided fast analysis and had an outstanding analytical performance with a wide linear range and a very low LOD. Along with its high sensitivity, results showed that this platform is quite useful for the detection of codeine selectively as it does not display a significant response to other possible interference compounds.

ACKNOWLEDGEMENTS

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➢ *ORAL PRESENTATION*

Determination of Antioxidant Activities of Sumac (*Rhus coriaria*) Extracts with Different Solvents

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Abstract
As a nutraceutical, sumac (Rhus Coriaria) was extracted by using different solvents of methanol, ethanol, and water. The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method of free radical scavenging capacity was used to determine the effects of solvent on antioxidant activities of the plant. The total phenolic content was studied by The Folin Ciocalteu Reagent method. The antioxidant activities of extracts exhibit minor changes in different solvents and varied in the range of 84.3–86.4 %. The total phenolic contents are affected by the selected solvent. The highest total phenolic content was determined at the liquid phase of water and it was estimated as 26.3 mg/g in gallic acid.
Keywords: DPPH, Solvent, Sumac, total phenolic content.

INTRODUCTION

Functional foods are the ingredients containing the health-giving-additives. There is a major increase in consuming functional foods and nutraceuticals. Phenolic compounds of the functional foods are responsible for the antioxidant effect. Antioxidants eliminate free radicals caused by factors such as radiation, toxic substances, stress, and air pollution. They prevent many diseases such as obesity, cell deaths, mutations, cancer, and heart conditions (Lobo et al., 2010; Gul et al., 2016; Kosar et al., 2007).

In daily life, vegetables and fruits are the main sources of phenolic compounds. There have been studied 4.000 – 6.000 medical plants with this purpose. Reis et al., indicated the excellent bioactivity features of mushrooms (Reis et al., 2017). Diankov et al., investigated the antioxidant activities of lemon peels (Diankov et al., 2011). Wildowati et al., analyzed the antioxidant features of clove, Indonesian cassia, coriander, nutmeg, and java cardamom (Wildowati et al., 2015). Ruch et al., studied the antioxidant fraction of Chinese green tea (Ruch et al., 1989).

Sumac (Rhus Coriaria) is a member of the Anacardiaceae family and grows in warm and mild-weather regions. It is a red-coloured plant that generally used in South Africa, North America, Turkey, and the Middle East. The seeds of the plant generally are dried and ground as pre-treatment. Although the chemical composition and biological activities are dependent on the region, the phytochemical studies on sumac indicate the high tanning, flavones, vitamins, and organic acid and content (Kosar et al., 2007; Tehrani et al., 2017; Wang and Zhu, 2017). Ciftci Yegin compared the antioxidant activities of the sumac spices taken from different regions (Yegin, 2017). Nagip studied the hypolipidemic effect of sumac and indicated the significant improvement in lipids profile, liver and kidney functions, and antioxidant parameters in hypercholesterolemic rats (Nagip, 2017).

The extraction procedure and the preferred treatment techniques are also effective in the antioxidant activities of compounds. Chan et al., analyzed the effects of the boiling, microwave, and blanching on the bioactivities of the antioxidant, anti-tyrosinase, anti-quorum sensing and antibacterial properties of the herbal spices (Chan et al., 2015). Abdelfadel et al., studied the effects of hot and cold extraction methods on the cumin, ginger, cinnamon and clove (Abdelfadel et al., 2015). Ereifej et al., experimented the effects of the extraction temperature on the antioxidant activities of herbal spices (Ereirej et al., 2016). Darmajana et al., investigated the effects of solvents of aquades, ethanol and glacial acetic acid on the antioxidant activity of white tea (Darmajana et al., 2017).

Although various studies reported the health effects of sumac, the literature lacks information on its antioxidant activity and phenolic content. The objectives of this study were to investigate the effects of the extraction solvent on the antioxidant activity of sumac by using the techniques of The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method and The Folin Ciocalteu Reagent method.

EXPERIMENTAL PROCEDURE

Materials and Method

Dried and ground sumac (Rhus coriaria) fruits were purchased from a local market in Mardin, Turkey. It was stored at -20°C before use. Ultrapure water (18.3 mΩ.cm) used in the experiments was supplied from a Human Power I+ Water Purification System. The ethanol (with the purity of 96%) and methanol (with the purity of 99%) were provided from Merck Chemicals.

The extraction solvents were selected as of water, ethanol and methanol. 2.5 g of sumac added to 50 ml of solvents and each solution was stirred on a magnetic stirrer at 400 rpm for 75 minutes. After the extraction was completed, the solution was filtered by using the 0.45 µm of PTFE filter paper (Figure 1).

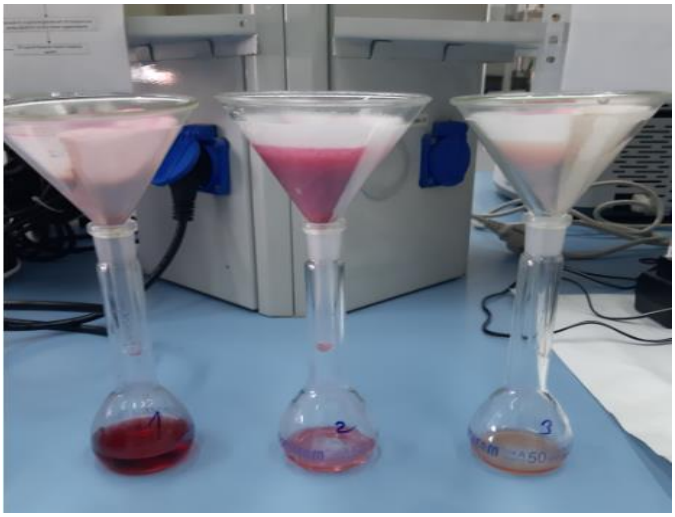


Figure 1. Extraction of sumac with different solvents

Perkin Elmer UV-Vis spectrophotometer (Figure 2) was preferred for the analyses of antioxidant activity and total phenolic content.

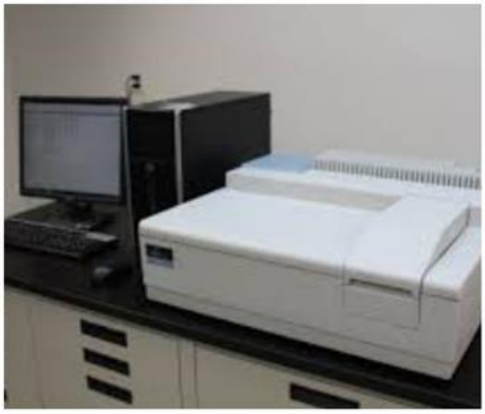
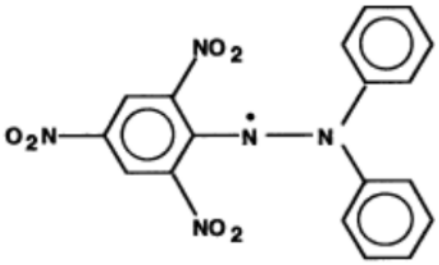


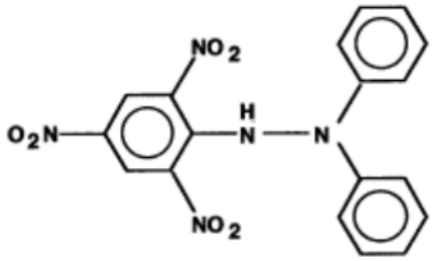
Figure 2. Perkin Elmer UV-Vis spectrophotometer

Determination of Antioxidant Activity

Free radical scavenging efficacy analysis was performed using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical. The method is based on the ability of extracts to deliver a proton or electron to lighten the purple DPPH solution (Figure 3). The low absorbance of the reaction mixture is indicative of high free radical removal activity.



1: Diphenylpicrylhydrazyl (free radical)



2: Diphenylpicrylhydrazine (nonradical)

Figure 3. Free radical and nonradical forms of DPPH (Molyneux, 2014)

In the stock solution preparation, methanol and water were mixed at the ratio of 3:2. The 0.022 g DPPH was added to 100 ml of this solution. To obtain a calibration curve, 2.5, 5, 7.5 and 10 ml of the stock solution were completed to 50 ml. For the DPPH analysis, 2 ml of each sample was added to 8 ml of DPPH solution. Samples were kept in the dark for 30 minutes at room temperature.

The calibration solutions and the samples were analyzed at 517 nm in UV analyses. The equation for free radical scavenging capacity was given in Eq. (1).

$$\%Act_{DPPH} = \frac{Abs_C - Abs_S}{Abs_C} \times 100 \qquad (1)$$

where, $\%Act_{DPPH}$ was the free radical scavenging capacity. The Abs_C and Abs_S were the absorbance values of control and samples, respectively.

Determination of Total Phenolic Content

The Folin-Ciocalteu (FCR) method is based on electron transfer, which measures the reduction capacity of phenolic antioxidant. The reagent (FCR) containing molybdenum VI is used and the color change was observed due to the reduction of Mo^{+6} to Mo^{+5} (Prior et al., 2005). The procedure is based on comparing the absorbance with a gallic acid standard curve, the results are expressed in gallic acid equivalents (GAE).

In the stock solution preparation, 0.05 g of gallic acid were dissolved in 100 ml ethanol. The calibration solutions were prepared by the mixture of dilution of the stock solution at the ratios of 0.05, 0.1, 0.2 ve 0.3 mg/ml, FCR reagent and Na_2CO_3. The samples were prepared in the same way except for gallic acid.

The calibration solutions and the samples were analyzed at 765 nm in UV analyses.

RESULTS and DISCUSSION

Antioxidant Activity Results

The calibration solutions for each solvent in DPPH analysis were presented in Fig 4.

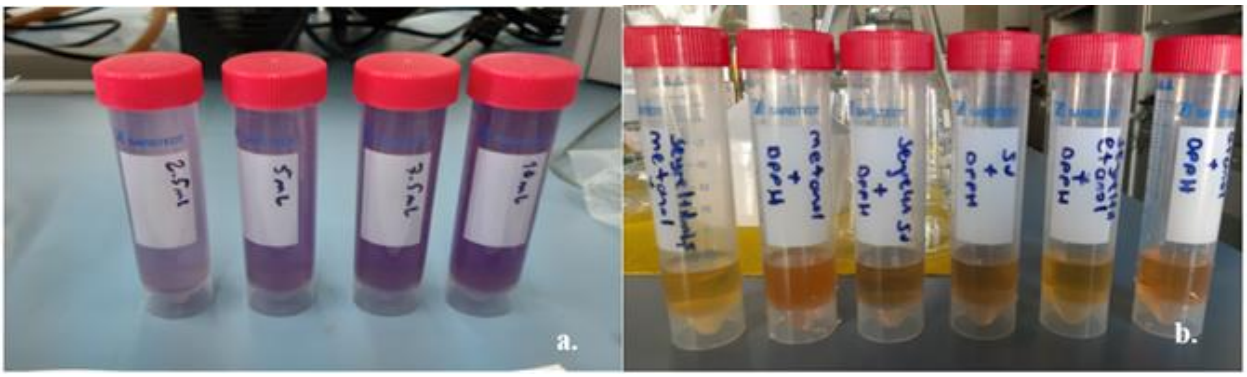


Figure 4. a. Calibration solutions and b. sumac extracts

Free radical scavenging acitvies of DPPH for the different extraction solvents were given in Table 1. In the comparison of solvent effects on the free radical scavenging capacities of DPPH, there were minor differences were determined. The highest free radical scavenging capacity (%Act $_{DPPH}$) was obtained in methanol extraction.

Table 7. Free Radical Scavenging Activity Results

Solvent		Absorbance	Concentration (mg/ml)
Methanol	Abs_C	0.3547	0.0225
	Abs_S	1.8970	0.1659
	$\%Act_{DPPH}$	86.3985	
Ethanol	Abs_C	0.1798	0.0053
	Abs_S	0.4879	0.0342
	$\%Act_{DPPH}$	84.3186	
Water	Abs_C	0.2295	0.0101
	Abs_S	0.8783	0.0716
	$\%Act_{DPPH}$	85.7860	

Total Phenolic Content Results

The calibration curves were obtained by using the mixture of diluted stock solution with FCR agent and Na_2CO_3. The correlation coeffitions were in the range of 0.9931 – 9.9974. The results of the total phenolic contents for each solvent was given in Table 2. According to the FCR analysis results in Table 2, the highest total phenolic content was seen in water extraction.

Table 2. Results of Total Phenolic Contents for Each Solvent

Solvent	Concentration (mg/g)
Methanol	2.58
Ethanol	4.29
Water	26.31

Phenolic compounds exhibit the high antioxidant and antiradical properties. Sumac species exhibit a high level of gallic acid concentration (Yegin, 2017). The total phenolic content (in gallic acid concentration) of the sumac taken from Turkey region higher than the other regions of Asia (Wang and Zhu, 2017).

CONCLUSION

Sumac (Rhus Coriaria) was a well-known medical plant. The extracts can be used for the bioactivities of antimicrobial, antibiotic, antioxidative and antitumorigenic. The process parameters play an essential role in the exraction of medical plants. As a medical plant, sumac was extracted by using different solvents of methanol, ethanol, and water.

The results of DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method of free radical scavenging capacity was varied between 86.3985% and 84.3186%. This minor change can be interpreted as the less effect of solvent on the antioxidant activity. The total phenolic content in gallic acid concentration was analysed by the Folin Ciocalteu Reagent method. The results showed that the total phenolic contents were sensitive to the solvent selection. The highest total phenolic content was determined at the liquid phase of water and it was estimated as 26.3 mg/g in gallic acid. The results indicate that water extraction of sumac can be useful for the biochemical and medical applications.

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➢ *ORAL PRESENTATION*

Kitosan Katkılı Yeşil Çam Kozalağı Ekstraktlarının Antikanser Etkilerinin Araştırılması

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Özet

Kanser, genomdaki birçok mutasyonun birikimi ile meydana gelen sistemik bir hastalıktır. Kanser tedavilerinde farklı tedavi yöntemleri kullanılmakta ve zaman içerisinde yüksek toksisite ve ilaç direncine sebep olmaktadır. Doğal ürünler ve bunların sentetik türevleri kanser tedavilerinde alternatif potansiyel taşımaktadır. *P. koraiensis* uzun yıllardır geleneksel tıpta birçok farklı hastalığın tedavisi için kullanılmaktadır. Ayrıca antibakteriyel ve antifungal gibi birçok biyolojik aktiviteler göstermektedir. Kitosan, uzun zincirli polimer türevi olan kitinin yaklaşık olarak yarısının deasetile edilmesiyle meydana gelmektedir. Kitosanın pH'a duyarlı olması kontrollü ilaç salınımında yaygın olarak kullanılmasını sağlamaktadır. Tümör hücrelerinin pH'sının sağlıklı hücrelerin pH'sından çok daha düşük olması kontrollü ilaç salınımını sağlamaktadır.

Bu çalışmada kitosansız ve kitosanlı yeşil çam kozalağı ekstraktlarının 24,48 ve 72 saatlik inkübasyonlar sonunda MCF-7, Vero ve HeLa hücreleri üzerinde meydana getirdikleri sitotoksik etkiler belirlendi. Ekstraktlar 400,200,100,50,25 µg/mL olarak hazırlanan dozlarda MCF-7 ve HeLa hücrelerine uygulanarak inkübasyon süreleri sonunda WST-8 hücre canlılığı belirleme kiti ile sitotoksisite testi yapıldı. Elde edilen bulgular sonucu kitosan katkılı yeşil çam kozalağı ekstraktlarının 48 ve 72 saatlik inkübasyonların sonunda MCF-7 hücreleri üzerinde 400 µg/ml ve 200 µg/mL dozlarda sitotoksik etki yarattığı belirlendi. Bu ekstraktın HeLa hücreleri üzerinde ise 24,48 ve 72 saatlik inkübasyonların sonunda 400ug/ml ve 200 µg/mL dozlarda sitotoksik etki yarattığı belirlendi.
Anahtar kelimeler: MCF-7, HeLa, Sitotoksisite, Kitosan, Çam Kozalağı Ekstraktı

Anti-Cancer Effects Of Kitosan Additive Green Pine Collar Extracts

Abstract

Cancer is a systemic disease that occurs with the accumulation of many mutations in the genome. Different treatment methods are used in cancer treatments and over time cause high toxicity and drug resistance. Natural products and their synthetic derivatives have alternative potential in cancer treatments. *P. koraiensis* has been used in traditional medicine for many years for the treatment of many different diseases. It also shows many biological activities such as antibacterial and antifungal. Chitosan is produced by deacetylating approximately half of the long chain polymer derivative chitin. The fact that chitosan is sensitive to pH makes it widely used in controlled drug release. The fact that the pH of the tumor cells is much lower than the pH of the healthy cells ensures controlled drug release.

In this study, cytotoxic effects of chitosan and chitosan green pine cone extracts on MCF-7, Vero and HeLa cells were determined after 24, 48 and 72 hours of incubation. The extracts were applied to MCF-7 and HeLa cells in doses prepared as 400,200,100,50,25µg/mL, and cytotoxicity was tested with the WST-8 cell viability determination kit at the end of the incubation period. As a result of the findings obtained, it was determined that chitosan-added green pine cone extracts had a cytotoxic effect on MCF-7 cells at doses of 400µg/ml and 200µg/mL after 48 and 72 hours of incubation. It was determined that this extract had a cytotoxic effect on HeLa cells at 400µg/ml and 200 µg/mL doses at the end of 24, 48 and 72 hours of incubation.
Keywords: MCF-7, HeLa, Cytotoxicity, Chitosan, Pinecone Extract

GİRİŞ

Kanser, genomdaki çoklu mutasyon birikimi ile meydana gelen sistemik bir hastalıktır (Loeb ve ark, 2003; Blagosklonny ve ark, 2005). Kanser hastalarında tedavi amacıyla kullanılan klasik yöntemler kemoterapi, radyoterapi ve cerrahi operasyonlar olarak sınıflandırılır (Akbayrak 1998; Kızılcı 1999; Kvols 2005; Çapar 2010). Kanser tedavilerinde kullanılan farklı tedavi yöntemleri zamanla yüksek toksisite ve ilaç direncine neden olmaktadır. Doğal ürünler ve bunların sentetik türevleri kanser tedavisi için alternatiftir. Ayrıca günümüzde kullanılan antikanser ilaçların %50'sinden fazlası doğal ürünlerden elde edilmiştir (Zhong ve ark, 2015).

Kore çamı olarak bilinen *Pinus koraiensis*, Doğu Asya'da ılıman ve serin ormanlarda yayılış göstermektedir (Piao ve ark, 2011, Zhang ve ark, 2017). *P. koraiensis* uzun yıllardır geleneksel tıpta birçok farklı hastalığın tedavisi için kullanılmaktadır (Kvols ve ark, 2005). Antibakteriyel ve antifungal gibi birçok farklı biyolojik aktivitelere sahiptir (Piao ve ark, 2011). Ayrıca Kore çamı tohumları antioksidan aktiviteye gösteren fenolik bileşikler bakımından fazlasıyla zengindir. Yakın zamanlarda yapılmış olan çalışmalarda *P. koraiensis* çam kozalaklarından elde edilen polifenollerin, çeşitli kanser hücrelerinin *in vitro* proliferasyonunu önemli ölçüde inhibe ettiği gösterilmiştir. Ek olarak *P. koraiensis*'in kaspaz aracılı apoptozu indüklediği belirtilmiştir (Lee ve ark, 2017). Kitosan uzun zincirli polimer türevi olan kitinin yaklaşık olarak yarısının deasetile edilmesiyle meydana gelmektedir. Kitosan glikozidik bağlarla bağlanan lineer omurgaya sahiptir (De la Fuente ve ark, 2008; Özkan ve ark, 2019; Wahba 2019). Kitosan biyolojik olarak uyumlu ve parçalanabilir özelliğe sahip olmasının yanı sıra anti-mikrobiyal aktivite de göstermektedir (Ullah ve ark, 2017; Wahba 2019).

A549, H1264, H1299 ve Calu-6 hücrelerinde yapılan çalışmada *P. koraiensis* çam kozalaklarından elde edilen sulu ekstraktların doza bağlı olarak hücre canlılığını azalttığı ve kaspaz3 aktivitesini indüklediği belirtilmiştir (Lee ve ark, 2017). Kitosan katkılı *Pinus merkusii* kabuğu ekstraktı nanopartikülleri (Nano-PMBE) ile yapılan çalışmada HeLa hücrelerinde hücre döngüsünü G0/G1 fazında durdurduğu, hücrelerde apoptozu tetiklediği, p53 ve kaspaz-9 ifadelerinde de önemli ölçüde artış meydana getirdiği belirlenmiştir (Proboningrat ve ark, 2019).

Bu çalışmada geleneksel tıpta birçok hastalığın tedavisinde yaygın olarak kullanılan yeşil çam kozaklarının kitosansız ve kitosanlı olarak hazırlanmış ekstraktralarının insan meme ve serviks kanseri üzerindeki sitotoksik etkileri incelenmiştir.

MATERYAL VE METOD

Bitki ekstraktlarının elde edilmesi: Kızılçam (*Pinus brutia* Ten.) kozalakları Antalya ili Döşemealtı ilçesinde bulunan çam ağaçlarından Ekim ayının ilk haftası toplanıldı. Toplanan kozalaklar homojenizatörden geçirilerek toz haline getirildi. Toz haline getirilen kozalaklar 500 g olacak şekilde tartıldı ve distile su ile 3 litreye tamamlandı. KOH ile pH 12'ye ayarlandıktan sonra termodinamik hassas ısı yöntemi ile 121 C'de 30 dakika ısıl işlem uygulandı. Oda sıcaklığına getirildikten sonra filtre kâğıdından geçirildi. +4 C'de saklandı. %1'lik kitosan eklendi ve spray dryer ile kurutuldu.

Hücre kültürü: MCF-7 (östrojen reseptör pozitif insan meme kanseri), HeLa (serviks kanseri) ve Vero (Maymun böbrek epiteli) hücreleri %10'luk FBS eklenmiş RPMI 1640 besiyerinde çoğaltıldı. Bütün hücreler %5 CO2'li atmosferde 37°C'de inkübe edildi. ATCC'nin tavsiye ettiği şekilde hücreler %0.25 tripsin, %0.03 EDTA karışımı ile kaldırılıp 1:2 ya da 1:3 oranında olacak şekilde pasajlanarak kullanılmayan hücreler %95 besiyeri ve %5 DMSO içerecek şekilde hazırlanan solüsyon içerisinde -80°C derin dondurucuda saklandı.

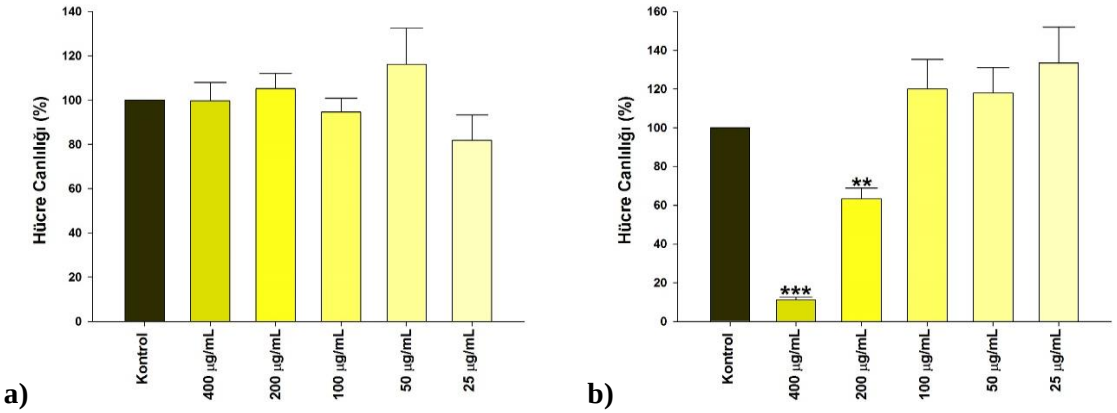
WST-8 hücre canlılığı testi: Hücre proliferasyonu ve sitotoksisite deneylerinde hücre canlılığını belirlemek amacıyla kullanılan WST-8 hücre proliferasyon kiti oldukça hassas kolorimetrik bir testtir. Çalışmamızda bu kit kullanılarak ilaçların sitotoksik etkileri araştırıldı. MCF-7, HeLa ve Vero hücreleri stoklardan açılarak küçük petri kaplarında çoğaltıldı ve petri kapları % 80-90 oranında dolunca tripsinizasyon işlemi ile kaldırılıp $1x10^4$ hücre/kuyucuk olacak şekilde 96 kuyucuklu steril plaklara ekildi. 24 saatlik inkübasyon süresinin ardından besiyerleri uzaklaştırıldı. İnkübasyon süresi sonunda %1 serum içeren besiyerinde hazırlanan farklı konsantrasyonlardaki kitosan katkısız ve katkılı çam kozalağı ekstraktları (25, 50, 100, 200, 250 ve 400 µg/mL) 96 kuyucuklu steril plaklara uygulandı. Uygulamayı takiben, başlangıçtaki hücre sayısını belirlemek için başlangıç zamanı (Time Zero) okuması yapıldı. Deneyler 24, 48 ve 72 saatlik inkübasyon süreleri sonunda her kuyucuğa 90 µl serumsuz besiyeri ve 10 µl WST-8 eklenerek sonlandırıldıktan sonra 4 saat 37°C'de inkübe edildi. İnkübasyon süresi sonunda plakların absorbans değerleri Elisa okuyucuda (Thermo Scientific Multiskan Go), 450 nm dalga boyunda ölçülerek kaydedildi.

İstatistiksel Analiz: Sitotoksisite testlerinden elde edilen deney sonuçlarındaki kontrol ve diğer gruplar arasındaki farklılık Graph-Pad InStat istatistik programında, Tek Yönlü Anova Testi ve ardından Dunnet Çoklu Karşılaştırma Testi kullanılarak değerlendirildi. Ekstraksiyonların IC50 değerleri Sigma Plot 10.0 istatistik programı kullanılarak belirlendi. Tüm veriler ortalama ± SEM değerleri halinde, Sigma Plot 10.0 programı kullanılarak grafik haline getirildi.

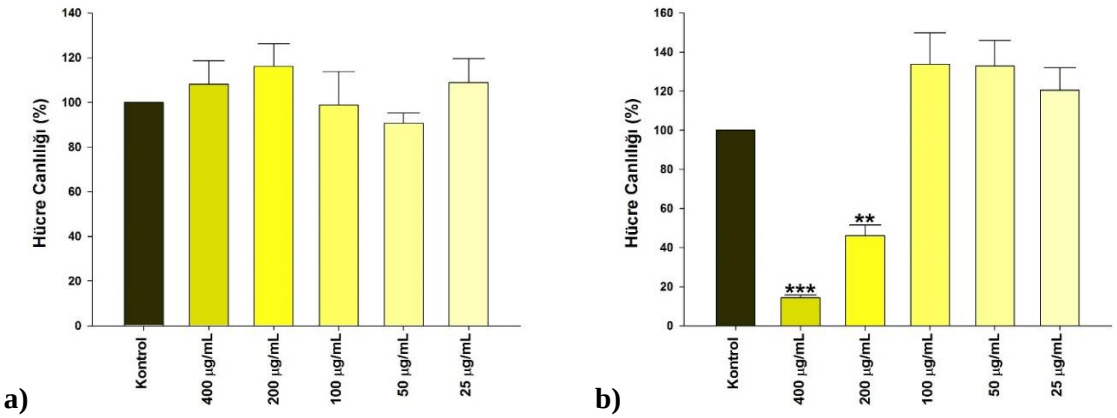
BULGULAR

MCF-7 Hücre Hattı

Kitosan ilave edilmemiş yeşil kozalak ekstraktı MCF-7 hücrelerinde hiçbir inkübasyon süresinde ve hiçbir dozda sitotoksik etki yaratmamıştır. Kitosan ilave edilen yeşil kozalak ekstraktı 24 saatlik inkübasyon sonunda hücrelerde sitotoksik etki yaratmazken, ekstrakt için 48 saat sonunda IC_{50} değeri 252.3 µg/mL olarak hesaplanmıştır **(Şekil 1.1).** 72 saat sonrasında ekstrakt için hesaplanan IC_{50} değeri 195.5µg/mL 'dir **(Şekil 1.2).**



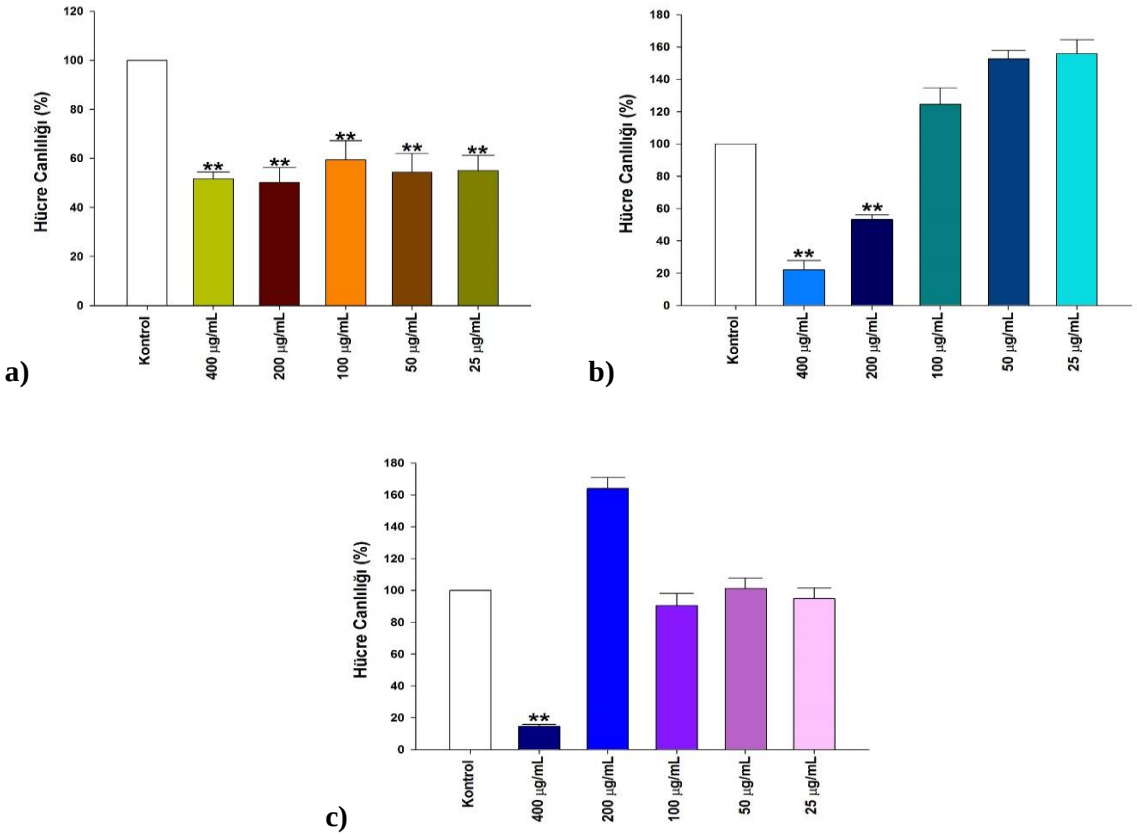
Şekil 1.1. a) MCF-7 hücre hattında yeşil çam kozalağı ekstraktının 48 saatlik inkübasyon süresinde farklı dozlardaki hücre canlılığına etkisi. b) MCF-7 hücre hattında kitosan katkılı yeşil çam kozalağı ekstratının 48 saatlik inkübasyon süresinde farklı dozlardaki hücre canlılığına etkisi.



Şekil 1.2. a) MCF-7 hücre hattında yeşil çam kozalağı ekstraktının 72 saatlik inkübasyon süresinde farklı dozlardaki hücre canlılığına etkisi. **b)** MCF-7 hücre hattında kitosan katkılı yeşil çam kozalağı ekstratının 72 saatlik inkübasyon süresinde farklı dozlardaki hücre canlılığına etkisi.

HeLa Hücre Hattı

Yeşil kozalak ekstraktı HeLa hücrelerinde 24,48 ve 72 saatlik inkübasyon sürelerinde hiçbir dozda sitotoksik etki yaratmamaktadır. Kitosan katkılı yeşil kozalak ekstraktları için 24, 48 ve 72 saatlik inkübasyon sürelerinde HeLa hücreleri için IC_{50} değerleri sırasıyla 200.5 µg/mL, 221.9 µg/mL ve 352.3 µg/ml olarak hesaplanmıştır **(Şekil 1.3.)**.



Şekil 1.3. Kitosan katkılı yeşil çam kozalağı ekstraktlarının farklı dozlarda HeLa hücre hatları üzerindeki etkileri **a)** 24 saatlik inkübasyon süresindeki hücre canlılığına etkisi **b)** 48 saatlik inkübasyon süresindeki hücre canlılığına etkisi **c)** 72 saatlik inkübasyon süresindeki hücre canlılığına etkisi.

TARTIŞMA

Elde edilen bulgular sonucunda kitosansız yeşil çam kozalağı ekstraktlarının HeLa hücreleri üzerinde uygulanan tüm dozlarda ve inkübasyon sürelerinde sitotoksik etkisinin olmadığı belirlenmiştir. Kitosan katkılı yeşil çam kozalağı ekstraktları HeLa hücreleri üzerinde 24 saatlik inkübasyon sonucu hücre canlılığında yaklaşık olarak %40, 48 saatlik inkübasyon sonucu ise %80 azalmaya sebep olduğu belirlenmiştir. Yeşil çam kozalağı ekstraktları MCF-7 hücrelerinde herhangi bir sitotoksik etki meydana getirmemektedir. Kitosan katkılı yeşil çam kozalağı ekstraktlarının MCF-7 hücrelerinde 48 ve 72 saatlik inkübasyon sürelerinde hücre canlılığını %80 oranında azalttığı görülmektedir.

Lee ve arkadaşlarının 2018 yılında insan akciğer kanseri hücreleri ile yapmış olduğu çalışmada, *Pinus koraiensis* çam kozalağı su ekstraktlarının kaspaz-3'e bağlı bir şekilde apoptotik hücre ölümünü indükleyerek p53'ten bağımsız olarak sitotoksik aktivite gösterdiği belirlenmiştir. Ayrıca çam kozalaklarının bileşenlerinin antimetastatik etkiye sahip olduğu belirtilmiştir (Lee, 2018). Farklı pinus türlerinin ve standartlarının hücre canlılığı üzerine etkilerinin incelendiği çalışmada özellikle *Pinus brutia ve Pinus sylvestris* türleri MCF-7 hücrelerinin canlılığı üzerinde sitotoksik etki yaratmadığı bildirilmiştir (Yeşil Çeliktaş, 2009). Ayrıca Yi ve arkadaşlarının yapmış olduğu farklı bir çalışmada çam kozalağı ekstraktlarının en düşük antiproliferatif etkiyi MCF-7 ve A-375 hücreleri üzerinde en yüksek etkiyi ise Lovo ve HeLa hücreleri üzerinde sergilediği bildirilmiştir (Yi ve ark, 2015). Çam kozalağı ekstraktları ile yapılan daha önceki çalışmalar ile

karşılaştırıldığında kitosan katkılı yeşil çam kozalağı ekstraktları MCF-7 ve HeLa hücrelerinde daha yüksek sitotoksik etki göstermektedir.

SONUÇ

Tüm veriler sonucunda; kitosansız yeşil çam kozalağı ekstraktlarının HeLa hücrelerinde denenen hiçbir dozda ve inkübasyon süresinde sitotoksik etki yaratmadığı belirlenmiştir. Kitosan katkılı yeşil çam kozalağı ekstraktları ise HeLa hücrelerinde 24 saatlik inkübasyon süresi sonunda denenen her dozda etkili olmuş ve IC_{50} değeri 200.5 µg/mL olarak belirlenmiştir. Bu ekstraktın 48 saatlik inkübasyon sonrasında Hela hücrelerinde 400 µg/ml ve 200 µg/ml dozlarda sırasıyla %80 ve %50 sitotoksik etki gösterdiği ve 72 saatlik inkübasyon sonrasında IC_{50} değerinin 352.3 µg/ml olduğu belirlenmiştir. Kitosansız yeşil çam kozalağı ekstraktları ve kitosan katkılı yeşil çam kozalağı ekstraktları, 24 saatlik inkübasyon süresi sonunda hiçbir dozda MCF-7 hücreleri üzerinde sitotoksiketki yaratmamıştır. Kitosan katkılı yeşil çam kozalağı ekstraktının 48 ve 72 saatlik inkübasyon sürelerinde 400 µg/ml ve 200 µg/ml dozlarda hücre canlılığı üzerinde sırasıyla %80 ve %40-50 oranında sitotoksik etki meydana getirdiği belirlenmiştir.

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➢ *ORAL PRESENTATION*

Effects of Hyaluronic Acid on Alloplastic Bone Graft Applied Rat Calvarial Bone Defect Model

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Abstract

Many reasons such as trauma, neoplasms, infections and congenital anomalies may cause calvarial bone defects. In order to overcome the inconvenience of osseous defects in the cranial area and stimulate bone regeneration, various graft types and therapies have been investigated. In the study, we aimed to investigate the effects of hyaluronic acid (HA) on alloplastic graft material applied rat calvarial bone defect model. Wistar male rats (n = 32) were divided into 4; defect group as control, defect+ HA treated , defect+ allograft, defect+allograft+HA group. Under anesthesia, frontal bone was opened and a periosteal flap was removed with periosteal elevator, and a circular full-thickness bone defect (8 mm) on the midline was created. In the defect area of groups 2 and 3, alloplastic bone graft material was placed, subcutaneous tissue was sutured. HA was applied locally on the defect area. All animals were sacrificed after 28 days and calvarial bones were removed, fixed with 10% formalin, decalcified with 5% EDTA, after routine histological preparations, 5 µm thickness sections were cut and stained with H-E. OPN and SPARC monoclonal antibodies were used for immunohistochemical analysis, examined under a light microscope. In defect group H-E sections, increase in mononuclear cell infiltration within the scar tissue in the area of trabeculae, degenerative changes in collagen fibrils and a significant increase in fibroblast cells were observed. In defect+HA group, intense inflammatory cells, and osteoblast cells, which enable bone formation, began to appear in acidophilic areas. In defect+graft group, new bone matrix development seemed to begin, bone trabeculae and osteoblast cells were observed in areas close to the graft. OPN/SPARC expression was positive in osteoblasts of defect group, in inflammatory cells of defect+HA group and in osteocyte cells of defect +graft and defect+graft+HA group, indicating new bone formation. In conclusion, HA+Graft application gave rise to comprehensive recovery with new bone formation in calvarial defect model.

Keywords: Calvarial defect, hyaluronic acid, immunohistochemistry, Osteopontin, rats.

INTRODUCTION

Trauma, neoplasms, infections and congenital anomalies may cause calvarial bone defects. Repairing of bone defects is a significant clinical problem. In order to overcome the inconvenience resulted from osseous defects in the cranial area and stimulate bone regeneration, various graft types and therapies have been investigated (Szpalski et al., 2010; Bosch et al., 1996). Effects of laser and ozone therapies on bone healing in the calvarial defects and alloplastic bone grafts have been used lately (Kazancioglu et al.,2013; Laçin et al., 2018). In the latter, new bone formation have been observed as a result of stimulation of osteoinductive and osteoconductive effect with graft materials that placed in the bone tissue. Bone defects that occur in the maxillofacial area may cause aesthetic and functional disorders so, deformities need to be reconstructed with bone grafts. Today, different graft materials are used for this purpose.

Allografts give osteoconductive strength to the bone and has bone-like biomechanical properties also prevent complications(Gupta and Maitra, 2002). In the calvarial defect model, and graft application procedure there is a 10-40% complication rate, including bleeding, nerve and vascular lesions, and postoperative pain (Younger and Chapman, 1989).

Hyaluronic acid (HA) also called hyaluronan is an anionic, nonsulfated glycosaminoglycan that forms one of the main components of the extracellular matrix domain (Shaharudin and Aziz, 2016) and can be found in

skin, joints, eyes and most other organs and tissues. Some of the HA's biological roles are tissue regeneration, adhesion, cell proliferation and differentiation and functioning of cells. It is known that HA application has a significant role in the adhesion and proliferation activities of cells during the wound healing period (Takeda et al., 2011). High amounts of HA is found in the skin. This content of HA in the skin is temporarily increased in granulation tissue during the wound healing process (Chen and Abatangelo, 1999). In gel form, HA attracts water and swells, this feature is useful in skin treatments as a dermal filler (FDA 2018).
A noncollagenous protein expressed in the bone matrix is Osteopontin (OPN). It is closely related to a series of cellular processes, such as cell signalling, cell adhesion, migration, and inflammation (Thurner et al., 2010), and adhesion of osteoclasts in bone surfaces during bone resorption (Reinholt et al.1990; Ikeda et al., 1992). Osteocytes express OPN under mechanical stress (Bailey et al., 2017) on the other hand, osteoblasts and odontoblast cells express Osteonectin (also known as secreted protein acidic and rich in cysteine- SPARC) which is a glycoprotein (Hamann et al., 2012) involved in initiating the bone and cartilage matrices mineralisation with its calcium binding property (Metsäranta et al., 1989), and that has affinity for collagen and modulate cell-matrix interactions (Wasi et al., 1984; Young et al. 1992; Lane and Sage, 1994). SPARC is highly expressed in tissues with new bone formation (Termine et al., 1981).
This study aimed to demonstrate the effects of hyaluronic acid applied to calvarial bone defect model recruited with alloplastic graft material in new bone development by immunohistochemical methods.

MATERIALS AND METHODS

Ethical Approval and Experimental Design

All experimental procedures were approved by the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (DUHADEK Ethical approval number: 2019/10) and performed in DUSAM Experimental Animals Unit. Animals were housed under a diurnal cycle at 25 °C and 65 %humidity, having free access to water and pellet diet. Wistar Albino rats weighing 250-300 g at the 8-10th weeks of age were used in the study.
Groups were as follows;

Group 1 Control (Defect group, n=8): A calvarial bone defect with a diameter of 8 mm was created, the defect was left empty without any procedure and the wound site was sutured, after 28 days rats were sacrificed, the calvarial bone was removed.
Group 2 Defect + Hyaluronic acid treatment (n=8): A calvarial bone defect with a diameter of 8 mm was created, 1-2 drops of Hyaluronic acid gel (16 mg/kg) was applied to the defect area once and sutured, after 28 days rats were sacrificed, the calvarial bone was removed.
Group 3 Defect + Graft (n=8): A calvarial bone defect with a diameter of 8 mm was created, allograft was applied to the defect and sutured, after 28 days rats were sacrificed, the calvarial bone was removed.
Group 4 Defect + Graft+ HA treatment (n=8): A calvarial bone defect with a diameter of 8 mm was created, allograft was applied to the defect mixing with hyaluronic acid gel and sutured, after 28 days rats were sacrificed, the calvarial bone was removed.

Anesthesia and Surgical Process

Before any surgical procedures, experimental animals had general anesthesia with 90 mg / kg intramuscular ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 10 mg / kg xylazine (Rompun, Bayer, Istanbul, Turkey). After the rats had general anesthesia, the calvarium area was shaved and then the subjects were placed on the fixing board. The surgical site was shaved and povidone-iodine solution (Poviiodex, Kimpur, Turkey) was applied in order to ensure antisepsis. After the necessary asepsis, antisepsis and sterilization conditions are met, local anesthesia was applied around the area to be operated with articaine solution (Ultracain-DS hoechst Marion Roussel, İstanbul,Türkiye) containing epinephrine (0,5ml; 1:200.000). The surgical area was isolated with sterile surgical drapes and the incision line was determined. A 2 cm long vertical dermal incision was made in the rat calvarium, the mucoperiostellar flap was removed and the calvarium was reached. After the calvarium sagittal suture was determined, a bone defect was created with a 5 mm diameter trefan bur equal to the suture. After the surgical procedures have been completed, periosteum with 4/0 glycolide based absorbable suture (Vicryl, Ethicon, Brussels, Belgium), then skin tissue was sutured with 4/0 silk sutures (Doğsan, Turkey) with a simple suture technique and the surgical area was closed. After the surgical procedure, a single dose of enrofloxacin (Baytril-K® 2. 5 mg / kg IM) was administered for infection control. Meloxicam (Maxicam ® 1mg / kg, IM) was injected to prevent postoperative pain. Biograft® HT (IFGL Bio Ceramics)

containing 40% β-tricalcium phosphate with 60% porous biphasic synthetic hydroxyapatite was used as allograft. This material is an alloplast with osteoconductive properties that has a granule size of 350 to 500 μm.

Histological staining procedures

Calvarial bones removed from rats were taken in 10% neutral formaldehyde solution for histopathological examinations, after fixing for 24 hours, they were kept in 10% formic acid + 10% sodium citrate solution for 15 days for decalcification, and underwent routine histological tissue follow-up and embedded in paraffin blocks. Sections of 4-6 μm were taken with a microtome (RM2265 rotary microtome; Leica, Germany) from the paraffin blocks and stained with Hematoxylin-Eosin, then examined under Light microscope (Zeiss, Imager A2, Germany).

Immunohistochemistry analyses

Remaining tissue sections were washed with distilled water after dehydrating in graded ethanol series. Slides were dried without damaging the tissue then marked with Dakopen (Dako, Glostrup, Denmark). In order to inhibit tissue endogenous peroxidase, 3% hydrogen peroxide (cat # TA-015-HP, Thermo Fisher Scientific, US) was applied for 20 min. Ultra V block (TA-125-UB, Thermo Fisher Scientific, US) was dropped to the sections for 8 min prior to the addition of the primary antibodies, which were incubated at +4° C overnight (Osteopontin monoclonal antibody, cat #MA5-17180, 1:100, Osteonectin-SPARC monoclonal antibody, cat # 33-5500, 1:100). Sections were washed with biotinylated secondary antibody (cat # TP-015-BN, Thermo Fischer Scientific, US) for 14 minutes. Streptavidin peroxidase (cat # TS-015-HR, Thermo Fischer Scientific, US) was dropped to sections. Chromogen DAB (catalog no: TA-001-HCX, ThermoFischer, US) was applied and counter stained with Harris hematoxylin and slides were mounted with entellan. Sections were examined under Light microscope (Zeiss, Imager A2, Germany)

Results

Histopathological observations

In the control (defect) group Haematoxylin-eosin staining sections; an increase in the mononuclear cell infiltration within scar tissue, local degenerative changes in collagen fibrils and a significant increase in fibroblast cells were observed in the area where the fractured bone trabeculae were located. Areas of necrosis were encountered. It was observed that healing has started in this region especially in the bone matrix (**Figure 1a**). In the Defect+HA group Haematoxylin-eosin staining sections, intense inflammatory cells in the defect area were observed, and osteoblast cells which enable bone formation, began to appear in acidophilic areas. It was observed that osteoclast cells were mostly located close to the main bone structure. A significant increase in leukocyte cells was observed in some areas (**Figure 1b**). In the Defect+ Graft group Haematoxylin-eosin staining sections, new bone matrix development had begun due to the increase in osteoclastic activity in the area where the main bone skeleton was located. Small bone trabeculae and osteoblast cells were observed in central area close to the graft. The matrix in the bone trabecula was found to be prominent in the central. Widespread leukocyte cells were found in other areas in a solitary fashion. Concentration was observed around the matrix with thickening of the collagen fibers (**Figure 1c**). In the Defect+Grafat+HA group sections, there was an increase in inflammatory cells in the graft area. The graft material replaced with bone trabecula. Osteoclasts were common and osteoblast cells were organized on the outer part of the trabeculae with newly produced bone matrix. Collagen fibers were located around the graft material, supporting the formation of new bone matrix (**Figure 1d**).

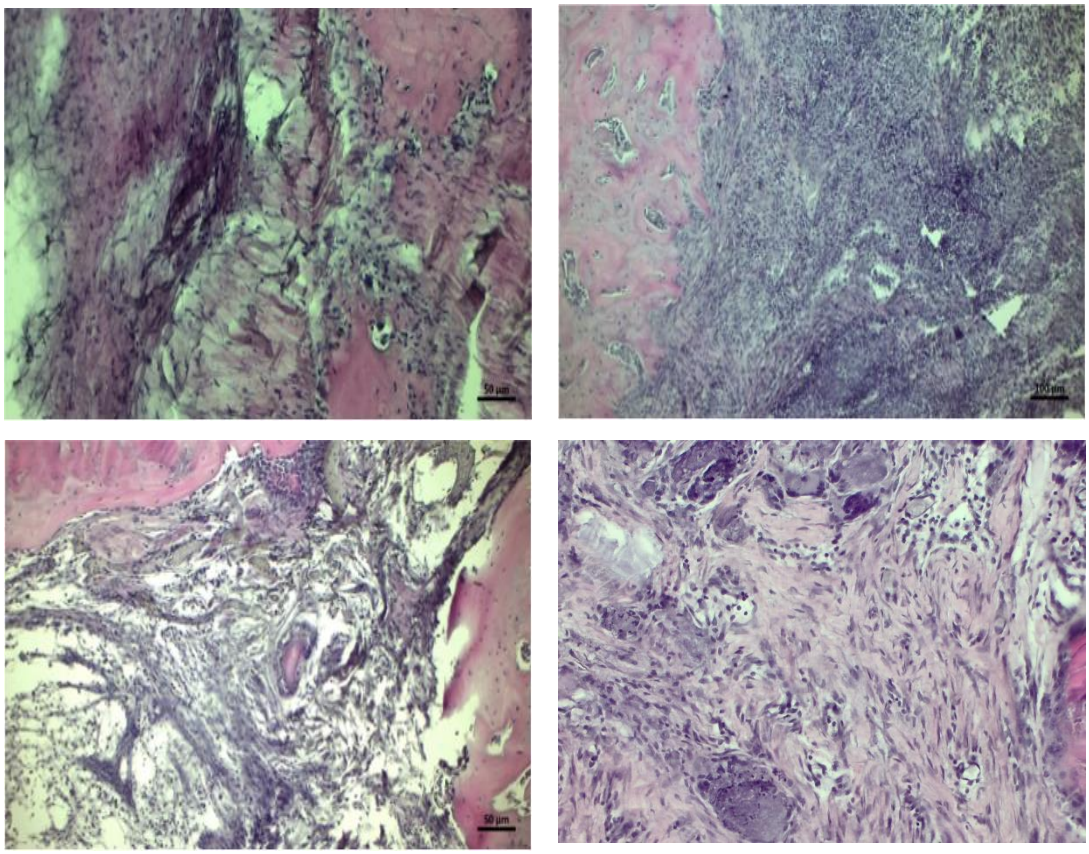

Figure 1. Haematoxylin-eosin staining of sections, **1a. Control (defect) group**; increase in mononuclear cell infiltration, degenerative changes in collagen fibrils, increase in fibroblast cells. **1b. Defect+HA group**; intense inflammatory cells in the defect area, osteoblasts began to appear in acidophilic areas, significant increase in leukocyte cells. **1c. Defect+ Graft group**; new bone matrix development with small bone trabeculae, osteoblasts in central area close to the graft. Widespread leukocytes in a solitary fashion. **1d. Defect+ Graft +HA group;** increased inflammatory cells and new bone trabecula formation in graft area, common osteoclast and osteoblast cells around trabeculae and lacunae, collagen fibers around graft material.

Immunostaining Results

***a.* OPN immunostaining**

In the control (defect) group OPN immunostaining sections, an increase in loose connective tissue, the formation of bone trabeculae and increased osteoblastic activity, the prominence of new bone trabeculae indicating the new bone formation. OPN expression was observed in these areas (**Figure 2a**). In the Defect+HA group OPN immunostaining sections, inflammatory cells were dense in the defect area, but OPN expression was negative. OPN immunoreaction was positive in fibroblasts and osteoblast cells of bone trabeculae. OPN protein was also expressed in some osteoblasts inside the bone trabeculae. In addition, positive OPN expression was observed in osteoclast cells close to the trabeculae (**Figure 2b**). In the Defect+ Graft group OPN immunostaining sections, positive OPN expression in some cells and fibroblastic activity were observed around new bone trabecula formation. In the periphery of small bone trabeculae within the graft area, positive OPN was expressed in a few osteoblast and osteoclastic cells (**Figure 2c**). In the Defect+Graft+HA OPN immunostaining sections, in the defect area, inflammatory cell infiltration was with small newly formed bone trabeculae. Osteoblastic activity was observed in the periphery of the trabeculae with increased collagen fiber increase. OPN expression was positive in fibroblast cells, showing bone matrix production (**Figure 2d**).

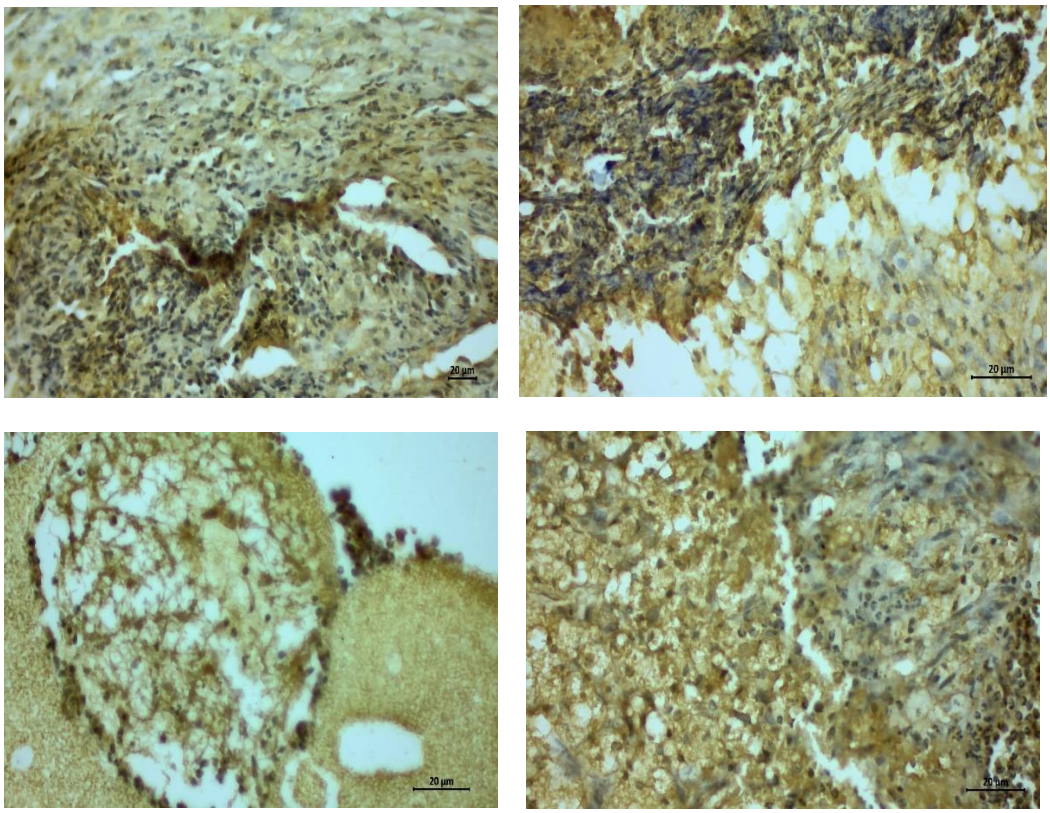

Figure 2. OPN immunostaining of groups. **2a. Control (defect) group**; increase in loose connective tissue, formation of bone trabeculae and increased osteoblastic activity, positive OPN expression. **2b. Defect+HA group**; inflammatory cells were dense in the defect area, negative OPN expression. Positive OPN immunoreaction in fibroblasts and osteoblast cells of bone trabeculae. OPN protein expression in osteoblasts of bone trabeculae. Positive OPN expression in osteoclast cells. **2c. Defect+ Graft group;** positive OPN expression in osteoblast and osteoclastic cells with high fibroblastic activity around newly formed bone trabecula. **2d. Defect+ Graft +HA group:** OPN expression mostly was shown in connective tissue cells and cells around new bone trabecula with high fibroblastic activity.

b. SPARC immunostaining

In the control (defect) group OPN immunostaining sections, inflammatory cells were evident in the defect area, small bone trabeculae and osteoblastic activity were prominent. SPARC expression was observed in osteoblasts. Small osteocyte cells were observed (**Figure 3a**).In the Defect+HA group OPN immunostaining sections, in the defect area, osteoblastic cell activity was observed significantly in terms of fibroblasts, and SPARC expression was found to be positive in inflammatory cells in this area. It was observed that the bone trabeculae has started to become prominent towards the main bone area (**Figure 3b**).In the Defect+ Graft group OPN immunostaining sections, It was observed that the inflammatory cells were dispersed in a solitary fashion and new bone trabeculae began to appear. SPARC expression was positive in osteoblastic activity and osteocyte cells indicating new bone formation (**Figure 3c**). In the Defect+Graft+HA group SPARC immunostaining sections, intense fibroblastic activity and SPARC expression was observed in the defect area. Inflammatory cells around small bone trabeculae and osteoblast cells showed mild SPARC expression **(Figure 3d).**

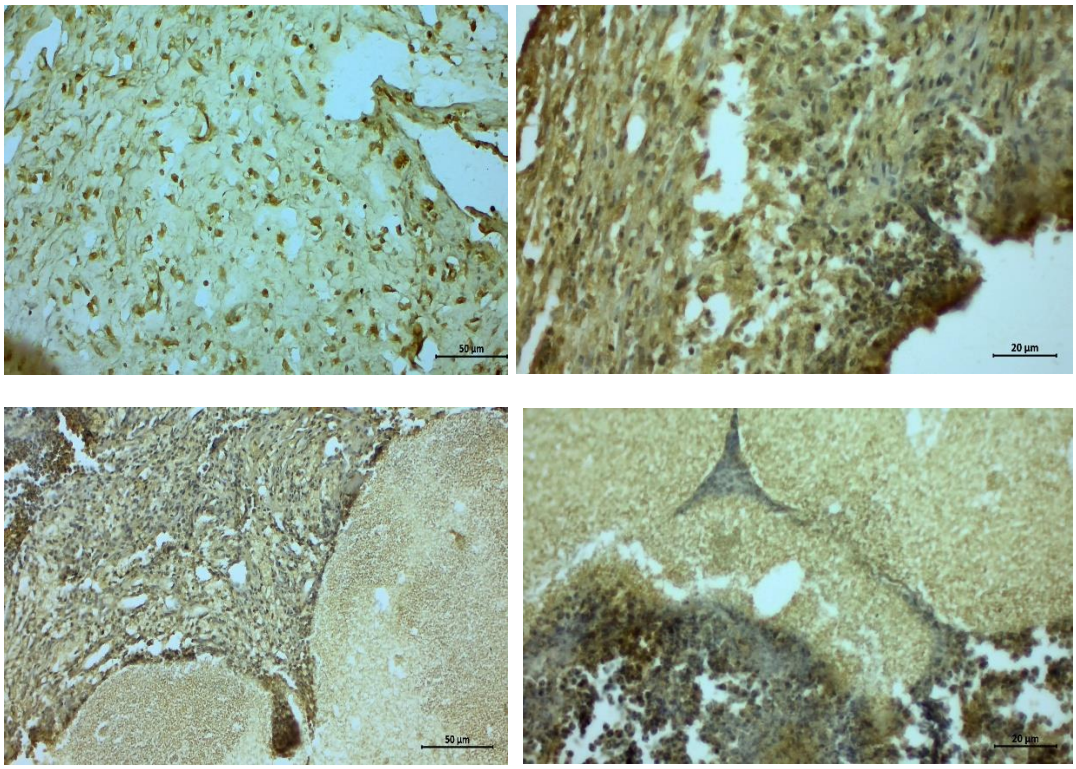

Figure 3. SPARC immunohistochemical staining of groups. **3a. Control (defect) group**; prominent inflammatory cells and osteoblastic activity. SPARC expression in osteoblasts. **3b. Defect+HA group**; significant osteoblastic cell activity, positive SPARC expression in inflammatory cells. **3c. Defect+ Graft group;** inflammatory cells dispersed in a solitary fashion, new bone trabeculae began to appear. Positive SPARC expression in osteoblastic activity and osteocyte cells indicating new bone formation. **3d. Defect+ Graft +HA group;** intense SPARC expression in the defect area. Inflammatory cells around small bone trabeculae and osteoblast cells with mild SPARC expression .

DISCUSSION

Bone defects may occur after trauma, infection, bone tumors or cysts, and orthognathic surgery. While minor defects in bone can be repaired by natural bone healing processes, large defects require reconstruction with grafts and implants using a variety of materials. For this purpose, different graft materials such as; alloplastic bone grafts, autogenous bone grafts, allogenic bone, and other synthetic materials have been used (Szpalski et al., 2010). While cortical grafts provide a durable and robust structure, they do not have the ability to increase osteogenesis in experimental and human studies. Therefore, there should be alternative supportive treatments that will increase osteoinductive activity and accelerate the formation of new bone. A study on autogenous tooth bone graft combined with platelet-rich fibrin in calvarial defects was performed by Kızıldag et al. (2019). The researchers have evaluated new bone formation using immunohistochemical staining(BMP-2), concluded that autogenous tooth bone graft combined with platelet-rich fibrin has accelerated bone healing in cranial defects. Kim et al. (2015) has studied composites of biphasic calcium phosphate (BCP), bone morphogenetic protein 2 (BMP-2), and mesenchymal stem cell (MSC) together in a rabbit calvarial defect model. Researchers indicated that osteocalcin immunoreactivity has increased with the treatment. Acar et al. (2015) has studied bone regeneration in a defect model of rabbit applied hydroxyapatite/biphasic calcium phosphate in combination with platelet-rich fibrin. According to their results, platelet-rich fibrin increases bone regeneration when used alone or in combination with hydroxyapatite/biphasic calcium phosphate. We are in the opinion that allograft application is a suitable model for calvarial bone healing experiments as we were able to observe accelerated regeneration of calvarial bone, granulation tissue formation, remodelling of osteogenic cells namely, new bone formation (Figures 1a-d). In another study, it has been stated that by adding high doses of hyaluronic acid to a biphasic bone material, decreased the proinflammatory tissue response and increased bone regeneration (Sieger et al.,2019). In a study on male Holtzman rats, The 1st molars were withdrawn and the left side was left to normal healing process as the control group, on the other hand hyaluronic acid was applied to the right side. It was observed that the trabecular bone has formed earlier and the bone matrix was more organized (Öztürk and Kahveci, 2017). Bone regeneration in rat calvarial defects

model with implantation of human bone marrow-derived mesenchymal stromal cell spheroids, osteocalcin/osteopontin were examined and formation of new, full-thickness bones at the implantation sites were observed (Suenaga et al., 2015). In our study, both OPN and SPARC expressions were positive in osteoblasts of the calvarial defect group, in inflammatory cells of defect+ HA group and in osteocyte cells of defect +allograft and defect+allograft+HA group, indicating new bone formation (Figures 2a-2d, 3a-3d). HA+allograft application seemed to cause comprehensive recovery with new bone formation in calvarial defect model.

We think that performing such experimental studies of implant application in teeth can make a significant contribution to human health in the dissemination of implant use.

CONCLUSION

Due to the excessive healing period in calvarial bone defects and the difficulty of a complete repair in these defects aesthetically and functionally, it is necessary to expedite the healing process and increase the effectiveness of applied techniques. For this purpose, the use of alloplastic graft material after calvarial bone damage in rats facilitated the closure of the defect area. In addition, the application of hyaluronic acid to the defect area, which induced new bone development and intercellular matrix formation, has increased osteogenic activity and maintained the rapid closure of the defect area. As a result of the combined use of both factors, early bone healing and new bone formation have been observed and proven by the changes in the distribution of osteopontin and SPARC biomarkers.

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➢ ***ORAL PRESENTATION***

Investigation of the Risk of Delayed Action to Mitigation of Nitrous Oxide Emissions from Agricultural Systems

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Abstract

According to the United Nations Population Fund, the human population has grown from 1.6 billion to 6.1 billion people during the last century. As a conclusion, extensive consumption of fossil energy resources has associated with a significant increase in the mass of greenhouse gases. Nitrous oxide (N_2O) is one of the greenhouse gases with tremendous global warming potential and the creation of reactive nitrogen compounds has increased by 120%. Increasing greenhouse gas emissions and decreasing fossil fuel sources has been posed new challenges to agriculture. Because of the potential future effects of global climate change, people want to produce renewable fuel to reduce greenhouse gas emissions, but as the same time N_2O emission increase because of feedstock production and fertilizer production. Therefore, decision-makers need to develop the potential of mitigation strategies to quantify and address the uncertainty of N_2O emissions, so the correct strategies to mitigate N_2O emissions from agricultural systems can be found. According to policymakers, in a risk condition, each day of delay is associated with a risk of increasing greenhouse gas emissions instead of declining, so actions also need to be timely. The purpose of this study is to investigate of the risk of delayed action to mitigate N_2O emissions from agricultural systems. In this context, the consequences of inaction, risk analysis methods, N_2O mitigation technologies, cost associated mitigation strategies were analyzed and examined through the appropriate methods. According to results, the impact of one single year of delaying abatement would cause around 1.9 GtCO2e of additional emissions globally in that year. Also, the average effective lifetime of infrastructure is 10 years in the greenhouse gas cost curve model, so the model shows us a delay of 10 years would cut the potential abatement in 2030 would fall from 38 to 19 GtCO2e.

Keywords: greenhouse gases, nitrous oxide emissions, mitigation technologies, risk assessment, delayed action

INTRODUCTION

Approximately two billion people were added to the world's population over the past two decades (Rubino et al. 2019). As a result, extensive consumption of fossil energy resources, including coal, oil, gas, has associated with a significant increase in the mass of greenhouse gases (GHG) (carbon dioxide, nitrous oxide, water vapor, methane, ozone, etc.), which are known to inhibit long-wavelength radiation from escaping into space, into the atmosphere. Unfortunately, with the rapid increase in the population of humans since the mid-1970`s, the creation of reactive nitrogen compounds has increased by 120% (Galloway et al. 2008; Smithson 2002). Nitrous oxide (N_2O) is one of the GHG with tremendous global warming potential (GWP) (Wang and Sze 1980) because of its long atmospheric lifetime (approximately 120 years) and heat-trapping effects. The atmospheric concentration of N_2O varied only slightly thousands of years before the industrial period, but it started to increase relatively rapidly toward the end of the 20th century (CHANGE 2007; IPCC. 2007). Especially, the atmospheric concentration of N_2O has risen approximately 18% in the past two hundred years and continues to rise (Figure 1).

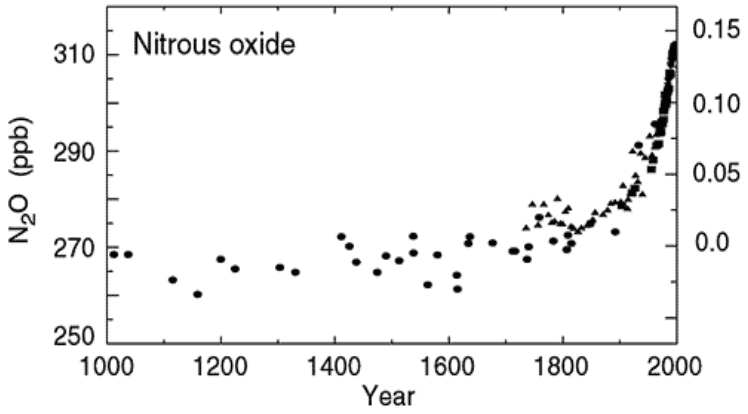

Figure 1. N_2O concentrations 1000 AD to 2000 AD (IPCC. 2007)

Based on the current U.S. GHG Inventory, N_2O contributes approximately 6.5% to total GHG emissions (in CO_2 equivalents) and N_2O emissions from agricultural soils account for more than 50% of the global anthropogenic N_2O flux (Hénault et al. 2019). Therefore, increasing GHG emissions and decreasing fossil fuel sources have been posed new challenges to agriculture (Fließbach et al. 2009). In this context, feedstock production for biofuels became a current issue worldwide. Nitrogen fertilization is increasing the amount of mineral nitrogen in soils and nitrous oxide emissions. Because the limiting factor for crop growth is the level of nitrogen availability, this is often a reason for lower crop yields in farming, but also a reason for lower emissions (Fließbach et al. 2009). On the other hand, using an extensive amount of nitrogen fertilizer to getting higher crop yields may lead to higher GHG emissions. Because of the potential future effects of global climate change, people want to produce renewable fuel to reduce GHG emissions, but at the same time, N_2O emissions increase because of feedstock production and fertilizer production. According to the low carbon fuel standard (LCFS), which seeks to reduce GHG emissions associated with fuel-powered vehicles considering the entire life cycle, in order to reduce the carbon footprint of fuels, this only occurs at the biorefinery, not feedstock production, N_2O estimations are too variable and uncertain. The key uncertainties in N_2O estimations are based on uncertainty in model input data and model structure (Del Grosso et al. 2010). Therefore, decision-makers need to develop the potential of mitigation strategies to quantify and address the uncertainty of N_2O emissions, so the correct strategies to mitigate N_2O emissions (e.g. using of nitrification inhibitors (such as 3,4-dimethylpyrazole phosphate (DMPP)); changing nitrogen source and application rate; fertilizer reduction; banded fertilization; wetland restoration and etc.) from agricultural systems can be found (Adler et al. 2012). When people are making decisions, they must determine not only which mitigation strategies to be chosen but also when they to be chosen. According to policy-makers, in a risk condition, each day of delay was associated with a risk of increasing GHG emissions instead of declining, so actions also need to be timely (Patalano and Wengrovitz 2007; McKinsey 2009). For example, if policymakers delay taking action, so global abatement action was to start 2020 instead of 2010, to keep global warming below 2 degrees Celsius would be nearly impossible according to Global Greenhouse Gas Abatement Cost Curve v 2.1. The impact of one single year of delaying abatement would cause around 1.9 GtCO2e of additional emissions globally in that year. Also, the average effective lifetime of infrastructure is 10 years in the greenhouse gas cost curve model, so the model shows us a delay of 10 years would cut the potential abatement in 2030 would fall from 38 to 19 GtCO2e (if the global abatement action starts in 2020 instead of 2010 (10 years of delaying), N_2O emissions will be 550 ppm instead of 480 ppm) (Enkvist, Dinkel, and Lin 2010; MacLeod et al. 2010; McKinsey 2009). As seen in Figure 2, the amount of reducing potential N_2O emission is also related to mitigation strategies. Therefore, appropriate N_2O emission mitigation strategies also should be examined since it is important as well as act now. In this context, the purpose of this study is to investigate the risk of delayed action to mitigate N_2O emissions from agricultural systems including the consequences of inaction, risk analysis method, and most used N_2O mitigation technologies analyzed and examined through the appropriate methods.

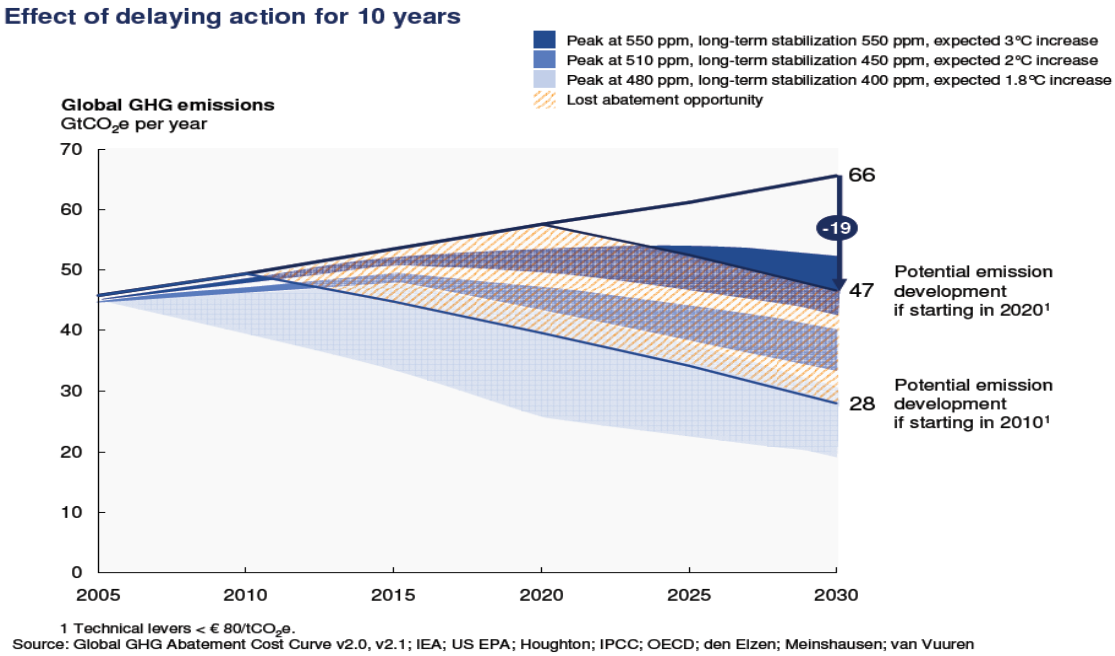

Figure 2. Effect of delaying action and adopting more granular N_2O emission estimates and N_2O mitigation strategies compared with single N_2O emission factors (Smithson 2002; McKinsey 2009)

The Risk Assessment Framework

In a simple definition, the risk is an inherent property of everyday human existence, so a key factor in all decision making. Risk assessment can be defined as the process of estimating both the probability that an event will occur, and the probable magnitude of its adverse effects such as economic, health-related, or ecological from a chemical or stressors over a specified time period (Gerba 2006; Olson and Gurian 2012). A formal risk assessment has four steps: hazard identification, exposure assessment, dose-response assessment, and risk characterization as shown in Figure 3.

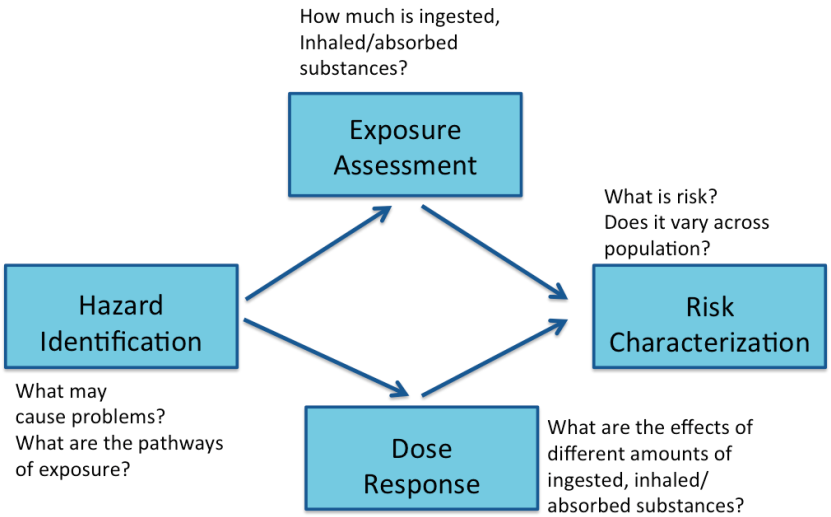

Figure 3. Risk assessment framework (Gerba 2006)

The Process of Risk Assessment

1- Hazard identification: Definition of the hazard is the potential for harm or an adverse effect on humans; for example, identifying chemical contaminants such as, a heavy metal, and documenting its toxic effects on humans. For N_2O, the substance can be absorbed through the skin as well as inhaled. Elevated concentration of N_2O in the air will be reached very quickly on loss of containment such as, with leaking.

2- Exposure assessment: Calculation exposure requires information on the concentrations of contaminants and the timeframe over which exposure occurs in target organisms. For example, finding the concentration of mercury in canned tuna products and determining the dose an "average" person would receive. For inhalation of N_2O, the reported intake/contact rate (IR) is 20 m^3/day; exposure frequency (EF) is 350 days/year; exposure duration (ED) is 30 years (EPA 1991).

3- Dose-response assessment: Quantifying the adverse effects arising from exposure to a hazard on the degree of exposure. For average daily dose (or chronic daily intake) of N_2O is normalized as milligrams of N_2O inhaled through the skin per kilogram of body weight per day (mg kg^{-1} day^{-1}).

$$\mathrm{CDI} = \frac{\mathrm{C\ x\ IR\ x\ EF\ x\ ED}}{\mathrm{BW\ x\ AT}} \qquad (1)$$

where CDI is chronic daily intake; C is concentration of chemical in each medium (e.g., mg/L for water or mg/m^3 for air); IR is intake/contact rate (L/day); EF is exposure frequency (days/year); ED is exposure duration (years); BW is body weight (kg); AT is averaging time (period over which the exposure is averaged-days). According to United States Environmental Protection Agency (EPA 1991), standard default exposure factors for inhalation of contaminants: IR is 20 m^3/day; EF is 350 days/year; ED is 30 years; BW is 70 kg; AT is 24500 days (350 days/year x 70 years). If global abatement action starts 2010, N_2O emissions will be 480 ppm. Therefore, chronic daily intake will be 587 mg $kg^{-1}day^{-1}$. However, if global abatement action starts 2020 instead of 2010 (10 years of delaying), N_2O emissions will be 550 ppm. Therefore, chronic daily intake will be 670 mg $kg^{-1}day^{-1}$.

4- Risk characterization: The process of determining the potential impact of a hazard based on the severity of its adverse effects and the amount of exposure (Gerba 2006). The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency added N_2O to the list of chemicals known to the state to cause cancer and reproductive toxicity for the purposes of Proposition 65. Previous studies on the adverse effect of N_2O have mostly focused on air pollution and risk for lung cancer (Raaschou-Nielsen et al. 2011; Pope Iii et al. 2002). However, other cancer types might also be associated with exposure to polluted air with high level of N_2O concentrations such as cancers of the mouth, pharynx, and larynx. Although further studies are required to confirm possible risks for other cancers, the study of Raaschou-Nielsen, Andersen and et al. 2011 showed that the risk for kidney cancer increased with increased N_2O concentration near the residence. Also, the substance may have effects on the bone marrow and the peripheral nervous system. High N_2O concentration may cause reproductive toxicity in humans. Beside the direct human health effects of N_2O, another concern is related to environment. When N_2O emissions reaches the stratosphere it destroy the ozone layer, as a result UV radiation level increased (Portmann, Daniel, and Ravishankara 2012). Air quality guidelines such as those from the World Health Organization (WHO), the United States Environmental Protection Agency (EPA) and the European Union (EN) are based on detailed studies designed to identify the levels that can cause measurable health effects. According to the scientists, breathing ground-level ozone can result in a number of adverse health effects including respiratory symptoms such as coughing, wheezing, throat irritation, and increasing the risk of skin cancer, lung cancer and also generally all types of cancer since when N_2O is nearer to the Earth's surface cause smog which has been linked to weakening of the immune system. As a summary, N_2O emissions may not have direct cancer effect. However, ozone depletion also should be considered if thinking about its important side effects.

N_2O gas Mitigation in Agriculture

According to the scientists, 37% of the earth's land surface was occupied by agricultural lands and agricultural activities are responsible 52% of global methane and 84% of N_2O emissions (Smith and Conen 2004). Agriculture releases significant amount of atmospheric N_2O which is generated by the microbial transformation of nitrogen in soils, and is often enhanced where available nitrogen exceeds plant requirements (Smith and Conen 2004; Cole et al. 1997; Paustian et al. 2004). Adler, Del Grosso et al. are reported that the major GHG contributors in the life cycle of a biofuel product are nitrogen fertilizer useage; N_2O emissions; harvesting (Adler et al. 2012). Their total contribution from N fertilizer useage and N_2O emissions accounted for 42-80% of the GWI (Global Warming Intensity) for feedstock production. Agricultural GHG emissions are complex, however the management of agricultural systems offers possibilities for mitigating GHGs in agriculture including reducing emissions; enhancing removals; avoiding emissions. Cropland management is

the one of the significant mitigation technologies since it focusses on agronomy, which aims to increase crop yields and reduce emissions (Follett 2001; Paustian et al. 2004); nutrient managements, which aims to apply nitrogen more precisely into the soil (Cole et al. 1997; Paustian et al. 2004; Monteny, Bannink, and Chadwick 2006); tillage management, which effects N_2O emissions directly since reducing soil tillage may promote atmospheric N_2O (Marland, McCarl, and Schneider 2001; Rauch et al. 2009); water management, which can reduce N_2O emissions promoting productivity (Monteny, Bannink, and Chadwick 2006); and land cover change since grassland can reduce N_2O emissions. In addition, management of land, organic soils, and manure can also reduce N_2O emissions if they are applied properly (Follett 2001). Efficient mitigation strategies should aim to provide enough nitrogen to satisfy plant demand, while minimizing excess nitrogen. Several challenges should be examined when considering about the most-effective GHG mitigation options including emissions which are under BAU conditions; the impacts of GHG mitigation strategies on crop yields and environmental impacts; interactions between different GHG technologies; and cost and benefit analysis for different mitigation options. There are two important N_2O mitigation strategies reported to reduce the GWI of bionergy feedstock production (based on corn grain) among all mitigation technologies, which are PCU (Polymer-coated urea) and nitrification inhibitors. Nitrogen source, application rate, application time, and placement are the key factors to reducing nitrogen inputs. Among them, nitrogen source and application rate are the most approprite methods for reducing embodied energy and N_2O emissions. This process is worked by gradual diffusion of nitrogen through the polymer coating dependant on soil moisture and temperature. It is required less energ so slow release rate of N is more precise than most slow-release products. In addition, release depends on coat thickness, chemistry, temperature, moisture. Although polymer-coated fertilizers have several advantages including high quality and consistent analysis, an elimination of a fertilizer impregnation stage, utility in cost-share programs, and decreased need, time and effort, it is often more expensive method than other forms of nitrogen. On the other hand, nitrification inhibitors, such as dicyandiamide and 2-ethynylpyridine, are applied to agricultural soil with nitrogen fertilizers to reduce nitrate leaching and N_2O emissions, and as result increase plant growth. However, the effectiveness of them decreases time after application to soils depending on soil temperature, soil moisture, soil pH and organic matter content(Edmeades 2003). Adler, Del Grosso et al. compared two of N_2O mitigation strategies to reduce the GWI of bioenergy feedstock production, based on corn grain (Adler et al. 2012). When PCU uses as a mitigation strategie, N_2O emissions reduce %14-58. On the other hand, if the nitrification inhibitor uses as a mitigation strategie, N_2O emissions reduce %31-44. This high range of percentages difference shows us N_2O emissions reductions in a mitigation strategie depends on many factors.

CONCLUSION

The study investigated the risk of delayed action to mitigate N_2O emissions from agricultural systems. The importance of N_2O emissions, consequences of inaction, risk assessment analysis for N_2O emissions, N_2O mitigation technologies have been studied. The average effective lifetime of infrastructure is 10 years in the greenhouse gas cost curve model shows us a delay of 10 years would cut the potential abatement in 2030 would fall from 38 to 19 GtCO2e. In this case, N_2O emissions will be 550 ppm instead of 480 ppm. Using this data, we processed a risk assessment analysis and found that if global abatement action starts 10 years of delay, the chronic daily intake will be 587 mg $kg^{-1}day^{-1}$ instead of 670 mg $kg^{-1}day^{-1}$. Due to the increased atmospheric N_2O emissions, several human health and environmental effects were summarized. Since the major N_2O emission is related to agricultural activities, several N_2O mitigation technologies were investigated and among them, polymer-coated urea and nitrification inhibitors were reported as two important N_2O mitigation strategies to reduce the global warming intensity. In summary, this study can provide awareness and knowledge to both policymakers and engineers on how to reduce N_2O emissions and decrease adverse effects on humans and the environment.

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➢ *ORAL PRESENTATION*

Spirulina platensis'in ykg-1 glioblastom hücre hattında apoptotik etkilerinin araştırılması

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Özet
Spirulina platensis , besin takviyesi olarak yaygın olarak kullanılan mavi-yeşil bir tatlı su yosunudur. Proteinler, karotenoidler, esansiyel yağ asitleri, çeşitli vitaminler (B vitamini kompleksi, E vitamini) ve mineraller (bakır, manganez, magnezyum, demir, selenyum ve çinko) açısından zengindir. Spirulina'dan elde edilmiş doğal bir ürün olan Phycocyanin, biliproteinlerden biridir. Bir çok farmakolojik çalışmalar, Phycocyanin'nin anti-kanser, antioksidan, antiinflamatuvar aktivite ve ışıkla indüklenen sitotoksisite gibi birçok işlevi olduğunu doğrulanmıştır. Kanserin önemli bir nedeni, hücre apoptozunun engellenmesinin gen düzenlemesi ile programlanmış olmasıdır. Phycocyanin'in, ROS üretimi ve iyi bilinen bir anti-apoptotik molekül olan Bcl-2'nin ekspresyonunu aşağı regüle ederek tümör hücrelerinde apoptozu indüklediği, mitokondriden sitozole sitokrom c salımını indüklediği bildirilmiş ve ayrıca Kaspaz 3 ve Kaspaz 8 aktivitelerini yukarı regülasyonu ile apoptotik hücre ölümüne neden olabileceği öngörülmektedir. Biz de çalışmamızda, spirulina'nın YKG-1 insan glia tümör hücreleri üzerindeki hücre yaşamı ve apoptoza olan potansiyel etkilerini araştırdık. Çalışmamızda YKG-1 hücreleri iki boyutlu kültür şartlarında DMEM besi yeri içerisinde %80 konfluensi sağlanana kadar kültüre edildi. Spirulina platensis 190 µg/ml olacak şekilde 48 saat boyunca hücrelere uygulandı. Uygulama sonunda hücreler %4 paraformaldehitte fikse edilerek Anti-Bax, Anti-Bcl, Anti-NF Kappa B ve Anti-Caspas 3 ile immünohistokimyasal olarak boyandı. Sonuçlarımıza göre Spirulina platensis uygulamasından sonra Bax/ Bcl oranının anlamlı bir şekilde Bax lehinde değiştiği ve NF kappa B ifadesinin azaldığı görüldü. Buna göre spirulina platensis'in 48 saat'lik uygulamasından sonra YKG-1 tümör hücrelerinde hücre yaşamını etkilediği ve hücre ölümünü tetiklediği düşünülmüştür.
Anahtar Kelimeler: YKG-1, Spirulina, kanser, apoptoz

Investigation of the apoptotic effects of Spirulina platensis on ykg-1 glioblastoma cell line

Abstract
Spirulina platensis is a blue-green freshwater algae commonly used as a nutritional supplement. It is rich in proteins, carotenoids, essential fatty acids, various vitamins (vitamin B complex, vitamin E) and minerals (copper, manganese, magnesium, iron, selenium and zinc). Phycocyanin, a natural product derived from Spirulina, is one of the biliproteins. Many pharmacological studies have confirmed that Phycocyanin has many functions such as anti-cancer, antioxidant, anti-inflammatory activity and light-induced cytotoxicity. An important cause of cancer is that the inhibition of cell apoptosis is programmed by gene regulation. Phycocyanin has been reported to induce apoptosis in tumor cells by downregulating ROS production and the expression of Bcl-2, a well-known anti-apoptotic molecule, inducing cytochrome c release from mitochondria to cytosol, and further up-regulation of Caspase 3 and Caspase 8 activities to cause apoptotic cell death. It is anticipated that the cause. In our study, we investigated the potential effects of spirulina on cell life and apoptosis on YKG-1 human glia tumor cells. In our study, YKG-1 cells were cultured under two-dimensional culture conditions in DMEM medium until 80% confluence was achieved. Spirulina platensis was applied to the cells for 48 hours at 190 µg / ml. At the end of the application, the cells were fixed in 4% paraformaldehyde and immunohistochemically stained with Anti-Bax, Anti-Bcl, Anti-NF Kappa B and Anti-Caspas 3. According to our results, it was seen that after Spirulina platensis application, Bax / Bcl ratio changed significantly in favor of Bax and NF kappa B expression decreased. Accordingly, it was thought that spirulina platensis affected cell life and triggered cell death in YKG-1 tumor cells after 48 hours of application.
Keywords: YKG-1, Spirulina, cancer, apoptosis

GİRİŞ

Kanser, dünya çapında yüksek sosyal ve ekonomik etkileri olan insan sağlığı için ciddi bir tehdit oluşturmaktadır. Bu nedenle, yeni antikanser ilaçların geliştirilmesi çok önemlidir. Son on yılda, yeni ilaçların araştırılması ve geliştirilmesi arttı ve doğa, anti-kanser bileşiklerin keşfi için uygun bir kaynak haline geldi(Alves ve ark., 2016). Mikro ve makro alg özleri, kanser önleyici özelliklerine sahip biyoaktif moleküllerdir.(Alves ve ark., 2016). Sitotoksik etkileri, örneğin insan hepatokarsinom hücre hattı (HepG2)(Kim ve ark., 2014), insan meme karsinom hücre hattı (MCF-7) (Erfani ve ark., 2015), aynı zamanda Caco-2 (Ahmadi ve ark., 2015) dahil olmak üzere insan kolon karsinom hücre hatları gibi çeşitli kanser hücre hatları için çalışılmıştır(Smieszek ve ark., 2017). Yüksek antioksidatif özellikler bağlamında en çok çalışılan alg, siyanobakteriler sınıfına ait, serbestçe yüzen filamentli mavi-yeşil bir mikroalg olan *Spirulina platensis*'tir(Wu ve ark., 2016). Spirulina platensis'ten saflaştırılmış bir deniz proteini olan C-Phycocyanin (C-PC), tümör oluşumunu inhibe etme işlevine sahip olduğu doğrulanmıştır(Jiang ve ark., 2018). In vitro çalışmalar, C-Phycocyanin'in sitotoksik etkisinin, mitokondriyal aktivite bozukluğundan kaynaklanabilecek tümörijenik hücre dizilerinin proliferatif kapasitesinde bir azalmayı içerdiğini göstermiştir (Koníčková ve ark., 2014).Ek olarak, Ismail ve ark. S. platensis'in Bax / Bcl-2 oranını artırarak hepatoselüler karsinom hücre hattı HepG2'nin apoptozunu indükleyebileceğini göstermiştir (Ismail ve ark., 2015). Bizde çalışmamızda spirulina platensis'in YKG-1 glioblastom hücre hattında potansiyel apoptoz etkilerini değerlendirmeyi amaçladık.

MATERYAL-METOD

Hücre Kültürü

ATCC den alınan YKG-1 glioblastom hücre hattı DMEM, %10 Fetal bovine serum (FBS), %1 L-Glutamin ve % 1 antibiyotik- (penisilin-streptomisin) içeren medyum ile dilüe edilerek kültür kabında çoğalmaya bırakıldı. Kültür kabı, %5 karbondioksit içeren, 37˚C nemli inkübatörde 2 gün boyunca inkübe edildi. iki grup olacak şekilde hücreler ayrıldı ve ilk gruba 48 saat boyunca spirulina ugulandı. İkinci grup, kontrol grubu olarak kabul edildi.

Immünositokimyasal Yöntem

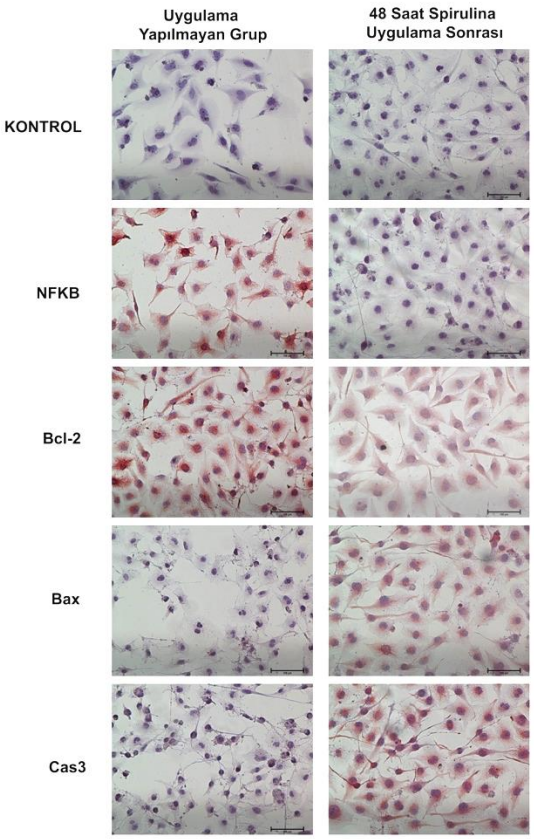
- Spirulina uygulamasından 24 saat sonra hücreler 1 kez PBS ile yıkandı ve %4'lük paraformaldehit ile 30 dk. fikse edildi.
- Fiksasyondan sonra %3'lük hidrojen peroksit (H2O2) uygulandı (5 dk). PBS ile 3x5dk olacak şekilde yıkandı.
- Permeabilizasyon için %0,1'lik Triton-X 100 15 dk inkübe edildi. PBS ile 3x5dk olacak şekilde yıkandı.
- 1 saat blocking uygulamasından sonra anti-Bax , anti-Bcl-2, anti NF kappa B ve anti caspas-3 antikorları 1:500 oranında dilüe edilerek 1 gece inkübe edildi.
- İnkübasyondan sonra sekonder antikorlar uygulanıp AEC kromojeniyle immunoreaktiviteleri görünürlüğü sağlandı.
- Kapatma mediumu ile kaplanarak kapatıldı ve fotoğrafları ışık mikroskobu ile çekildi.

İstatiksel Analiz

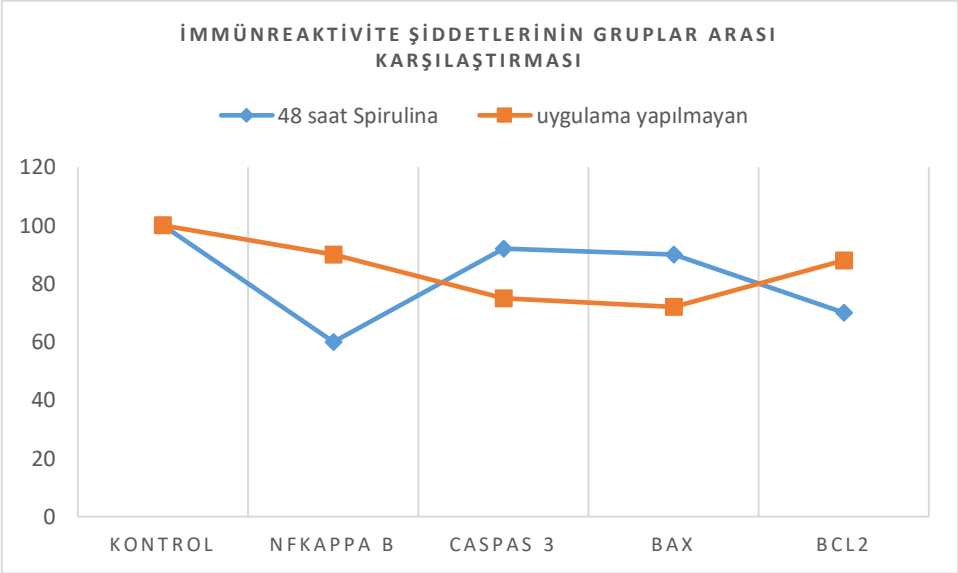
İstatistiksel analiz İmmohistokimyasal boyama sonuçlarında immunorektivite şiddetleri toplam alanda 500 hücre sayıldı. İmmün reaktivite şiddetleri; hafif (+), orta (++) , şiddetli (+++) olarak değerlendirildi.

BULGULAR ve TARTIŞMA

Elde edilen verilere göre YKG-1 glioblastom hücrelerinin 48 saat spirulina uygulamasından sonra yapılan immünositokimyasal boyama da Bax/Bcl-2 oranında anlamlı olarak Bax lehine azaldığı, kaspaz- 3 ekspresyonunda arttış ve NF kappa B ekspresyonunun da ise azalma olduğunu gördük.



Resim 1: Spirulina uygulaması yapılan ve yapılmayan grupların mikroskobik görüntüleri(x20)



Şekil 1: İmmünreaktivite Şiddetlerinin Gruplar Arası Karşılaştırması

Kanser, düzensiz hücre büyümesi ve durmadan proliferasyona uğrayan bir grup hastalıktır. Erken teşhis nedeniyle genel sağkalım süresi biraz artmış olsa da kansere bağlı mortalite dünya çapında ikinci en büyük ölüm nedenidir(Stewart ve Kleihues, 2003). Glioblastomas multiforme (GBM) en çok yetişkinlerde yaygın görülen malign primer beyin tümörüdür. Astrositik kökenli yüksek dereceli bir gliomdur ve yüksek vaskülarite ve yaygın lokal invazivlik ile karakterizedir(Uchinokura ve ark., 2006). Önceki çalışmalar, fikosiyaninin anti-kanser aktivitesini hücre apoptozunu ve hücre döngüsü tutuklamasını indükleyerek gösterdiğini göstermektedir(Tantirapan ve Suwanwong, 2014). Apoptoz, hücre proliferasyonunun tamamlayıcı bir mekanizmasıdır. İki ana apoptotik yol vardır: biri, endojen sinyalin nihayetinde Kaspaz-9 ve Kaspaz-3'ü aktive ettiği mitokondriyal / sitokrom C (endojen) yoludur; diğer yol, sonunda Caspase-8 ve Caspase-3'ü aktive eden

hücre zarı yüzey ölüm reseptörü (eksojen) yoludur (Kaufmann ve Earnshaw, 2000). Kaspaz-3 apoptotik sinyal yollarının çoğunda aktive olur ve Kaspaz-3 sonunda apoptozu indükler(Creagh ve ark., 2003). Mekanik olarak, C-fikosiyanin, apoptoz ve hücre proliferasyonunu modüle ederek anti-kanser etkisini gösterir. C-fikosiyanin'in, ROS üretimi ve iyi bilinen bir anti-apoptotik molekül olan Bcl-2'nin ekspresyonunu aşağı doğru düzenleyen ve ayrıca mitokondriden sitozol ve PARP bölünmesine sitokrom C salınımını indükleyerek tümör hücrelerinde apoptozu indüklediği gösterilmiştir(Liao ve ark., 2016). Sonuçlarımıza göre, 48 saat'lik spirulina uygulamasından sonra YKG-1 hücreleri Bax/Bcl2 oranında Bax lehinde azalması bize hücrenin apoptoza gittiğini düşündürmektedir. Cas3 ekspresyonundaki artış bu kanıtı destekler niteliktedir. Yapılan bir çalışmada, C-fikosiyanin ile tedavi edilen MDA-MB-231 hücrelerinde doz bağımlı olarak Bcl-2 protein seviyesinin aşağı regüle olduğu ve Fas ve Cas 3'ün seviyelerinin arttığı bulunmuş ve C-fikosiyaninin MDA-MB-231 hücreleri üzerindeki apoptozu tetikleyerek antitümör etkisi olduğu gösterilmiştir (Jiang ve ark., 2018). Çalışmamızda, YKG-1 hücrelerine 48 saatlik spirulina uygulamasının ardından, Jiana ve arkadaşlarının çalışmalarına benzer şekilde, hücrelerdeki Cas 3 ekspresyonunda arttığı bulunmuştur ve bu sonuçlar literatürü destekler şekildedir. Nükleer faktör-κb (NF-KappaB), enflamasyon, bağışıklık, hücre proliferasyonu, farklılaşma ve hayatta kalma dahil olmak üzere çeşitli biyolojik süreçlerde kritik roller oynayan bir transkripsiyon faktörleri ailesinden oluşur (Oeckinghaus ve Ghosh, 2009). C-fikosiyanin'in NF-KappaB aktivitesini azaltarak karaciğer ve pankreas kanseri üzerinde antineoplastik bir etki yaptığı bulunmuştur (Liao ve ark., 2016; Nishanth ve ark., 2010). Ek olarak, Bingula ve ark. C-fikosiyanin'le tedavi edilen A549 hücre hattında NF-KappaB ekspresyonunun azaldığını tespit etmiştir (Bingula ve ark., 2016). C-fikosiyanin, NF-KappaB yolu aracılığıyla çoklu fizyolojik aktivitelerin düzenlenmesine katıldığı bildirilmiştir (Zhu ve ark., 2016). Kanserin başlaması, metastazı, gelişimi ve tedaviye dirençte NF-KappaB 'nin rolü son yıllarda özellikle dikkat çekmiştir (Xia ve ark., 2014). Çalışmamızda, NF-KappaB primer antikorunu kullandık ve boyama sonuçlarımıza göre Zhu ve arkadaşlarının çalışmasının tersine YKG-1 hücrelerinde Spirulina uygulamasının NF-KappaB ekspresyonunda anlamlı bir değişikliğe neden olmadığını gördük. NF-KappaB sonuçlarında bir farklılık olmayışı Spirulina uygulamasının YKG-1 hücrelerinde NF-KappaB yolağına etki etmemesi yada uygulama süresini yetersizliğinden kaynaklı olabileceğini düşünmekteyiz.

SONUÇ

Çalışmamızın sonuçları spirulina'nın 48 saat boyunca YKG-1 hücrelerine uygulanmasıyla Bax/Bcl-2 oranının Bax lehinde arttırdığı dolayısıyla Cas 3 ekspresyonunu da arttırdığı fakat NF-KappaB yolağına etki etmediğini immünohistokimyasal tekniklerle göstermiştir. C- fikosiyanin'in kanser'e karşı apoptoza sürükleyici etkilerinin olduğu görülmektedir. Yapılacak ileri çalışmalarla saf C-fikosiyanin uygulamasının farklı dozlar ve sürelerle araştırılması gerekmektedir.

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➢ *ORAL PRESENTATION*

Gebelik döneminde kullanılan sertralin, fluoksetin ve escitaloprom'un fetal kemik gelişimi üzerine olan etkilerinin araştırılması

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Özet
Depresyon, yaşam süresince yaygınlık oranı %2-15 arasında değişen, küresel bir sağlık problemidir. Selektif serotonin gerialım inhibitörleri (SSRI) depresyon, anksiyete bozuklukları, obsesif-kompulsif bozukluk gibi psikiyatrik hastalıkların tedavisinde sıklıkla kullanılan ilaçlardır. Psikotrop ilaçların güvenliği ve psikiyatrik bozuklukların tedavisinde kullanılmalarıyla ilgili çelişkili bulgular mevcuttur. Bazı çalışmalar, serotoninin kemik fizyolojisi üzerindeki rolünü göstermiştir. Serotonin, osteoklast farklılaşmasını düzenler, osteoprotegerin (OPG) miktarını arttırır ve osteoklast aktivasyon faktörü RANKL'Yİ azaltır, oysa fluoksetin ters etkilere neden olur. Yaptığımız çalışmada her grupta 5'er Wistar Albino türü dişi rat olmak üzere dört grup oluşturuldu. 1. Grup Kontrol grubu, 2. Grup Sertralin grubu, 3. Grup Fluoksetin grubu, 4. Grup escitaloprom grubu olarak düzenlendi. Deneklere 16 gün boyunca oral yoldan SSRI tipi antidepresanlardan olan sertralin 5mg/kg/gün, fluoksetin 10mg/kg/gün ve escitalopram 10mg/kg/gün olarak verildi. Ratlarda embriyogenesisin bitip organogenesis'in başladığı dönem olan 16. günde anne ratlar genel anestezi altında sezeryanla yavrulardan ayrıldı. Operasyon sonucunda çıkarılan yavrular %4 PFA ile fikse edildi. Rutin parafin takip sonrasında alınan 5µ kesitler immünohistokimyasal olarak SOX-9, WNT, BMP-4 ve Osteokalsin antikorlarıyla, histokimyasal olarak ise Hematoksilen-Eozin ile boyandı. Sonuçlarımıza göre, histokimyasal boyamalar değerlendirildiğinde kemik yapısında farklılık olduğu görüldü. İmmunohistokimyasal sonuçlara göre SOX-9 ve WNT ekspresyonlarında anlamlı farklılık gözlenmezken BMP-4 ve Osteokalsin ekspresyonlarında görülen azalma nedeniyle Fluoksetin verilen grupta ki yavrularda kemik gelişiminin olumsuz yönde etkilenebileceği düşünülmüştür.
Anahtar kelimeler: gebelik, SSRI, kemik gelişimi, fetüs

Abstract
Depression is a global health problem, with a lifetime prevalence of 2-15%. Selective serotonin recovery inhibitors (SSRIs) are medications often used to treat psychiatric diseases such as depression, anxiety disorders, and obsessive-compulsive disorder. There are conflicting findings about the safety of psychotropic drugs and their use in the treatment of psychiatric disorders. Some studies have shown the role of serotonin on bone physiology. Serotonin regulates osteoclast differentiation, increases the amount of osteoprotegerin (OPG), and reduces the osteoclast activation factor RANKL, whereas fluoxetine causes adverse effects. In our study, four groups were formed in each group, including 5 Wistar Albino female rats. 1. Group Control Group, 2. Group Sertraline Group, 3. Group fluoxetine Group, 4. The group was organized as the escitaloprom group. Subjects were given sertraline 5mg/kg/day, fluoxetine 10mg/kg/day and escitalopram 10mg/kg/day, which were SSRI-type antidepressants orally for 16 days. 16, which is the period when embryogenesis ends and Organogenesis begins in rats. during the day, the mother rats were separated from the offspring by caesarean section under general anesthesia. Puppies removed as a result of the operation were fixed with 4% PFA. 5 KES sections taken after routine paraffin follow-up were immunohistochemically stained with Sox-9, Wnt, BMP-4 and osteocalcin antibodies, and Histochemically with Hematoxylene-eosin. According to our results, when histochemical staining was evaluated, there was a difference in bone structure. According to immunohistochemical results, no significant differences were observed in Sox-9 and Wnt expression, while due to the decrease in BMP-4 and osteocalcin expression, it was thought that bone development may be negatively affected in offspring in the fluoxetine group.
Key words: pregnancy, SSRI, bone development, fetüs

GİRİŞ

Depresyon, yaşam süresince yaygınlık oranı %2-15 arasında değişen, küresel bir sağlık problemidir. Selektif serotonin gerialım inhibitörleri (SSRI) depresyon, anksiyete bozuklukları, obsesif-kompulsif bozukluk gibi psikiyatrik hastalıkların tedavisinde sıklıkla kullanılan ilaçlardır. Psikotrop ilaçların güvenliği ve psikiyatrik bozuklukların tedavisinde kullanılmalarıyla ilgili çelişkili bulgular mevcuttur(Lee et al, 2007). Çalışmamızda kullandığımız ilaçlardan escitalopram daha yeni dönemde piyasaya sürüldüğü için elde edilen toksisite verileri, az ama gelişmiş teknolojilerden dolayı daha güvenli görülmektedir. Yine de çoğu SSRI için yayınlanmış çalışmaların metodolojisi, sınırlamaları, tutarsızlığı göz önüne alındığında, gelişmekte olan fetüs üzerinde zararlı etkileri tüm ilaçlar için en azından oldukça belirsizdir. Bazı çalışmalar, serotonin'in kemik fizyolojisi üzerindeki rolünü göstermiştir. Serotonin, osteoklast farklılaşmasını düzenler ve osteoprotegerin (OPG) miktarını arttırır aynı zamanda Osteoklast aktivasyon faktörü RANKL'ı azaltır, oysa fluoksetin ters etkilere neden olur(Battaglino et al, 2004)(Gustafsson et al, 2006). Yaptığımız çalışmamızda gebelik döneminde kullanılan antidepresanlardan sertralin, fluoksetin ve escitalopram'ın embriyonik dönemdeki ratların erken kemik gelişimi üzerinde olası toksisite risk ve sonuçlarını değerlendirmeyi amaçladık.

MATERYAL-METOD

Hayvanlar

Bu çalışma için öncelikle Afyon Kocatepe Üniversitesi Hayvan Deneyleri Etik Kurulundan onay alındı. Hayvan deneyleri için kullanılan tüm yöntemler, 'Ulusal Sağlık Enstitüsü Laboratuar Hayvanlarının Bakımı ve Kullanımı' protokollerine uygun olarak düzenlendi. Çalışmamızda üç tanesi çalışma grubu, bir tanesi ise kontrol grubu olan her grupta Wistar Albino türü dişi rat olmak üzere 5 hayvan kullanıldı. Ratlar çalışma süresince Deney Hayvanları Merkezinde bakılıp takip edildi. Standart koşullar altında 12 saat karanlık, 12 saat ışık ve 25 ± 2 ° C sıcaklık altında bakıma tabi tutuldu. Hayvanlar yeterli miktarda yiyecek ve suya serbestçe erişebildi ve çalışma sonunda anne ratlar genel anestezi altında sezeryanla yavrulardan ayrıldı.

Deney Grupları

Grup 1: Kontrol grubu. Ratların 16 gün süreyle normal gıdalarına ilaveten günde 1 kez 1 cc SF(Serum fizyolojik) gavajla verildi.
Grup 2: Sertralin grubu. Ratların 16 gün süreyle normal gıdalarına ilaveten günde 1 kez 1 cc SF'de çözünmüş 5 mg/kg/gün gavajla verildi.
Grup 3: Fluoxetine grubu. Ratların 16 gün süreyle normal gıdalarına ilaveten günde 1 kez 1 cc SF'de çözünmüş 10 mg/kg/gün gavajla verildi.
Grup 4: Escitaloprom grubu. Ratların 16 gün süreyle normal gıdalarına ilaveten günde 1 kez 1 cc SF'de çözünmüş 10 mg/kg/gün gavajla verildi.

Kimyasallar

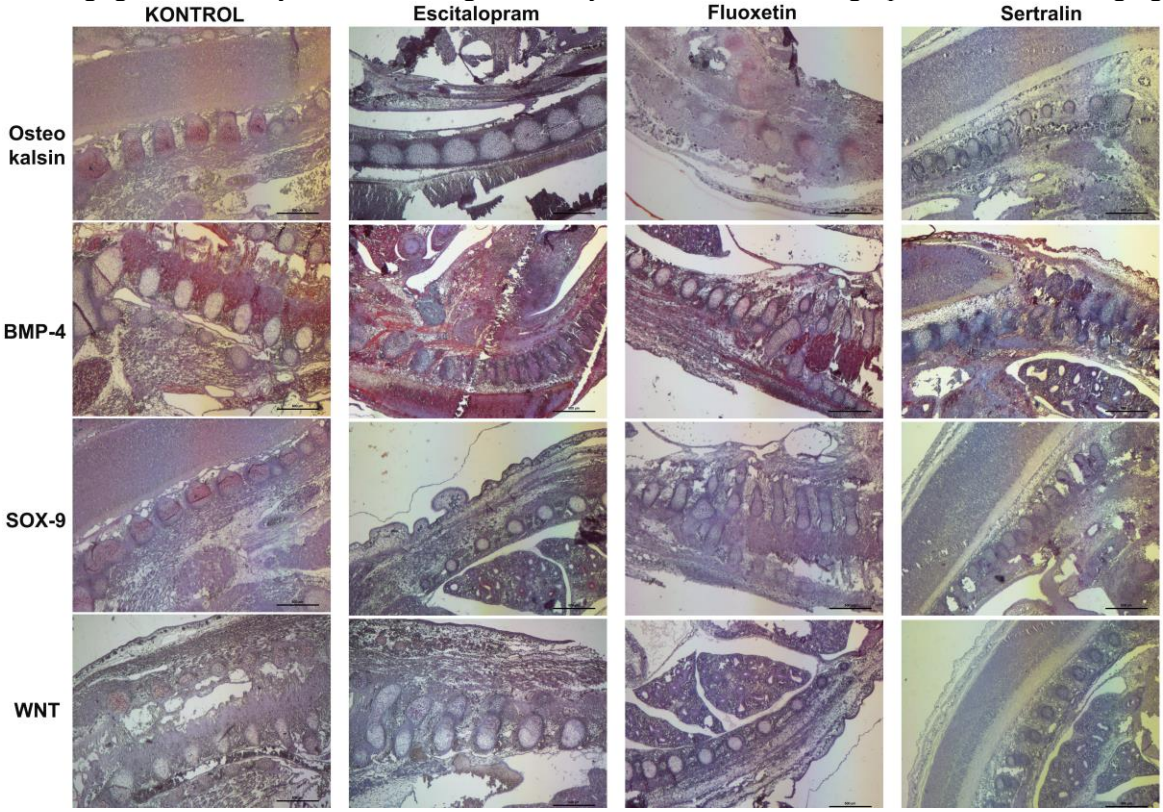
Sertralin, Fluoxetine, Escitallopram

Histopatoloji

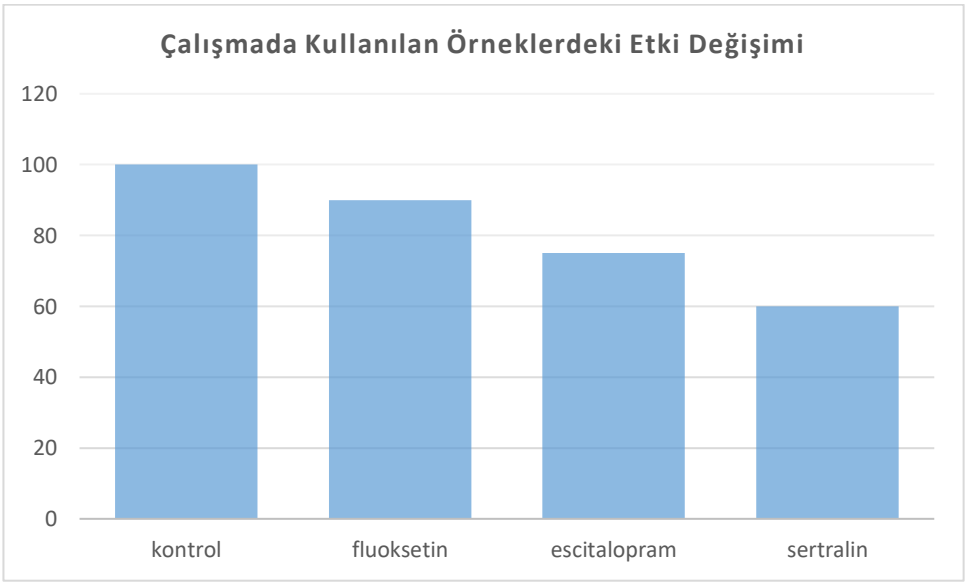
Deney sonunda anne ratlar hamile kaldıkları günün 16. gününde anne ratlar genel aneztezi altında yavru ratlardan ayrıldı. Alınan örnekler %4'luk paraformaldehit içerisine alındı. Rutin histolojik takibe alınan dokular, ardından parafine gömülerek blok haline getirildi. Daha sonra Parafin bloklardan 5 µm kalınlıkta kesitler klasik ve pozitif şarjlı lamlara alınacaktır. Alınan örneklerin histokimyasal olarak hematoksilen-eozin ile boyandı ve immunohistokimyasal olarak da SOX-9, WNT, BMP-4 ve Osteokalsin antikorlarıyla boyanıp değerlendirilmesi yapıldı.

BULGULAR ve TARTIŞMA

Sertralin'e maruz kalan yavrularda Osteokalsin, SOX-9 ve WNT ekspresyonlarında kontrol grubuna göre belirgin bir azalma tespit edildi. Çalışmada kullanılan diğer SSRI'ların da kontrol grubuna kıyasla en az etkilenen grubun fluoxetin grubu olduğu tespit edildi. Fetal SSRI maruziyetinde BMP-4 ekspresyonunda fark olmadığı görüldü fakat yine de kontrol grubuna kıyasla sertralin verilen grupta bir azalma olduğu gözlendi.



Resim 1: Grupların immünohistokimyasal boyamaların ışık mikroskobi görüntüleri (H&E X4)



Şekil 1: Çalışmada Kullanılan Örneklerdeki Etki Değişimi

Gebelik dönemi biyolojik, fizyolojik ve psikososyal değişikliklerin ortaya çıkması nedeniyle, depresyon ve anksiyete gelişmesi veya var olan rahatsızlığın ilerlemesi bakımından risk içeren bir dönemdir (Evans ve ark., 2001). Yapılan çalışmalara göre depresyonun hamilelik döneminde görülme sıklığı %7,4 ile 12,7 arasında değiştiği belirtilmiştir(Bennett ve ark., 2004; Lancaster ve ark., 2010). Gebelikte depresyonun hem anne, hem de yenidoğan üzerinde olumsuz etkileri bulunmaktadır. Depresyon, preklampsi (Kurki ve ark., 2000), prematür

doğum, fetüs gelişiminde gerilik ve düşük doğum ağırlığı (Hollins, 2007; Patel ve Prince, 2006), yenidoğan gelişiminde gerilik (Deave ve ark., 2008) gibi durumlarda risk faktör olarak kabul edilmektedir. Tüm bu riskler göz önüne alındığında depresif bozukluğu olan gebelerin hızlı ve etkin tedavisi gerekmektedir. SSRI'lar, hem anne hem de bebek üzerinde diğer antidepresanlara kıyasla daha az yan etkiye sahip oldukları için sık sık hamilelik ve emzirme döneminde reçete edilir (Tran ve Robb, 2015). Hayvan çalışmalarında ve insan popülasyonlarında SSRI'ya maruz kalma artmış osteoporoz ve kırık riski ile ilişkilendirilmiştir (Rauma ve ark., 2016; Warden ve ark., 2005). Hamilelik ve emzirme sırasında SSRI maruziyetinin, kemirgen modelinde doğumdan 3 ay ve 9 ay sonra maternal trabeküler kemik kütlesini azalttığını (Weaver ve ark., 2018) ve ek olarak, SSRI'lara maruz kalan bebeklerin daha kısa ve daha küçük bir baş çevresine sahip olduğu görülmüştür(Dubnov-Raz ve ark., 2012). Başka bir çalışmada ise SSRI verilerek büyüyen farelerin kemik mineral birikiminin azaldığı gösterilmiştir (Warden ve ark., 2005). Yapılan bir çalışmada, fluoksetine gebelik ve laktasyonel maruziyet, farelerde kemik mineralizasyonunu, trabeküler kemik hacmi fraksiyonunu ve femoral uzunluğu azaltmıştır (Weaver ve ark., 2019). Fluoksetin ve escitalopram FDA ilaç sınıflanma gruplandırılmasında C grubunda dahil iken sertralin FDA'daki bazı kaynaklara göre B bazı kaynaklara göre C grubunda fakat Avustralya ilaç sınıflandırılması TGA'a göre henüz kategorize edilmemiştir. Yaptığımız çalışma, escitalopram'ın C grubuna dahil edilmesine rağmen embryonik kemik gelişimi üzerine olumsuz etkilerinin olduğunu, bu olumsuz etkilerin de WNT ve BMP-4 sinyal yolaklarını baskılayarak gösterdiğini düşündürmektedir. Ayrıca hem escitalopram'ın hem de sertralin'in embriyolarının morfolojik olarak değerlendirildiğinde kontrol grubuna göre doku harabiyeti ve bir miktar gelişim geriliğine sebep olduğu saptanmıştır.

SONUÇ

Çalışmamızda elde ettiğimiz sonuçlara göre embriyogenez dönemi içinde fluoxetin, sertralin ve escilatopram uygulanan ratların kemik gelişimlerinde başta sertralinde olmak üzere gelişimsel bozuklular ortaya çıkmaktadır. Bu nedenle gebelik dönemlerinde gebelere ilaç kullanımının olabildiğince sınırlı tutulmasının tercihan az miktarda veya hiç ilaç vermeden değişik psikoterapiler uygulanmasının daha etkin olacağını düşünmekteyiz.

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➢ *ORAL PRESENTATION*

Characterization of Probiotic Bacteria from Honeybee gut

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Abstract

The microbial flora associated with the digestive system of honeybees is very important as they aid in food digestion, provide essential nutrients, protect the host from pathogens, detoxify harmful molecules, and increase host immunity. The bee samples (*Apis mellifera anatolica*) were taken from Aegean Agricultural Research Institute Bee Centre. The digestive system was removed by dissecting the bee samples under aseptic conditions in a laminar cabin. Extracted intestinal samples were homogenized in 10 mL of phosphate buffered saline (PBS). For the isolation of bacteria, the spreading plate method was applied on MRS agar medium and incubated under anaerobic conditions for 3-4 days. Gram staining and catalase test were applied to the obtained isolates. In order to determine the probiotic potentials of the isolates, acid and bile salt tolerance tests were applied. Among 15 isolates 5 isolates designated as M13, M4, M3, M11, M2 which showed high probiotic potential were selected for further analysis. After 16s rDNA sequencing , the sequence of bacterial isolates were submitted to 16s rDNA sequence database at the National Center for Biotechnology Information (NCBI). According to the analysis, isolates identified as M13 (*Lactobacillus helsingborgensis* 99.42%), M4 (*Lactobacillus melliventris* 98.8%), M3 (*Lactobacillus helsingborgensis* 99.24%), M11 (*Lactobacillus helsingborgensis* 99.23%) and M2 (*Bifidobacterium asteroids* 98.91%).

Keywords: . Honeybee, Lactic acid bacteria, Probiotics, 16s rDNA

INTRODUCTION

Microorganisms in the intestines of animals can benefit their hosts by helping digest food, detoxifying harmful molecules, providing essential nutrients, protecting against pathogens and parasites, and improving development and immunity (Engel ve Moran, 2013).

As social insects, honey bees possess a unique community of gut microbiota. There are three main phyla that dominate the gut microbiota of honey bees: Proteobacteria (*Gilliamella*, *Parasaccharibacter* and *Frischella*), *Actinobacteria* (*Bifidobacterium*), and Firmicutes, some of which are lactic acid bacteria (Lamei, 2018).

The lactic acid bacteria which produce lactic acid as an end product are non-respiring, non-spore-forming cocci or rods Gram-positive bacteria (Quinto et al.,2014).

Some types of LAB can be developed as potential probiotics. This is due to their ability to produce bioactive compounds such as lactic acid, acetate and formic acid, ethanol, enzymes, benzoate, antimicrobial peptides (AMPs), free fatty acid, and volatile compounds (Parichehreh et al., 2018). The digestive tract of honey bees has been found to show potential results among several isolated symbiotic LAB strains, some of which could be developed as probiotics (Berasategui et al., 2016).

According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are living microorganisms that can provide health benefits to the host when given in sufficient quantities. Probiotic microorganisms are often obtained from traditional sources such as dairy products. On the other hand, the use of probiotics from non-traditional sources such as non-intestinal sources, non-dairy fermented food products, and various parts of the digestive tract from animals will potentially increase. (Mathialagan et al., 2018).

Material and methods

Sampling and bacterial isolation

The bee sample obtained from breeding colonies belonging to the Efe Bee genotype (*Apis mellifera anatolica*) registered by the Aegean Agricultural Research Institute located in Izmir, Turkey.

The samples were stored in sterile falcon tubes containing 10 ml normal saline (Tajabadi et al., 2011) and immediately taken to the laboratory. Bee samples were dissected separately on a sterile petri dish and the entire gastrointestinal tract removed aseptically under laminar flow (Olofsson and Vasquéz 2008). The gastrointestinal system were homogenized in normal saline. For lactic acid bacteria isolation, gastrointestinal samples were pooled and smeared on MRS (de Man, Rogosa and Sharpe) agar medium by serial dilution and incubated at 37 ° C for 3-4 days under anaerobic conditions (Tajabadi et al., 2011). To obtain pure bacterial isolates colonies with different morphologies purified by streaking culture method. For primary screening, Gram staining, spore-formation, motility, nitrate redution and catalase tests were performed.

Determination of acid tolerance

In order to determine acid tolerance, MRS and M17 broths pH adjusted to 2.5 using 1 N sterile hydrochloric acid (HCL, Sigma Aldrich, USA) to create an environment similar to gastric acidity conditions. Cultures activated twice (18 hours) in MRS and M17 broths were centrifuged at 10000 rpm for 10 minutes. The bacterial cell pellets obtained were suspended using 7 mL sterile saline and in test tubes with a pH value of 2.5 in 10 ml. MRS was inoculated into broths at 1% and incubated for 3 hours at 37 ºC. Subsequently, the viability of the cultures was followed by serial dilutions and incubation at 37 ° C for 48-72 hours (Klingberg et al. 2005, Prasad et al. 1998).

Determination of bile salt tolerance

For the bile salt tolerance test, 7 ml of MRS and M17 broths with 0.3% (w / v) Oxgall (Bile bovine, Sigma-Aldrich, USA) were inoculated with 1% of the isolates. Later, viable bacteria counts were done after 48-72 hours of incubation at 37 ° C (Liong and Shah 2005).

DNA isolation and 16s rDNA PCR amplification

For DNA extraction, commercial DNA extraction kit based on spin-colon was used (Norgen Biotek). 28-F 5'-AGAGTTTGATCCTGGC TCAG-3' and 1512-R5'-ACGGCTACCTTGTTACGACT-3' universal primers used for 16s rDNA amplification. (Hassan et al., 2014; Weisburg et al., 1991). PCR conditions were as follows: initial denaturation at 95C° for 3 minutes followed by 40 cycles: 95 ° C for 30 seconds, annealing at 55 ° C for 30 seconds and extension at 72 ° C for 1 minute and final extension at 72 ° C for 10 minutes. Then, the PCR products were carried out using safe dye in electrophoresis in 0.8% agarose gel. Product sizes were compared using a 1 KB DNA ladder.

16s rDNA sequence analysis and identification

The 16s rDNA sequencing was carried out at Izmir Institute of Technology BIOMER centre. The obtained DNA sequence chromatograms were analyzed in FinchTV program. The *16S rRNA* gene sequences were matched with the available sequences using the Basic Length Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).Then, Mega 7 program was used to create the phylogenetic tree.

Results

Bacterial isolation and screening

For primary screening, a total 15 colonies which showed Gram-positive, non-motile cells, non-spor forming, catalase and nitrate negative charachteristics were selected. The isolates were kept in glycerol at -70°C for further analysis.

Determination of acid and bile tolerance

Acid and bile tolerance (pH 2.5 MRS Broth) viability values after 72 hours are given at Table 1. 5 isolates designated as M2, M3 , M4, M11 and M13 which were tolerant to acid and bile selected for further identification.

Table1. Acid and bile salt tolerance

Isolate	Acid tolerance	Bile salt tolerance	
	Viability cfu/ml (pH:2,5)	Viability cfu/ml	pH
M2	$3,80.10^3$	$4,62.10^5$	5,20
M3	$4,40.10^3$	$5,01.10^5$	5,45
M4	$4,10.10^3$	$4,70.10^5$	5,10
M11	$3,90.10^3$	$4,02.10^5$	5,25
M13	$4,30.10^3$	$5,02.10^5$	5,51

16s rDNA sequence analysis and identification

16S rRNA gene sequence analysis BLAST results are given at Table 2. The phylogenetic tree was constructed using Mega 7 software (Figure 1).

Table 2. Homology search results for 16S rRNA gene sequences

Isolate	Geneus and species	Homology(%)
M13	*Lactobacillus helsingborgensis*	99.42
M4	*Lactobacillus melliventris*	98.82
M3	*Lactobacillus helsingborgensis*	99.24
M11	*Lactobacillus helsingborgensis*	99.23
M2	*Bifidobacterium asteroides*	98.91

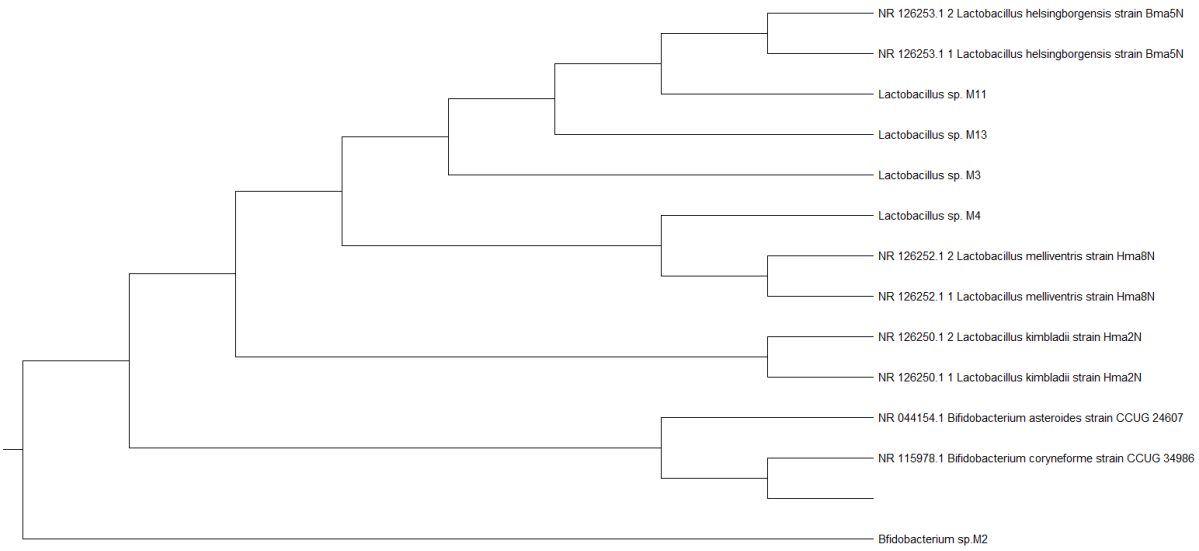

Figure 1. Phylogenetic tree constructed using Mega 7 software.

Discussion

This study was designed to isolate and identify the LAB bacteria with probiotic potential from honetbee gastrointestinal tract. A total of 5 isolates were identified which belongs to *Lactobacillus* (4 isolates) and *Bifidobacterium* (1 isolate).

One study in Egypt reported to have identified LAB bacteria from *Apis mellifera carnica* as *Enterococcus faecalis* MG890204, *Enterococcus faecalis* KX073783, *Enterococcus faecalis* EU594564, Lactobacillus brevis MH191230 and *Lactobacillus casei* KT273339. (Elzeini et al., 2002). Another study performed in Iran isolated *Lactobacillus plantarum, Enterococcus hirae, Enterococcus faecium* and *Lactobacillus pentosus* from *Apis mellifera meda.* According to their findings *Lactobacillus plantarum* and *Lactobacillus pentosus* were predominant which another study reported similar findings (Tajabadi et al., 2020).

As a symbiotic lactic acid bacterial (LAB) microbiota in the honey stomach *L. helsingborgensis* and *Lactobacillus melliventris* first discovered in 2005 (Olofsson & Vásquez, 2008). Other studies also isolated *L. helsingborgensis* from, gut, feces and bee bread of *Apis mellifera*. (Olofsson et al., 2014). Lamei (2018) studied 7 different *Lactobacillus* species (*Lactobacillus apinorum, L. mellifer, L. apis, L. helsingborgensis, L. melliventris, L. kimbladii and L. kullabergensis*) and two different *Bifidobacter* (*Bifidobacterium asteroides and B. coryneforme*) species in the study of lactic acid bacteria on *Apis mellifera*. Although the common colonization area of lactic acid bacteria in honey bees is known as the honey stomach, it has been observed that bacteria belonging to the genus *Lactobacillus, Bifidobacterium* and *Enterococcus* can also be isolated from the middle (ventriculus) and hind gut. (Suyabatmaz et al., 2020).

Mathialagan et al. (2018) studied honey stomach in different honey bee breeds (*Apis mellifera, A. cerena indica, A. florea, A. dorsata* and *Tetragonula iridipennis*) to investigate the diversity of naturally occurring probiotic lactic acid bacteria (LAB) associated with honey bees. The results showed a rich variety of LAB in the analyzed samples; A total of 42 isolates belonging to 6 genera were obtained. Percentages of isolated strains on total microflora are as follows; *Enterococcus* (23.8%), *Micrococcus* (18.8%), *Streptococcus* (13.8%), *Pediococcus* (13.8%), *Lactobacillus* (13.8%), *Lactococcus* and *Leuconostoc* (10.0%).

CONCLUSION

In this study 5 bacterial isolates belonging to the *Lactobacillus* and *Bifidobacterium* have been isolated from *Apis mellifera anatolica* gastrointestinal tract which showed probiotic potentials.

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kullabergensis sp. nov., isolated from the honey stomach of the honeybee Apis mellifera. *International journal of systematic and evolutionary microbiology*, *64*(Pt 9), 3109.

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➢ *ORAL PRESENTATION*

Fruit juice flavored kombucha drink: Investigation of metabolic activities and bioactivity of novel probiotic beverage candidate

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Abstract
In recent years, fermented beverage consumption is one of the rising trends in nutrition. Kombucha is a popular traditional fermented beverage due to its beneficial effects on health. Although, black tea infusion is used in kombucha fermentation, nowadays kombucha teas flavored with herbal tea infusions or fruit juices are preferred. In this study, kombucha drinks are flavored with orange (*Citrus sinensis*) and pomegranate (*Punica granatum*) juices in different quantities to investigate not only metabolic activities and bioactivity but also sensory profiles. In this perspective, bacterial cellulose formation, antibacterial effects, antioxidant activities and pH changes of drinks were analyzed. Sensory analysis was also conducted. According to the results, addition of fruit juice promotes the cellulose production. Moreover, concentration of the fruit juice and the quantity directly affects the antimicrobial activity. Pomegranate flavored kombucha beverages had higher antimicrobial activity against bacterial strains (n=6) than orange flavored beverages. The antimicrobial activity of pomegranate flavored kombucha was statistically significant than orange flavored kombucha ($p<0.05$).
Keywords: Kombucha fermentation, antioxidant activity, antibacterial activity, cellulose production

INTRODUCTION

Protecting personal microbiota via healthy diet draw attention of almost all consumers in global manner (Selhub et al., 2014). As a part of this, traditional fermented foods and beverages are rediscovered and frequently used in healthy diet due to their benefits (Marco et al., 2017). One of the most popular rediscovered traditional fermented beverage kombucha tea is becoming increasingly popular in Western countries (Pure and Pure, 2016). Symbiotic relationship of various yeast and bacteria lived in a specific cellulosic matrix called “tea fungus” or “ Scoby” (Marsh et al., 2014; Villarreal-Soto, et al. 2018) is main actor for kombucha beverage formation. For the fermentation of this beverage sweetened black tea is generally used. As a result of the fermentation process, metabolites such as organic acids, amino acids, antibiotics and various micronutrients are formed in the beverage (Güldane et al., 2017). Due to all of these aforementioned constituents kombucha is effective against metabolic diseases, psoriasis, constipation, indigestion, hypertension, antioxidant, constipation, antimicrobial, antihyperglycemic, chronic diseases (Neffe-Skocińska et al., 2017).

Nutrient content in the tea also determines the taste, beneficial effects and healing properties of beverage. All these features depend on the fermentation period and composition of the starter culture This beverage is classified as a probiotic due to its metabolic effects like maintaining the balance of pH in the body and regulatory effects on intestinal flora (Neffe-Skocińska, et al., 2017;), as well as a natural antibiotic because of its antimicrobial activity (Sievers et al., 1995). Although this probiotic beverage has many beneficial effects, researchers are focused on improving these effects by using various aromatic plants and fermentation media rich in antioxidant, vitamins, nutrients, and antibacterial substances (Ayed and Hamdi 2015). There can be find many studies which is focused on increasing the antioxidant and antimicrobial activity of fermented kombucha beverages via using different herbs and substrates (; ; Pure and Pure 2016; Ayed et al. 2017).

In the present study, antioxidant capacities, antibacterial activities, pH changes, cellulose productions, and sensory features of traditional kombucha beverages aromatized with orange (*Citrus sinensis*) and pomegranate (*Punica granatum*) juices were investigated. For this purpose, orange and pomegranate juices were freshly prepared and used in kombucha fermentation as substrates. Also, this study aims to investigate the effect of the concentrations of the fruit juices on the bioactivities of the fermented products. The main reason of choosing orange as substrate is directly related with consumer preferences and pomegranate is directly related with its nutritional value (Vinson et al., 2000;).

MATERIALS AND METHODS

Substrate media preparation and fermentation conditions.

Fresh orange and pomegranates were washed and squeezed and blended with black tea infusions prepared according to protocol described in Marsh et al. (2014). As a culture media, black tea infusions were blended fresh fruit juices in five different concentrations (0%, 25%,50%,75% and 100%.) with a 100 ml final volume.

For preparation of inoculated samples, 10% soup and 2% Scoby of previously 14 day grown culture was used for each media. For non-fermented samples, prepared culture media were not inoculated. Both fermented and non-fermented samples were incubated at RT for 14 days. On day 14, the soup of samples are subjected to bioactivity analysis. For cellulose production measurements, samples were collected at day 14.

Measurements of Bioactivity Assays

pH variation during fermentation

The pH values of the samples were measured with pH meter (Isolab, Wertheim, Germany) on sampling days**.**

Antibacterial Activity Tests

Six different well-defined bacteria were subjected into antibacterial activity test by using the disc diffusion method (Bauer 1966). *Klebsiella pneumoniae* (ATCC 13883), *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (DSM 22648), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 35218) was tested against antimicrobial effect of both fermented and non-fermented samples. For antibacterial activity test, 60 µl of sterile samples were impregnated into empty antibiogram discs (Bioanalyses Ankara, Turkey) as a test group and 20 µL of 30 µg/mL Ampicillin (Sigma Aldrich, St. Louis, MO, USA) and 20 µL of 30 µg/mL Kanamycin (Cayman chemical, Michigan, ABD) were impregnated into antibiogram disks as a control group for each test organism. Inoculated and disc placed petri dishes were incubated for 24 h at 37 ° C and inhibition zones were measured after incubation. Mean and standard deviation of three replicates were calculated.

Antioxidant capacity measurement via DPPH assay

2,2 -Diphenyl-1-picrylhydrazyl (DPPH) assay was applied for antioxidant capacity described by Von Gadow et al. (1997) was used. Absorbances were measured via spectrophotometer (V-5000 Optical Instruments, China) at 516 nm. Antioxidant capacities of each sample were calculated, and inhibition percentage was calculated.

Cellulose production measurement

In order to measure dry weight of cellulose production, pellicles from inoculated samples were collected and processed according to protocol defined by Keshk and Sameshima (2005).

Sensory analysis

In order to perform sensory analysis, protocol described by Irigoyen et al. (2005) was used with slight modifications. Blind test was applied to twenty participants who selected from the Akdeniz University campus and aged between 18-45. Due to tests blind nature, attendants were not informed about the content of the drinks. Participants evaluated the drinks in 5 point hedonic scale in which 1 represent to very bad to 5 states very good. The points they gave express the degree of acceptability in acidity, taste, appearance, odor and overall assessment

Statistical analysis

All values were expressed as mean ± S.D. All statistical analyses were evaluated with One-way ANOVA by using IBM SPSS 22 software. Statistical significance was set at $P< 0.05$.

RESULTS and DISCUSSION

Measurements of Bioactivity Assays

pH variation during fermentation

As can be seen from Table 1, fermentation causes decrease in the pH of the fermented samples; whereas it causes an increase in the pH of the non-fermented samples. This decline in the pH explained by conversion of sugar to organic acids as a natural result of fermentation (Abd El-Salam 2012). The addition of the pomegranate juice to the culture media caused more dramatic decline in pH than orange juice addition. There are many studies which shows the duration of fermentation plays an important role in the pH variations of kombucha samples. In their study, Marsh et al. (2014) stated that pH of 10-day old fermented kombucha beverages are ranging from 3 to 3.50. Cardoso et al. (2020) explains this situation via predominance of acetic acid and lactic acid bacteria in the samples. On the other hand, Nummer (2013) stated that in terms of microbial

safety the highest pH should be 4.2 and in terms of consumer safety the minimum pH should be 2.5. In this manner, novel probiotic drink candidates in this study complete the requirements in terms of microbial and consumer safety.

Table 8. pH variations during fermentation

		Fermented					Non-fermented				
Fruit Juice Quantity		0%	25%	50%	75%	100%	0%	25%	50%	75%	100%
Orange	Day 0	3,27	3,44	3,49	3,52	3,53	5,41	3,75	3,68	3,64	3,61
	Day 14	2,59	2,82	3,11	3,08	3,12	5,13	3,77	3,71	3,72	3,73
Pomegranate	Day 0	3,27	3,24	3,26	3,25	3,23	5,41	3,46	3,38	3,33	3,29
	Day 14	2,59	2,88	2,65	2,89	2,91	5,13	3,53	3,44	3,4	3,39

Antibacterial Activity Tests

0-day samples did not show any antibacterial activity. It was found that pomegranate flavored kombucha beverages had higher antimicrobial activity than orange flavored beverages (Table 2, Table 3). The antimicrobial activity difference between pomegranate and orange flavored kombucha beverages were statistically significant ($p<0.05$). In their study Vinson et al. (2000) concluded that although orange juice was widely preferred by consumers, because it lacks the plasma antioxidant ability, it is not capable of protecting lower-density lipoproteins from oxidation This also may explain the lower antibacterial activity of orange flavoured kombucha in this study.

Generally, %25 orange juice flavored kombucha had highest antibacterial activity and inhibited the growth of 5 bacterial strains. On the other hand, the highest antibacterial activity observed among pomegranate samples was belong to %100 pomegranate juice samples and showed antibacterial activity against all 6 strains used in this study. This is followed by %50 and %75 flavored kombucha samples. Whereas except 75% pomegranate juice flavored kombucha beverage, all concentrations of pomegranate juice flavored kombucha cause increase in the antimicrobial activity on *E. coli.*

Table 9*:* Antimicrobial activity of 14-day fermented orange juice flavored kombucha beverages

	0%	*25%*	*50%*	*75%*	*100%*	*Kan*	*Amp*
E. coli	6±0	6±0	6±0	6±0	6±0	25±2.2	16±2.9
K. pneumonia	6.75±0.5	8±0	7±0	7±0	7±0	27.75±2.1	15.5±1.3
B. cereus	6.2±0.5	10±1.4	9±0	9±1.4	9±0	25±0.8	10.7±5.5
S. epidermidis	6±0	11±1.41	7.5±0.71	9±0	7±0	26.7±1.2	15±1.6
S. aureus	6±0	7.5±2.1	6±0	6±0	6±0	25.5±1	12.2±4.3
P. aureginosa	7.5±1.9	7±1.4	7±1.4	7.5±0.7	6±0	21±1.1	9.25±2.2

(Kan: Kanamycin, Amp: Ampicillin)

Table 10*:* Antimicrobial activity results of 14-day fermented pomegranate juice flavored kombucha beverages

	0%	*25%*	*50%*	*75%*	*100%*	*Kan*	*Amp*
E. coli	6±0	7.5±0.7	8±1.4	7.5±2.1	11.5±2.1	25±2.2	16±2.9
K. pneumonia	6.75±0.5	6.5±0.7	7±1.4	6.5±0.7	6.5±0.7	27.75±2.1	15.5±1.29
B. cereus	6.2±0.5	6±0	7.5±0.7	8±0	9±1.4	25±0.8	10.7±5.5
S. epidermidis	6±0	6±0	9.5±0.7	10±0	10.5±0.7	26.7±1.2	15±1.6
S. aureus	6±0	6±0	9±0	8±0	10.5±0.7	25.5±1	12.2±4.3
P. aureginosa	7.5±1.9	7±0	8±0	9±0	10±0	21±1.1	9.25±2.2

(Kan: Kanamycin, Amp: Ampicillin)

Antioxidant capacity measurement via DPPH assay

Theantioxidant capacity of pomegranate juice flavoured kombucha beverages showed higher antioxidant activity than orange juice flavoured ones ($p<0.05$) (Figure 1). This situation is directly related with the higher antioxidant capacity of pomegranate than orange. For orange juice flavoured samples, 50% and 100% juice containing fermented products showed statistically significant difference from day 0 samples (Figure 1-A). For pomegranate juice flavoured samples, 25%, 50% and 75% juice containing fermented products showed statistically significant difference from day 0 samples (Figure 1-B). Antioxidants has great importance due to their capability of free radical balancing in the cell (Yamaguchi et al., 2009). The variations in antioxidant activity is investigated in many perspectives such as effect of preparation of beverages (Chu and Chen, 2006), effect of additives (Shahbazi et al., 2018) and duration of fermentation (Vitas et al., 2019). Moreover, it is showed that microbial composition of starter culture is another parameter which defines the properties of fermented product (Amarasinghe et al., 2018). It is stated that fermented samples have higher antioxidant capacity than non-fermented ones because of the structural modifications of polyphenols in tea done by bacteria and yeast enzymes. In this manner, it could be concluded that addition of fruit juices and fermentation cause an increase in the antioxidant activity of beverages.

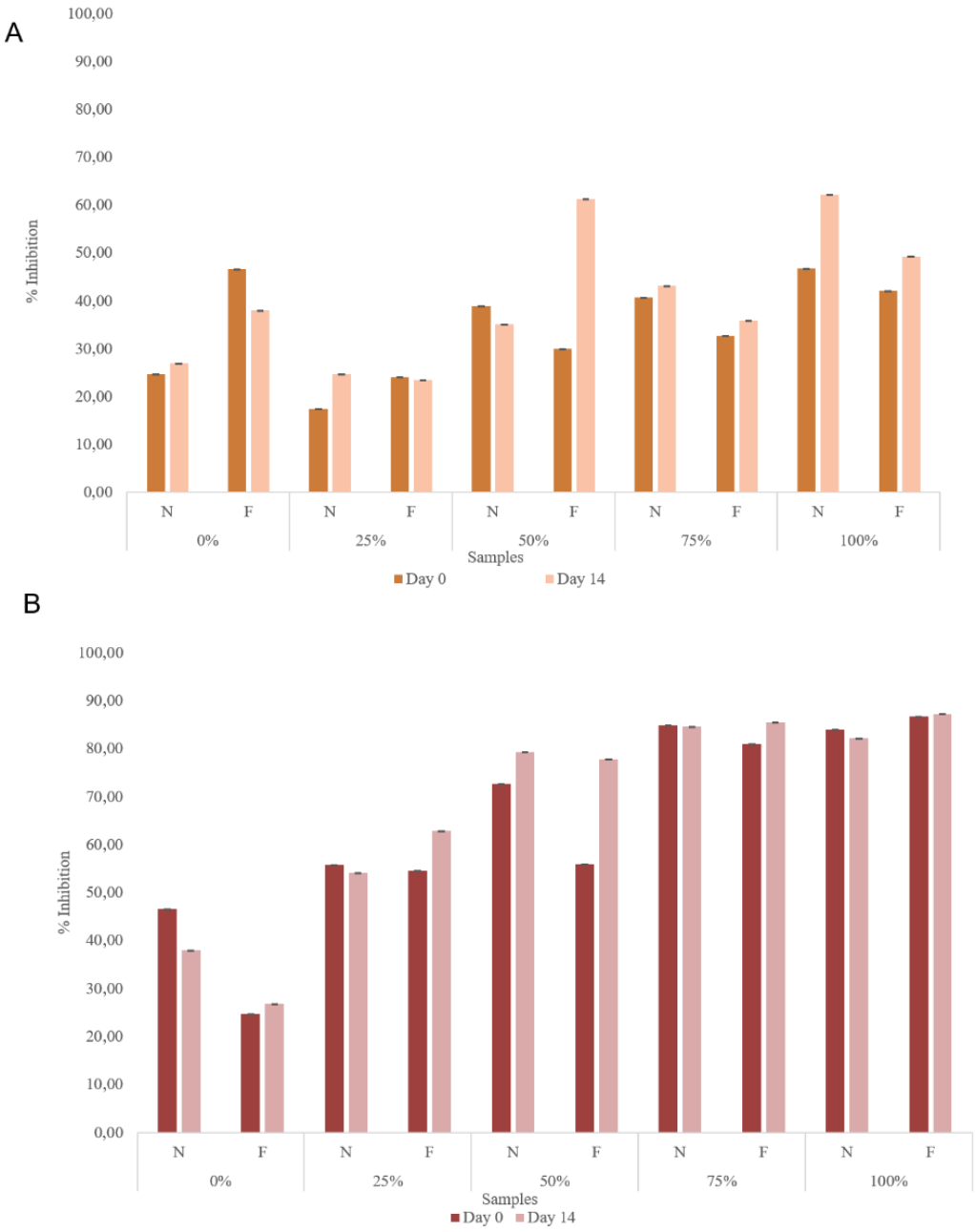

Figure 11. Antioxidant capacity of (A) orange (B) pomegranate flavored kombucha beverages and non-fermented samples (N:Non-fermented samples; F: Fermented samples)

Cellulose production measurement

Dry weight of day 0 and day 14 samples were shown in the Figure 2. It can be said that addition of fruit juice had a positive effect on the production of cellulose. Pomegranate flavoured kombucha samples had highest cellulose production compared with orange flavoured samples($p<0.05$). there was a positive correlation between fruit juice concentration and cellulose production of both fruit juice flavoured samples. In the literature, starter media composition is defined as another parameter for cellulosic layer formation. For example, Goh et al. (2012) shows that sugar concentration directly affects the cellulose yield. Also, Hassan and Al-Kalifawi (2014) states that lower black tea amount in the medium promotes the cellulose formation.

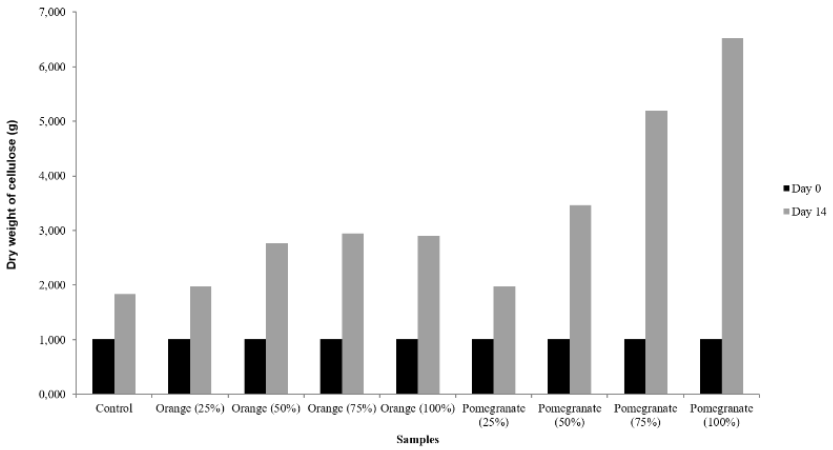

Figure 12. Dry weight of cellulose collected at day 0 and day 14

Sensory analysis

Participants evaluated novel beverages as bright colored, tasty and balmy (Figure 3). Because of the addition of fruit juice softens the acidic taste of traditional kombucha, fruit juice flavored kombuchas were found to be tastier. Orange is one of the *Citrus* family members which is known for its acidic nature. Although participants found the orange juice flavored samples are the least acidic, these beverages were evaluated as the least tasty ones. 75% pomegranate juice flavored kombucha was evaluated as the tastiest sample. The increase in the concentration of fruit juice in the drinks caused the smell of the drinks to be more appreciated by the participants. All fruit juice flavored beverages were evaluated as better than traditional kombucha. Among the samples the odor of the 100% pomegranate juice sample was evaluated as the best. In terms of viscosity, orange juice flavored beverages were evaluated less viscous than pomegranate juice flavored ones. Also, as an overall assessment, participants prefers pomegranate flavored kombucha beverages than orange flavored ones. Most probably this situation was directly related with the sugar amount of the juices and organic acid production. Ulusoy and Tamer (2019) stated that fermentation causes decrease in the sugar amount of the beverage and Ayed el al. (2017) showed that production of organic acids results in vinegary taste.

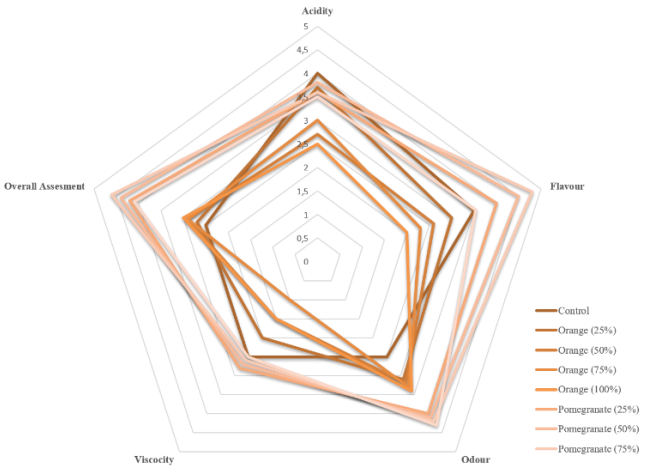

Figure 13. Hedonic ratings of sensory evaluation

CONCLUSION

The aims of this study were developing novel probiotic beverages via fruit juice addition to traditional kombucha and investigation of the metabolic activities and bioactivity of these novel probiotic beverages. For this purpose, orange (*Citrus sinensis*) and pomegranate (*Punica granatum*) juices in different concentrations were added to kombucha culture and fermented for 14 days. Then, bacterial cellulose formation, antibacterial effects, antioxidant activities and sensory analysis of the samples were done. According to our results, addition of fruit juice to kombucha tea has an positive effect on the bioactivity and sensory properties of the drink. As a further research, determination of optimal fermentation time, sucrose, black tea and fruit juice concentrations and detailed examination of ingredients beneficial for human health of these products are strongly recommended.

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➢ ***ORAL PRESENTATION***

Health Promoter: Kombucha, and Its Antioxidative, Anti-diabetic and Anti-carcinogenic properties

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Abstract

Kombucha is a China originated fermented tea beverage is getting popular with its health promoter potential nowadays. It includes organic acids, vitamins, minerals, and phenolic compounds. Different substrates are utilized for enrichment of tea fermentation. Kombucha is a remarkable traditional fermented tea, including organic acids, vitamins, minerals, and phenolic compounds. It is also preferred because of its preventive and therapeutic properties. Kombucha has been expressed with its antioxidant, anti-microbial, anti-biotic effect, immune system supportive, anti-carcinogenic, anti-diabetic, anti-obesity, hypercholesterolemic, hypoglycemic, anti-microbial, laxative properties and known to support for weight loss by accelerating metabolism. In the scope of this study, the antioxidative, anti-diabetic, anti-obesity and anti-carcinogenic properties of Kombucha were evaluated in terms of recent studies.

Keywords: Kombucha, antioxidant effect, anti-diabetic effect, anti-obesity effect, anti-carcinogenic effect

INTRODUCTION

In recent years, the effects of food on human health have overcome their satisfying properties; the bioactive components in their content and their effects are getting more and more attention every day. Kombucha is a China originated fermented tea beverage, a combination of the symbiotic system by acetic acid bacteria (*Bacterium gliconicum, Acetobacter aceti, Acetobacter xylinum, Glucobacter oxydans, Acetobacter pasteurianus,* etc.) and yeasts (*Zygosaccharomyces*, *Saccharomyces* spp., *Pichia* spp., *Torulopsis* spp., *Brettanomyce*s spp.) (Chu and Chen 2006). According to sources, it is claimed that the name Kombucha originates from the words of "*Kombu*" meaning seaweed in Japanese and "*Cha*" meaning tea (Jarrell et al., 2000), but according to other sources, the name "Kombu" comes from the name of the physician who brought this beverage to Japan for the first time (Jayabalan et al., 2014).

Kombucha consists of two parts as the cellulosic layer that formed on the surface and the liquid medium. The cellulose layer on the surface of the tea form by Acetic acid bacteria, consist of yeast and bacteria cell masses present in consortium (Chen and Liu 2000). In aerobic fermentation, substrates such as tea and coffee constitute the nitrogen source required for the development of SCOBY (Symbiotic Culture of Bacteria and Yeast) cells with compounds such as caffeine and theophylline, and the added sugar (7-15%) represents the carbon source (Essawet et al., 2015). After preparing the content of Kombucha, the container is covered with a cheese-cloth that allows air passage and left to fermentation at 25-28 °C for 7-12 days, by taking care to protect from sunlight. Kombucha's yeasts break the carbohydrate source with the invertase enzyme and produce ethyl alcohol from the glucose they obtain. Symbiotic bacteria produce acetic acid from ethyl alcohol produced by yeasts (Dutta and Gachhui, 2006; İleri et al., 2010). Also, acetic acid bacteria use gluconic acid to produce acetic acid, and glucose to produce ethanol. For this reason, the pH value and taste of Kombucha are acidic due to organic acids formed during fermentation (Dufresne and Farnworth, 2000). One of the most important metabolic activities in Kombucha is the production of cellulose by the acetic acid bacteria presents in content (Römling and Galperin, 2015).

Alongside with the content of Kombucha varies according to the substrates and fermentation conditions, generally, it contains acetic acid, lactic acid, gluconic acid, polyphenols, probiotic compounds, ethanol, ethyl gluconate, tea components such as catechins, theaflavins, flavonols, hydrolytic enzymes such as amylase, invertase, and elements such as Ca, Cu, Fe, Na, K, Mn, Ni, Zn (Essawet et al., 2015; Watawana et al., 2015).

ANTIOXIDATIVE PROPERTIES OF KOMBUCHA

Phenolic compounds prevent the damage of cells in the human body by reacting with free radicals, forming chelates with metals, converting hydroperoxides into a more stable form, creating a synergistic effect with other reducing agents, thus showing an antioxidant effect in the body (Rice-Evans et al., 1997). Fermented foods attract attention with their rich content of antioxidative and phenolic components that help protect the body from harmful metabolites. With fermentation, the shelf life of the products is extended and we are able to benefit for a longer time, while at the same time, new products with enriched content and improved functional properties are obtained. Fermented products have been reported in various studies to support the immune system, play an auxiliary role in the prevention of cancer and the treatment of diseases, reduce the symptoms of osteoporosis, have antioxidant, antimicrobial, anti-cholesterol and probiotic properties, and their effects on health have also been revealed (Farad et al., 2010).

Kombucha is usually obtained by fermenting various tea leaves (black, green, white, etc.) with the addition of sugar; bioactive content and taste are enriched with different substrate additions. Several studies have evaluated the enrichment of substrates such as black carrot (Yildiz et al., 2019), cherry laurel, blackthorn, and red raspberry (Ulusoy and Tamer, 2019) snake fruit (Zubaidah et al., 2018), red grape juice (Ayed et al., 2017) and African mustard leaves (Rahmani, et al., 2019) in Kombucha production.

Kombucha's bioactive potential is dedicated for tea leaves' chemical composition. Tea polyphenols are known as flavanols formed by catechins that are most of the important and characteristic in Kombucha. Important catechins are consist of epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (ECGG), catechin, and gallocatechin that form the distinctive taste of the fermented tea (Dufresne and Farnworth 2000). The flavonoids and phenolic acids are the determined major phenolic compounds in the Kombucha samples produced with black and green tea leaves. The most abundant phenolic compounds determined were gallocatechin 3-O-gallate/epigallocatechin 3-O-gallate, gallocatechin isomer 2/epigallocatechin, catechin, 5-O-galloylquinic acid, quercetin 3-O- rhamnosyl-rhamnosyl-glucoside isomer 2 and quercetin 3-O-glucosyl-rhamnosyl-galactoside isomer 2 in both green and black tea fermented Kombucha samples (Cardoso et al., 2020). Besides the composition and concentration of phenolics in Kombucha, metabolites such as ascorbic acid and other organic acids produced during fermentation can also alter the antioxidant capacity (Malbaša et al., 2011). Also, total phenolic compounds increase gradually over the fermentation period. The degradation of complex polyphenols to small molecules by fermentation also increases the antioxidant content (Jayabalan et al. 2014) and the bioaccessible potential of these components.

ANTI-DIABETIC AND ANTI-OBESITY PROPERTIES

Diabetes mellitus is a global health problem caused by various factors, including low physical activity, obesity, sedentary life-style, and intake of excessive calorie including diets. It was first seen thousands of years ago, and its incidence increased rapidly over the years. Diabetes is a hyperglycemia, dyslipidemia, and glucosuria characterized metabolic disease. The basis of the abnormalities in protein, fat and carbohydrate metabolism observed in diabetes is the lack of insulin effects on target tissues. Besides the metabolic problems, complications such as atherosclerosis, neuropathy, nephropathy, and retinopathy develop due to exposure to long-term hyperglycemia (Lin. 2010; Meng et al., 2019).

Type-I diabetes is caused by insulin deficiency, which develops with autoimmune pancreatic destruction and constitutes 5-10% of *diabetes mellitus*. Type-II diabetes is characterized by insulin resistance and impaired insulin secretion, constituting 90-95% of *diabetes mellitus* (Abdulfatahiet al., 2012; Meng et al., 2019). Hyperglycemia and hyperlipidemia leading to increased reactive oxygen species and oxidative stress production. Hyperglycemia activate pathways including protein glycation, glucose autoxidation, and oxidative glycated protein degradation (Ceriello, 1999; Patgett et al., 2013). Increasing oxidative stress in diabetes leads to diabetic complications and multiple organ damage. Pancreatic β-cells are known to be one of the structures most susceptible to oxidative stress and are thought to be caused by the hyperglycemia toxic effects (Donalth et al., 1999; Ihara et al.,1999; Bhattacharya et al., 2013). Another cause of oxidative stress in diabetes is

hyperlipidemia and changes in antioxidant mechanisms therefore it is important to evaluate both glycemic control and lipid levels and antioxidant mechanisms together in diabetic patients (Ojiako, 2015).

Many antioxidant foods have been researched to be protective against diabetic oxidative stress, and some antioxidants have an important role in reducing oxidative stress in *diabetes mellitus*. Fermented food has been attracting consumers as healthy food to known for its curative and preventive effect on various chronic diseases, including diabetes. Recent studies revealed that the fermentation process contributes to the antibacterial, anti-inflammatory, anti-obesity, anti-allergy, antioxidant, anti-hypertensive and anti-diabetic properties of bioactive metabolites by improving their bio-activities (Bhattacharya et al., 2013; Zulkawi et al., 2018).

Kombucha is a remarkable traditional fermented tea, including organic acids, vitamins, minerals, and phenolic compounds. Considering the properties of Kombucha, several investigations have already been conducted on its nutritional properties (Mousevi et al., 2020). Kombucha has been shown an inhibitory effect of α-amylase and lipase activities in pancreas and plasma for suppressing increased blood sugar levels in alloxan-induced diabetic rats. The pancreatic α-amylase inhibition is known as the therapeutic approach for the prevention and control of post-prandial hyperglycemia in non-insulin-dependent diabetic patients by means of reducing the uptake of releasing glucose from starch by those enzymes. Increased lipase activity in diabetes leads to increases in triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C) concentrations in plasma by stimulating lipid absorption. Kombucha treatment provides a significant reduction in LDL-C and TG concentrations in plasma. Moreover, while diabetes was determined to induce a remarkable reduce in the plasma high-density lipoprotein-cholesterol (HDL-C) level, Kombucha supplements observed to revert this decrease (Aloulou et al., 2012).

It has been expressed that the blood glucose levels were significantly decrease and serum insulin levels were significantly increase in Kombucha treatment diabetic groups. Bhattacharya et al., (2013) determined that significant reduction in total cholesterol (TC) and TG, and significant increment HDL-C of Kombucha given to diabetic rats. In this study, it was revealed that Kombucha normalizes the impaired metabolism by reducing the increased weight loss in diabetes (Shenoy, 2000).

Increased serum TC, TG and HDL-C levels in *diabetes mellitus*, an important risk factor for the development of atherosclerotic heart disease. Several studies have reported that kombucha supplements may improve diabetic dyslipidemia by reducing serum lipid levels (Marintim, 2003; Rochette et al., 2014). Experimental data support that antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activity were significantly increased in Kombucha groups versus to the diabetic groups (Bellassoued et al., 2015).

Obesity is a serious health problem worldwide. The prevalence of obesity is rising among all ages with changes of dietary fat intake and lifestyles. Obesity is a major risk factor for T2DM leading to destruction of insulin receptors causing insulin resistance. Diabesity describes the association between T2DM and obesity, as a worldwide health problem, due to their serious complications and extremely high prevalence, especially cardiovascular, respectively micro- and macro-angiopathy linked with accelerated vascular aging leading to microvascular dysfunction and atherosclerosis (Lazar et al., 2019). Bellassoured et al., (2015) stated the anti-hypercholesterolemic effect and antioxidant properties of Kombucha in obese rats.

ANTI-CARCINOGENIC PROPERTIES

Cancer, as one of the leading causes of death around the world, is a multifactorial disease that originate from genetic and environmental factors. The treatment of cancer involves chemotherapy, radiation, targeted therapy and immunotherapy which have numerous side effects. It is also very important to prevent tumorigenesis as much as treatment. The extensive research has been conducted for finding safe and efficacious chemopreventive phytochemicals with fewer side effects to avoid different types of cancer. Recently, the possible chemopreventive ability of Kombucha has become very popular. The evidence has come from mostly *in-vitro* studies that kombucha can have protective role against many human cancers through different mechanisms (Jayabalan et al., 2008; 2014).

Kombucha consumption has been claimed to have anti-carcinogenic potential according to personal observations and testimonials. The anti-carcinogenic effect of Kombucha was also claimed by a population study conducted in Russia in 1951, it has been suggested that Kombucha consumption may correlated with high resistance to cancer development (Dufresne and Farnworth, 2000). The anti-proliferative activity of kombucha beverages prepared from black tea and winter savory tea on three cancer cells lines including cervix

epithelial carcinoma (HeLa cells), colon adenocarcinoma (HT-29), and breast adenocarcinoma (MCF-7) was investigated by using the sulforhodamine B (SRB) colorimetric assay. It has been reported that both kombucha beverages have similar anti-proliferative activities on three cancer cell lines and the anti-proliferative ability of winter savory tea was increased by the combination with kombucha compared to simple water extracts (Cetojevic-Simin et al., 2008).

The anti-invasive and cytotoxic effects of Kombucha were studied by Jayabalan and colleagues (Jayabalan et al., 2011). The treatment of cancer cell lines with an ethyl acetate fraction of black tea kombucha samples which included vitexin and dimethyl 2-(2-hydroxy-2-methoxypropylidene) malonate involved cytotoxic impacts on human osteosarcoma (U2OS) and human renal carcinoma (786-O) cell lines. It has also been reported that it considerably reduced the host cell invasion by cancer cells and cell motility of human lung carcinoma (A549), 786-O cells and U2OS cells and human lung carcinoma (A549) cells, and decreased the activities of matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) in 786-O cells and A549 cells (Jayabalan et al., 2011). Another *in-vitro* study conducted by Srihari and colleagues (Srihari et al., 2013), they evaluated the anti-angiogenic effect of lyophilized Kombucha extracts in human androgen-independent prostate cancer cell line (PC-3), and concluded that it significantly inhibited the angiogenesis of PC-3 cells by downregulating the angiogenesis stimulators/regulators expressions, such as cyclooxygenase-2, human inducible factor-1 gene expression, interleukin-8, matrix metalloproteinases, and vascular endothelial growth factor (Srihari et al., 2013).

Moreover, Kombucha exhibited cytotoxic effects against A549 and epidermoid carcinoma (Hep-2) (Deghrigue et al., 2013) and Caco-2 colorectal cancer cells (Kaewkod et al., 2019). There is only one *in-vivo* study published about the protective effects of Kombucha, and accordingly, the consumption of ginger including Kombucha could balance multi-antioxidant factors in different tissues in the murine breast cancer model (Salafzoon et al., 2017).

CONCLUSION

It was concluded that Kombucha, which has hypoglycemic and hypolipidemic properties, is effective in protecting against increased oxidative stress diabetes mellitus and can be used as a treatment/support agent in the treatment diabetes. The anticarcinogenic properties of Kombucha which have high antioxidant capacity, may be related to the downregulation of cancer pathways, interaction with numerous molecular targets involved in cancer prevention and regulation of immune system. Besides, there is a deficiency in scientific evidence based on *in-vivo* tumor models and human clinical studies. Further studies should be conducted to determine the chemo-preventive potential and anticarcinogenic properties of Kombucha.

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➢ *ORAL PRESENTATION*

Assessment of Some Heavy Metals in Roadsides with Various Traffic Volumes in Lahijan City-Iran

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Abstract
Heavy metal is a general term applied to a metal or metalloid, which has atomic density greater than 4g/cm^3 (at least 5 times or more than water). Toxic heavy metals are serious environmental contaminants due to their none-degraded or none-destroyed properties. In this paper, we analyzed the pollution of heavy metal in roadside dust in Lahijan city (located in Guilan province in Iran northern). This work focused on road dust aspects in roadside soils of two sites: along road with dense traffic (20 street, high traffic volume) and road with lower traffic, a local road (20 street, low traffic volume). Road dust samples (20 in total) were collected under stable weather conditions during May and June of 2020. Road dust samples were collected and analyzed for their variety of lead (Pb), Zinc (Zn), Nickle (Ni), Cobalt (Co) and Cadmium (Cd) concentrations by ICP-OES. The results showed that all heavy metal contents except Cd, are higher than suitable values in pointed soils. High amounts of these metals can have terrible effects on health especially carcinogenic influences.
Keywords: Heavy metals, roadside, analysis, Lahijan, health.

INTRODUCTION

The development of human societies has created many problems for the environment. Roads, plastics, industrial effluents, and sewage have polluted and caused many problems for vegetation, animals, and humans. One of the most important chemical contaminants is heavy metals, causing irreparable damage. Human activity increases the level of heavy metals pollution in the environment. Because these compounds are not metabolized in the body, they can be stored in body tissues such as muscles and bones. Heavy metals have the potential to cause diseases such as mental retardation, hearing impairment, immune system dysfunction, brain diseases, blindness, muscle weakness, and cancer. Roads are usually rich in Pb, Zn and copper. The presence of these metals on the road is usually due to leaded gasoline, tire wear, corrosion of roadside safety fences, and wear of brake linings. Also, the source of Ni and chromium in road dust is probably due to corrosion of vehicular parts. Moreover, heavy metals can enter the environment through natural paths, such as mineral erosion, wind, river, groundwater, and volcanic activity.

In this study, the concentrations of heavy metals such as lead (Pb), Zinc (Zn), Nickle (Ni), Cobalt (Co), and Cadmium (Cd) in road in Lahijan city (north of Iran) were investigated using inductively coupled plasma atomic emission spectroscopy (ICP-OES).

MATERIALS AND METHODS

Study Area

The study areas were selected from Lahijan city center. All samples were randomly selected from several areas, where most of the vehicles running on these roads use gasoline and diesel. A large number of people traveling daily on these roads are subjected to its dusty environment.

Measurements and Characterization

A PerkinElmer (Shelton, CT, USA) Optima 3300 DV ICP-OES instrument was used for determinations.

Preparation of samples

At each of these locations, dust samples were collected within 0.5 m distance from the edge of the pavement. These surface soil samples were taken from the top (0-2) cm of soil. At each sampling point, three sub-samples were taken and then mixed to obtain a bulk sample. Such a sampling strategy was adopted in order to reduce the possibility of random influence of urban waste not clearly visible. Samples were placed in plastic bags, carefully labeled, and taken to the laboratories for analysis. Soil samples were digested with HCl, NHO3, and H2O2 according to U. S. EPA 3050B method.

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Tables and Figures

Figure captions and table headings should be sufficient to explain the figure or table without needing to refer to the text. Figures and tables not cited in the text should not be presented. The following is an example for Table 1. Figure and table captions should be in Times New Roman font and 11 point font size.

Table 11. Mean concentration of metals (mg/kg) in street dust (dense traffic).

Heavy metal	Concentrations
Pb	832.4
Zn	722.3
Ni	102
Co	36.23
Ca	2.8

Table 2. Mean concentration of metals (mg/kg) in street dust (lower traffic).

Heavy metal	**Concentrations**
Pb	332.7
Zn	278.4
Ni	23.36
Co	12.14
Ca	0.2

RESULTS and DISCUSSION

The results of heavy metals from the samples are given in Tables 1 and 2. The results showed that all heavy metal contents except Cd, are higher than acceptable values in natural soils. The average concentration of Pb was 832.4 mg/kg. Pb is mainly caused by car exhaust and vehicle emissions, eg tire wear, bearing wear. This high concentration of lead is mainly due to the use of non-standard gasoline. The average concentration of Zn was 722.3 mg/kg, which is due to the use of Zn compounds as antioxidants and as detergent/dispersants improving agents for motor oil. We believe that the source of Ni in street dust is due to the corrosion of vehicular parts. The high rate of corrosion and wear from old vehicles (due to the use of worn-out cars in Iran) plying these roads could have accounted for the significant levels of anthropogenic contributions of Ni in the road dust. The average street concentration of Co was 36.23 mg / kg, which was acceptable value. The mean Cd concentration has been found in the street 2.8 mg/kg. Cd is a relatively rare heavy metal, which occurs naturally in combination with other metals. Cd has been observed in road dust due to its presence in automobile fuel and in soil. Prolonged exposure to Cd can affect a variety of organs with the kidney being the principal target. Due to the weather in Lahijan, which is mostly rainy in the year, there is a concern that these heavy metals will enter surface water and groundwater. In northern Iran, agricultural products are also irrigated from surface and groundwater, raising concerns that these metals may enter the food chain.

CONCLUSION

In summary, the average concentration of heavy metals in roadside soils of Lahijan city (northern Iran) was investigated. The results showed that the amount of heavy metals studied is high in some cases and threatens health. Due to the rapidly increasing population of Lahijan city, the pollution status along this roads is expected to increase in the coming years. Some protective measures such as the use of public transport, conversion of liquid fossil fuel to gaseous fuel, and having more green areas are suggested to combat this problem.

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➢ *ORAL PRESENTATION*

Koyun kıkırdak ve granüloza hücrelerinin dondurulmasında kullanılan farkı dozlarda dimetil sulfoksit (DMSO) ve gliserolün hücre canlılığı üzerine etkilerinin incelenmesi

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Özet
Hücrelerin biyobankalarda dondurularak saklanması bilimsel araştırmalar sırasında geriye dönebilme veya kaybolan türleri bu hücreleri klonlamada kullanarak geriye getirebilme olanağı sunar. Ancak dondurma işlemi hücreler için öldürücü olabilir ve bu yüzden donma ve çözünme sırasında hücrelerin herhangi bir zarara uğramaması amacı ile dondurma solüsyonu içerisine kriyoprotektan adı verilen çeşitli kimyasal maddeler eklenmektedir. Çalışmada koyun primer kültür ile elde edilen kıkırdak ve granüloza hücre hatları ile çalışılmıştır. Yavaş dondurma tekniği ile, kriyoprotektan olarak seçilen DMSO ve Gliserolün farklı dozları (%5-10) denenmiş, bu maddelerin hücrelerin canlılığı ve proliferasyonu üzerindeki etkileri akış sitometrisi ve MTT analizleri ile değerlendirilmiştir. Sonuç olarak, DMSO uygulanan tüm deney gruplarında canlılık oranı gliserol uygulanan deney gruplarına göre daha yüksek bulunmuştur. Bununla birlikte akış sitometrisinde hücre canlılıkları düşük bulunan gliserol gruplarının 24 saat kültürü sonrasında MTT sonuçlarına bakıldığında bazı deney gruplarında proliferasyonun artmış olduğu gözlemlenmiştir. Tüm veriler ışığında DMSO'nun koyun kıkırdak ve granüloza hücrelerini dondurmanın olumsuz etkisinden gliserole oranla daha iyi koruduğu sonucuna varılmıştır.
Anahtar Kelimeler: : Hücre Kültürü, Kriyoprezervasyon,Yavaş Dondurma, Kriyoprotektan, MTT Analizi, Akış Sitometrisi

Investigation of the effects of different doses of dimethyl sulfoxide (DMSO) and glycerol on cell viability used in freezing sheep cartilage and granulosa cells.

Abstract
Freezing and preserving cells in biobanks offers the opportunity to return the lost species during scientific research or to restore the lost species by using these cells in cloning. However, the freezing process can be lethal to the cells, so various chemical substances called cryoprotectants are added to the freezing solution in order to prevent any damage to the cells during freezing and thawing. In the study, cartilage and granulosa cell lines obtained from sheep primary culture were studied. Different doses (5-10%) of DMSO and Glycerol selected as cryoprotectants were tested with slow freezing technique, and the effects of these substances on cell viability and proliferation were evaluated by flow cytometry and MTT analysis. As a result, the viability rate was found to be higher in all experimental groups treated with DMSO compared to the experimental groups treated with glycerol. However, when the MTT results of glycerol groups with low cell viability in flow cytometry after 24 hours of culture were examined, it was observed that proliferation increased in some experimental groups. In the light of all data, it was concluded that DMSO protects sheep cartilage and granulosa cells better than glycerol from the negative effects of freezing.
Keywords: Cell Culture, Cryopreservation, Slow Freezing, Cryopretectants, MTT Analysis, Flow Cytometry

GİRİŞ

Hücre dondurmanın amacı, ilerleyen dönemlerde ihtiyaç duyulabilecek hücrelerin saklanabilmesi için dondurulmuş hücre stoklarının oluşturulmasıdır (Heyligers ve Klein-Nulend 2005, Cui ve ark. 2007, Loi ve ark. 2008). Dondurulmuş biyolojik materyallerin saklandığı gen bankaları (kriyo-bank, biyobankalar) gen kaynaklarının koruması için oluşturulan programların temel öğelerinden birini oluşturur. Birçok tür üzerine başarıyla sonuçlanan klonlama çalışmalarından sonra, somatik hücre nükleer transferi de tehlike altındaki

memeliler için koruma programlarının ayrılmaz bir parçası olarak önerilmiştir (Wildt ve Wemmer 1999, Ryder 2002, Andrabi ve Maxwell 2007, Arat ve ark. 2011, Comizzoli 2017, Selokar ve ark. 2018). Klonlamanın nesli tükenmekte olan sığır ırklarının kurtarılmasında nasıl yardımda bulunacağına dair mükemmel bir örnek, yıllar önce bildirilmiştir (Wells ve ark. 1998). Bu nedenle, koruma programlarının bir parçası olan kriyobankaların sadece gamet ve embriyo bulundurmak yerine, somatik hücrelerinde (vücut hücresi) depolandığı yerler olması gerektiği vurgulanmaktadır (Leon-Quinto ve ark. 2009). Arat ve ark (2011), Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) tarafından hazırlanan Hayvan Genetik Kaynakları için ilk Küresel Eylem Planı (FAO 2007) ve gen kaynakları koruma klavuzu (FAO 2012) çerçevesinde önerildiği gibi hücre stoklarını da içeren hayvan gen kaynakları bankasının (http://www.turkhaygen.gov.tr/,) Türkiye'de kurulmakta olduğunu ve bu bankada saklanan dondurulmuş materyaller ile ilk Boz ırk klon sığırlarını üretiklerini rapor etmişlerdir. Benzer şekilde Hindistan'da kurulan somatik hücre bankasındaki dondurulmuş hücreler kullanılarak manda klonlanmıştır (Selokar ve ark. 2018).

Uygulanmakta olan tüm dondurma yöntemlerindeki temel prensip, donma ve çözünme sırasında oluşabilecek hücre içi buz kristallerinin oluşmasının önüne geçilmesini sağlayarak, hücrelerin buz kristallerinden zarar görmelerinin önüne geçmektir (Gage 1979). Hücrelerde oluşabilecek bu zararların önlenmesi için hücre içi sıvının, hücre duvarından geçebilen ve hücrelere olabildiğince zararsız olan kriyoprotektan (donmadan koruyan) maddeler ile yer değiştirmesi hedeflenmektedir (Cetinkaya ve Arat 2011, Stolzin ve ark. 2012). Hücre kültürlerinin dondurulmasında en çok kullanılan kriyoprotektanın DMSO ve gliserol olduğu ancak hücre içine daha iyi nüfuz etmesinden dolayı daha başarılı olan DMSO'nun tercih edildiği belirtilmektedir (Freshney 2005). Bununla birlikte kroprotektanların dondurma prosedüründeki başarısı hücre tiplerine göre farklılık gösterebilir.

Sunulan bu çalışmada farklı dozlardaki kriyoprotektanlar ile hazırlanmış dondurma solüsyonlarının, koyun kıkırdak ve granüloza hücrelerinin canlılığı üzerine etkileri akış sitometrisi ve MTT analizleri ile araştırılmıştır.

MATERYAL VE METOD

Materyal

Çalışma materyalini mezbahada kesilen koyunların farklı dokularından elde edilen kıkırdak ve granüloza hücreleri oluşturmaktadır.

Primer Kültür

Henüz kesilmiş koyuna ait kulak dokusu, ve ovaryumları %5 Antibiyotik-antimikotik (Sigma-A5955) içeren DPBS (Sigma- D5652) içerisinde laboratuara getirilmiştir. Hücre izolasyonu ve kültürü daha önce açıklandığı gibi yapılmıştır (Arat 2011). Özetle; laboratuvara getirilen ve +4 °C'de saklanan dokular %5 antibiyotik-antimikotik içeren DPBS ile yıkandıktan sonra steril bistüri ucu ile küçük parçalara ($1mm^3$) ayrılarak 35mm kültür petrilerine ekilmiştir. Doku parçalarının yapışmasının ardından tüm dokuların yüzeyini kaplayacak şekilde % 15 fetal buzağı serumu (FCS), %1 antibiyotik (Biochrom-A2213) içeren hücre kültür medyumu (DMEM/F12) (Gibco- 32500-035) ile kaplanmıştır. Ekim yapılan doku petrileri 37°C ve %5 CO_2 içeren inkübatöre yerleştirilmiştir. Kültür başlangıcından 10-12 gün sonra doku parçalarından hücre üremeleri incelenerek kültür ortamının medyumu değiştirilmiştir. Kültür kabını kaplayan hücreler büyük doku parçaları uzaklaştırıldıktan sonra %0.25 tripsin-EDTA (Gibco-25200-056) solusyonu ile kaldırılıp 60 mm'lik kültür petrilerine transfer edilmiştir (Arat 2011).

Ovaryumların follikülerinden aspire edilen garanulosa hücreleri de 35 mm'lik kültür kaplarına ekildikten sonra aynı kültür ortamında kültür kaplarını kaplayana kadar kültüre edilmiş, ardından tripsinlerenek kaldırılmış ve 60 mm 'lik kültür petrilerine transfer edilmiştir(Arat 2011).

Hücrelerin dondurulması

Pasajları yapılan hücreler tamamen konfluent olduklarında bir kısmı kontrol grubu için tekrar ekilirken bir kısmı deney grubuna uygun olarak dondurulmuştur. Hücreler 60 ml'lik petri kaplarını tamamen kapladığında 5 ml dPBS ile yıkanarak, 0.5 ml %0.25 Tripsin-EDTA solüsyonu eklenmesi ile 5 dakika boyunca inkübatörde bekletilerek petri kaplarından kaldırılmıştır. Kabin içerisine alınan petrilere tripsinin inaktive edilmesi için eklenen tripsin miktarının en az iki katı kadar kültür medyumu eklenerek santrifüj tüplerine alınmış ve 1000 rpm'de 5 dakika boyunca santrifüj edilmiştir. Santrifüj sonrası üst kısımda kalan süpernatant atılarak hücreler, 0.5 ml besiyeri ile sulandırılmış ve kriyotüplere aktarılmıştır. Bu kriyotüplerin her birinin üzerine daha önce

deney gruplarına göre farklı konsantrasyonlarda 2x olarak hazırlanan ve +4ºC bekletilen dondurma solusyonlarından 0.5 ml ilave edilmiş ve hücreler 1 ml dondurma medyumu içinde MrFrosty içine alınıp -80 ºC derin dondurucuda 24 saat bekletilerek dondurulmuştur.

Hücrelerin çözülmesi

Daha önce anlatıldığı şekilde dondurulmuş ve -80 ºC derin dondurucuda bekleyen kriyotüpler, laboratuvara getirilerek hemen 37ºC su banyosunda 30-50 saniye bekletilmesi ile çözündürülmüştür. Çözündürme işlemi tamamlanan hücreler santrifüj tüplerine alınarak 5 dakika boyunca 1000 rpm'de santrifüj edilmiş ve üst kısımda kalan süpernatant atılmıştır. Hücreler, 1 ml medyum ile sulandırılmış ve her bir deney grubuna ait hücrelerin sayımı yapıldıktan sonra bir kısmı MTT analizi için alınarak 96 kuyulu petri kaplarına ekilmiş ve geri kalanı akış sitometrisi analizi için Anexin-V ile boyanmıştır.

Deney Grupları

Kontrol deney grupları

Pozitik kontrol olarak, primer kültür ile elde edilen, koyuna ait olan (kıkırdak, granüloza) hücreler dondurma işlemine tabi tutulmadan inkübatörde %10 FBS, %1 antibiyotik içeren DMEM-F12 medyumda kültüre edilmiş ve konfluent olduklarında diğer deney grupları ile birlikte akış sitometrisi ve MTT analizleri yapılmıştır. Negatif kontrol olarak primer kültür ile elde edilen, koyuna ait olan (kıkırdak, granüloza) hücreler kriyoprotektan kullanılmadan dondurulmuş işlemin sonunda çözülerek akış sitometrisi ve MTT analizleri yapılmıştır.

DMSO ve gliserol karşılaştırması deney grupları

Koyuna ait granüloza ve kıkırdak hücreleri DMSO ve gliserolün %5, %10 konsantrasyonlarında MrFrosty içinde -80 ºC'de derin dondurucuda 24 saat bekletilerek dondurulmuş ve daha sonra çözülerek analiz edilmiştir.

Dondurma medyumu final konsantrasyonları

- %5 DMSO içeren dondurma medyumu (%5 DMSO, %55 DMEM, %40 FBS)
- %10 DMSO içeren dondurma medyumu (%10 DMSO, %50 DMEM, %40 FBS)
- %5 gliserol içeren dondurma medyumu (%5 gliserol, %55 DMEM, %40 FBS)
- %10 gliserol içeren dondurma medyumu (%10 gliserol, %50 DMEM, %40 FBS)

MTT analizi

Her deney grubuna ait örneklerin hücre sayıları belirlendikten sonra, 96 kuyulu petri kabına her bir örnekten 15.000/kuyu hücre olacak şekilde üçer kuyuya ekim yapılmıştır. Her bir örnek için pozitif kontrol hücreleri de aynı sayıda üçer kuyuya ekilmiştir. Ayrıca üçer kuyuda boş bırakılmıştır. Böylece donmuş çözülmüş bir kriyotüp içindeki hücreler için 96 kuyucuklu petride, 3 adet örnek, üç adet pozitif kontrol, üç adet boş kuyu oluşturulmuştur. Ekimi tamamlanan petri kapları 24 saat boyunca inkübatörde kültüre bırakılmıştır ve bu bekleme süresi sonunda her bir kuyucuğa 20 µl MTT boyası eklenerek (işlem karanlıkta yapılır) hücreler 37ºC'de 3 saat boyunca karanlıkta inkübe edilmiştir. Daha sonra her bir kuyuya 100 µl DMSO eklenerek oda sıcaklığında 20 dakika süre boyunca karanlıkta bekletilmiş ve renk değişimleri 540 nm dalga boyunda ELISA cihazında okutulmuştur. ELISA cihazında okutulduktan sonra elde edilen sayısal verilere aşağıdaki formül uygulanarak hücre canlılık oranları hesaplanmıştır (Kılıç ve ark. 2012).

$$\text{Hücre Proliferasyon Yüzdesi} = \frac{A-B}{C-B} \times 100$$

A: Deney grubundaki değer
B: Boş kuyu değeri
C: Pozitif Kontrol grubundaki değer

Akış sitometrisi analizi (flow sitometri)

Sayım için 1 ml medyum içinde bulunan ve bir kısmı MTT analizi için alınmış olan hücreler, 5 dakika boyunca 1000 rpm'de santrifüj edilmiş, medyum atılmış ve hücre peleti soğuk PBS(1 ml) ile sulandırılmış ve tekrar santrifüj edilmiştir. Soğuk PBS ile yıkama işlemi iki kez tekrarlanmıştır. Santrifüjden çıkan hücrelerdeki süpernatant atılarak hücreler üzerine 195 µl Binding Buffer ve 5 µl Annexin V FITC eklenerek 10 dakika boyunca oda sıcaklığında (karanlıkta) inkübe edilmiştir. Bekleme süresi sonunda tekrar santrifüj edilerek (1000 rpm, 5 dakika) üst kısımda kalan süpernatant atılmıştır. Sonrasında hücreler üzerine 190 µl Binding Buffer eklenmesinin ardından hafif pipetleme yapılarak 5 µl RNaseA ve 10 µl PI (Propidium Iodide) eklenmiş ve karanlık ortam şartları sağlanarak 10 dakika boyunca oda sıcaklığında inkübe edilmiştir. Bekleme süresi

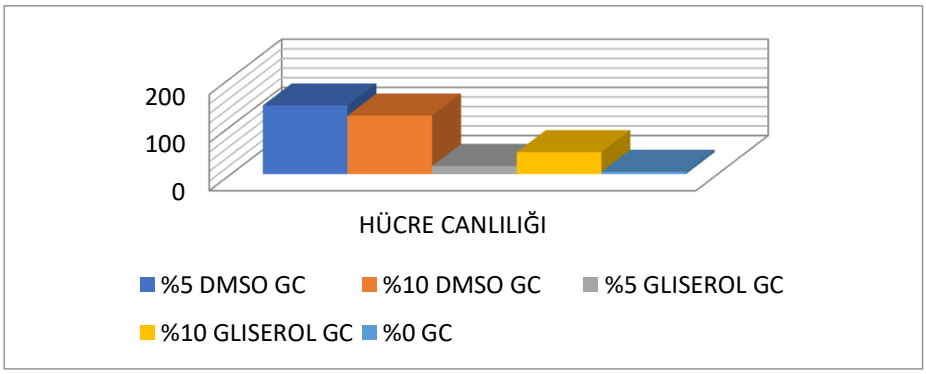
sonunda tüpler tekrar santrifüj edilerek süpernatant atılmış ve hücreler üzerine 500 µl dPBS eklenmesi ile birlikte hafif pipetleme yapılmasının ardından hücreler flow sitometri tüplerine aktarılarak BD FACS Calibur Flow Cytometer cihazında okutularak analiz tamamlanmıştır.

İstatistiksel analizler

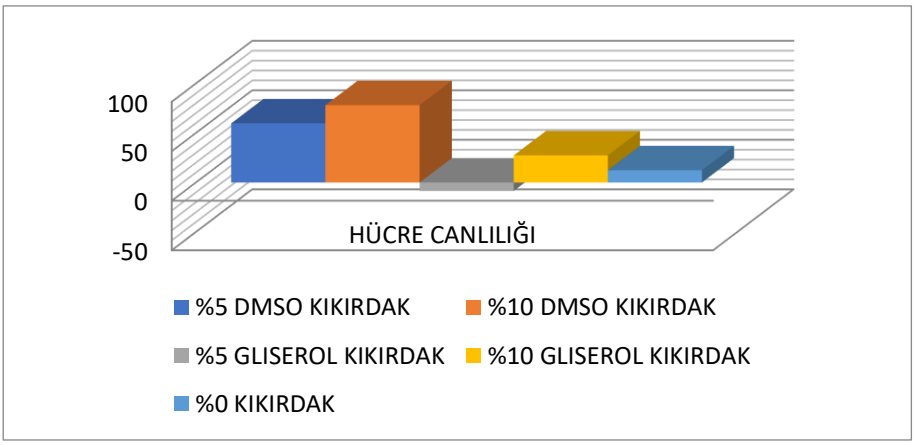
Bu tez çalışmasında her bir grup üç kere birbirinden bağımsız olarak tekrar edilmiştir. Akış sitometrisi ve MTT analizleri tamamlandıktan sonra SPSS (***S**tatical **P**ackage fort he **S**ocial **S**ciences*) programı yardımı ile istatistiksel analizler hesaplanmıştır. SPSS programında One-Way ANOVA (tek yönlü varyans analizi) testi kullanılarak deney grupları arasında bulunan fark Duncan önemlilik testine göre değerlendirilmesi yapılmıştır ($P<0.05$).

BULGULAR ve TARTIŞMA

Canlı materyallerin dondurulmasında yavaş dondurma, hızlı dondurma ve vitrifikasyon adı verilen farklı yöntemler kullanılmaktadır. Hücre dondurulmasında genelde tercih edilen yöntem yavaş dondurmadır (Freshney 2005, Morris 2007). Birçok laboratuvar, kontrollü yavaş bir dondurma yaklaşımı kullanarak iyi sonuçlar bildirmiştir. Bununla birlikte hücre dondurulmasında farklı yöntemlerin karşılaştırıldığı çalışmalar vardır. Duran ve arkadaşları (2001) tarafından yapılan bir çalışmada, Hep-2 ve tavşan böbrek hücrelerine çeşitli dondurma yöntemleri denenmiş ve hücre canlılığına olan etkileri araştırılmıştır. Araştırıcılar hücrelerin dondurulmasında kriyoprotektan olarak %5-10 oranlarında DMSO, gliserol ve Rowe karışımlarını (içeriği %4.2 w/v sorbitol, %35 v/v gliserol) farklı dondurma yöntemlerinde denedikleri çalışmalarında, yavaş dondurma metodunda hücreleri ilk olarak +4ºC'de 30 dakika, -20ºC'de 30 dakika ve -70ºC'de bir gece boyunca beklettikten sonra sıvı azota geçirmişler, orta hızda dondurma metodunda ilk önce +4ºC'de 30 dakika, kristalizasyonun başlaması için -20ºC'de 30 dakika boyunca beklettikten sonra sıvı azota bırakmışlar, hızlı dondurma metodunda ise hücreleri +4ºC'de 30 dakika boyunca beklettikten sonra direkt sıvı azota aktarmışlardır. Yapılan bu çalışmada üç farklı dondurma yönteminden çıkan sonuçlar karşılaştırıldığında en yüksek hücre canlılığının yavaş dondurma metodu ile sağlanmış olduğu görülmüş, %10 konsantrasyonunda kullanılan DMSO, gliserol ve Rowe karışımı sonuçlarına bakıldığında en yüksek canlılık oranını DMSO'nun sağladığını belirtilmiştir. Cetinkaya ve ark. (2009) tarafından yapılan bir çalışmada ise sığır kas ve kıkırdak hücrelerinin dondurulmasında yavaş dondurma tekniği ve vitrifikasyon tekniğini karşılaştırılmıştır. Kriyoprotektan madde olarak DMSO tercih edilmiş ve %5-10 oranları denenmiştir. Araştırıcılar en iyi sonucun 1ºC/dk ısı düşmesi ile yavaş dondurma yönteminden alındığını dondurma hızının daha yavaş gerçekleştiği gruplarda başarı azalırken, hızın arttırıldığı gruplarda başarı oranının hücre tipine göre değişkenlik gösterdiğini belirtmişlerdir. DMSO konsantrasyonları karşılaştırıldığında ise %10 oranının uygulandığı grupların daha fazla başarı sağlamış olduğu belirtilmiştir. Cetinkaya ve Arat (2011)'ın yaptığı başka bir çalışmada kas ve kıkırdak hücreleri kontrollü dondurma makinesinde ısının 0.5, 1, 2 C/dk düşürülmesi ile yavaş dondurulmuş erişkin kıkırdak hücrelerinin canlılık oranları sırasıyla % 60.8, %78.3, %80 olarak bulunmuş ancak aralarında istatistiksel fark tespit edilmediği bildirilmiş, ve vitrifikasyon uygulanan erişkin kıkırdak hücreleri ile de yavaş dondurma sonuçlarına benzer canlılık oranı elde edilmiştir. Ancak aynı çalışmada kas hücrelerinde en yüksek canlılık oranı (%74) 1 C/dk ısı düşmesi ile dondurulan hücrelerden elde edilirken, vitrifikasyon uygulanan hem kas, hemde fetal kıkırdak (sırasıyla %49, %33) hücrelerinde canlılık oranı düşmüştür. Naaldijk ve ark. (2013) uyguladıkları kontrollü hızlı dondurmanın, 1°C/dk kontrollu yavaş dondurmadan ve vitrifikasyondan daha düşük hücre verimi ile sonuçlandığını belirtmişlerdir. Ayrıca Xu ve ark. (2012) mezenkimal kök hücrelerde hızlı dondurmanın (5-10 ° C / dak) hücre iskeletine yavaş dondurmadan (1 ° C / dak) daha fazla zarar verdiğini belirtmişlerdir. Baust ve ark (2007), hücrelerin kontrollu dondurma makinesi kullanarak ısının –80 ° C'ye kadar –1 ° C / dk düşürülmesi ile yavaş dondurulmasının, donmamış hücrelere kıyasla eşdeğer hücre verimi ile sonuçlandığını belirtirlerken, Naaldijk ve ark (2013) Mr. Frostry'de dondurdukları hücrelerden elde ettikleri verimin kontrollü dondurma makinesinde donduralan hücrelere oranla daha yüksek olduğunu bildirmişlerdir. Bu tez çalışmasında da %5 ve % 10 DMSO ve gliserol konsantrasyonları ile genelde daha iyi sonuç verdiği belirtilen 1°C/dk kontrollu yavaş dondurma yöntemi tercih edilmiş, yavaş dondurma işlemi için Mr. Frostry kullanılmış, diğer çalışmalarla benzer şekilde en yüksek canlılık oranı %10 DMSO konsantrasyonundan elde edilmiştir Şekil 1, 2).



Şekil 1. Koyun granüloza hücrelerinde MTT analiz sonuçları



Şekil 2. Koyun kıkırdak hücrelerinde MTT Analizi sonuçları

Graham ve ark. (2015) tavukdan elde ettikleri kırmızı kan hücrelerinin dondurulmasında gliserol, DMSO ve HES çözeltilerinin farklı konsantrasyonlarda etkilerini incelemişlerdir. Gliserol (%20), DMSO (%10), HES (%7.5, %11.5, %20) konsantrasyonlarını uygulayarak dondurmayı gerçekleştirmişlerdir. Çalışma sonrasında nekroz ve geç apoptoz değerlerine bakıldığında % 10 DMSO konsantrasyonunda %3 ve % 20, gliserol konsantrasyonunda %1 gibi düşük değerler gözlemlenirken HES konsantrasyonlarında % 60-80 aralığında değerler bulunmuştur. Ayrıca taramalı elektron mikroskobu ile inceleme sonucu en fazla membran değişikliği % 20 HES konsantrasyonunda ortaya çıkmıştır. Bu çalışmada farklı konsantrasyonlarda DMSO ve gliserol kullanılarak dondurma işleminin yapıldığı deney gruplarında erken apoptotik hücre oranı %1'in (%0.02 - %0.23) altında bulunmuştur. DMSO konsantrasyonlarında geç apoptotik hücre oranı %3.39- %8.63 arasında değişmiştir. Bu oranlar gliserol gruplarında %0.72- % 3.92 arasında bulunmuştur. Ancak nekrotik hücre oranları apoptotik hücre olanlarından yüksek bulunmuş ve en yüksek nekrotik hücre oranları gliserol gruplarında (%22.02-% 65.49) görülmüştür. Bu oranlar DMSO grupların %7.19-%25.10 arasında değişmiştir. Çalışmamız değerlendirildiğinde DMSO birçok koşulda %10 konsantrasyonunda başarılı bulunmuştur. Apototik hücre oranlarına karşın nekrotik hücre oranlarının daha yüksek olması dondurma protokollerinin hücreye verdiği mekanik hasarın kriyoprotektanın verdiği toksik/apoptotik etkiden daha fazla olduğunu göstermektedir (Tablo 1,2).

Tablo 1. Koyun granüloza hücrelerinde akış sitometrisi analiz sonuçları

Grup	Hücre*	Kriyoprotektan	Nekrotik Hücre Oranı (%)	Geç Apoptotik Hücre Oranı	Canlı Hücreler Oranı	Erken Apoptotik Hücre Oranı
1	K-GC	%5 DMSO	16.99 ± 0.37^{d}	4.21 ± 0.04^{b}	78.69 ± 0.32^{c}	0.10 ± 0.00^{b}
2	K-GC	%10 DMSO	8.13 ± 0.95^{e}	8.63 ± 0.94^{a}	83.11 ± 1.05^{b}	0.13 ± 0.05^{b}
3	K-GC	%5 GLISEROL	65.49 ± 0.28^{b}	0.72 ± 0.33^{c}	33.37 ± 0.28^{e}	$0.07\pm0.01^{b.c}$
4	K-GC	%10 GLISEROL	26.72 ± 0.34^{c}	1.71 ± 0.11^{c}	71.46 ± 0.33^{d}	$0.09\pm0.01^{b.c}$
5	K-GC	%0	90.34 ± 0.05^{a}	5.02 ± 0.05^{b}	4.40 ± 0.11^{f}	0.23 ± 0.01^{a}
6	K-GC	Pozitif Kontrol	6.15 ± 0.07^{f}	1.21 ± 0.13^{c}	92.60 ± 0.20^{a}	0.02 ± 0.00^{c}

$^{a-f}$ Sütunlarda farklı harflerle belirtilen değerler istatistiksel olarak birbirinden farklıdır.$P<0.05$

Tablo 2. Koyun kıkırdak hücrelerinde Akış Sitometrisi Analiz Sonuçları

Grup	Hücre*	Kriyoprotektan	Nekrotik Hücre Oranı (%)	Geç Apoptotik Hücre Oranı	Canlı Hücreler Oranı	Erken Apoptotik Hücre Oranı
1	K-KR	%5 DMSO	25.10±0.07^{c}	3.58±0.13^{b}	71.20±0.19^{d}	0.10±0.01^{b}
2	K-KR	%10 DMSO	7.19±0.11^{d}	3.39±0.17^{b}	89.38±0.24^{b}	0.02±0.00^{c}
3	K-KR	%5 GLISEROL	44.35±1.82^{b}	3.92±0.16^{b}	51.62±1.70^{e}	0.10±0.03^{b}
4	K-KR	%10 GLISEROL	22.02±0.34^{c}	2.49±0.24^{b}	75.290.64^{c}	0.09±0.01$^{b.c}$
5	K-KR	%0	86.55±0.92^{a}	10.11±1.11^{a}	3.10±0.22^{f}	0.23±0.03^{a}
6	K-KR	Pozitif Kontrol	4.24±1.48^{d}	0.46±0.14^{c}	95.23±1.63^{a}	0.05±0.00$^{b.c}$

*K-KR: koyun kıkırdak hücresi, $^{a-f}$Sütunlarda farklı harflerle belirtilen değerler istatistiksel olarak birbirinden farklıdır. $P<0.05$

TEŞEKKÜR

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➢ ***ORAL PRESENTATION***

Isolation and Characterization of Chlorogenic Acid from *Inula heterolepis* and Quantification in Some Plants Growing in Erzincan, Turkey

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Abstract

The chlorogenic acid (CA) was purified using preparative HPLC after fractionation of the methanol extract of Inula heterolepis over Sephadex LH-20 for the first time. The structure was elucidated using NMR spectra. The CA content of both *I. heterolepis* and other plants that grow in Erzincan was quantified using HPLC-UV at 330 nm. Chlorogenic acid was not detected in 11 plants including *Onobrychis galegifolia, Onobrychis nitida, Ebenus macrophylla, Thymus convulutus, Chrysathesium stellerioides, Thymus pectinatus, Origanum acutidens, Rhinanthus angustifolius, Thymus vulgaris, Alchemilla erzincanensis,* and *Scrophularia libanotica.* In addition to *Inula heterolepis, Scorzonera aucherina, Helichyrsum arenarium, Helichyrsum arenarium* subs. *erzincanicum, Trachomitum venetum, Achillea teretifolia, Helichrysum plicatum, Tanacetum balsamita,* and *Vinca sonerii*, chlorogenic acid content was found in various amount ranged from 9.60 to 48.92 mg/g extract.

Keywords: Inula heterolepis, chlorogenic acid, HPLC quantification

INTRODUCTION

Genus *Inula* (Asteraceae) is distributed throughout all the world. Many species of this genus are being used by the local people for different purposes such as loss of appetite, headaches and hemorrhoids. Some species of this genus have been reported to have anticancer, antibacterial, cytotoxic and anti-inflammatory activities. *Inula* species contains various significant bioactive secondary metabolites including sesquiterpenes, lactones, flavonoids, glycosides and phenolic compounds (Seca et al., 2014). *Inula heterolepis*, a perennial herbaceous plant mainly grown in the Eastern Mediterranean region, is used topically in folk medicine by local people and known as "kaya andızı" (Fakir et al., 2009). According to the best knowledge, this is the first study to determine the chlorogenic acid content of *Inula heterolepis*. In previous studies, two germacranolides were isolated from *I. heterolepis* (Bohlmann et al., 1982). In present study, from the aerial parts of *Inula heterolepis*, chlorogenic acid was separated and purified using several chromatographic techniques such as sefadex LH-20 and preparative-HPLC, and was quantified in *Inula heterolepis* and some plants that grown in Erzincan region using HPLC-UV instrument. Chlorogenic acid is a caffeic acid ester of quinic acid and, most abundant phenolic acid found in green coffee. It has large number of biological activities including antioxidant, antibacterial, anti-microbial, hepatoprotective, antipyretic, anti-obesity, antiviral, anti-hypertension (Naveed et al., 2018).

MATERIALS AND METHODS

Plant material

Plant materials were collected from various locations in Erzincan. The plants used in this study are as follows: *Onobrychis galegifolia, Onobrychis nitida, Ebenus macrophylla, Inula heterolepis, Thymus convulutus, Scorzonera aucherina, Helichyrsum arenarium, Trachomitum venetum, Chrysathesium stellerioides, Achillea teretifolia, Helichyrsum arenarium* subsp. *erzincanicum, Helichrysum plicatum, Thymus pectinatus, Origanum acutidens, Rhinanthus angustifolius, Thymus vulgaris, Alchemilla erzincanensis, Scrophularia*

libanotica, Tanacetum balsamita and Vinca sonerii. All plant materials were collected at inflorescence stage at 15 July-15 August 2018. Plant samples were authenticated by Prof. Dr. Ali Kandemir and the specimens were deposited at herbarium.

Isolation and characterization of chlorogenic acid from *Inula heterolepis*

150 g of well-grounded aerial parts of I. heterolepis were successively extracted with 500 mL of methanol for 24 hours at room temperature. The extraction process was repeated four times. The solvents were filtrated and evaporated at 60 °C under vacuum to give crude methanol extract (12 g). The crude extract was chromatographed over Sephadex LH-20 using methanol as mobile phase. 20 fractions were collected. According to the TLC analysis 1-10 (A), 11-17 (B) and 18-20 (C) fractions were combined. The C was applied to sephadex LH-20 and eluted with 65:35 ($MeOH:CHCl_3$). The subfracitons were collected 12 fractions in 1 mL volumes. The 4-10 fractions were combined and further purified using preparative HPLC. Preparative separation parameters as follows: detection wavelength; 235 nm, mobile phases; water and ACN, flow rate; 8 mL/min, injection volume; 2.0 mL with 200 mg/mL concentration, oven temperature; ambient, mobile phase program; linear gradient form 100:0 (Water:ACN) to 50:50 (Water:ACN) for 30 minutes. Injection was repeated five times. The peak observed in 23-25 min was collected and evaporated to the dryness to give 120 mg with high purity of chlorogenic acid .

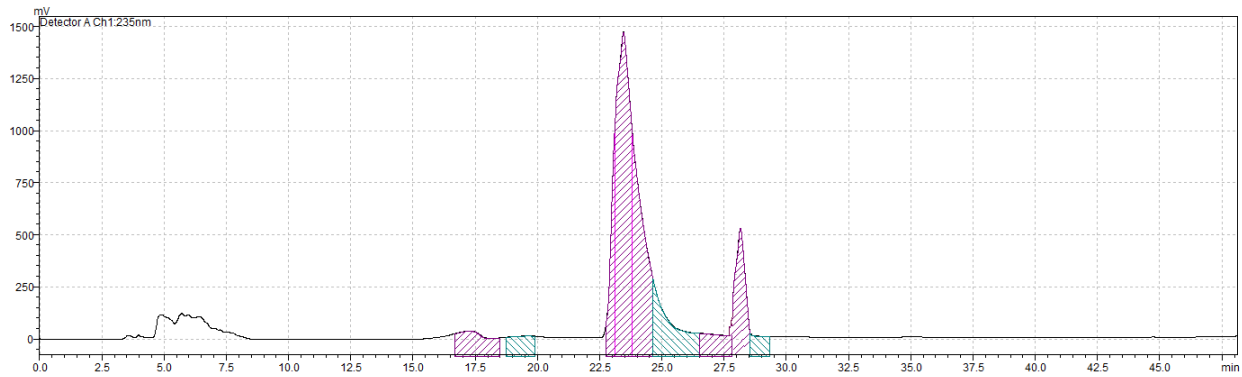

Figure 1. Preparative HPLC chromatogram of CA

Quantification of CA

Ten mg of CA accurately weighed and solved in 10 mL of methanol to get a 1000 ppm stock solution. Five concentrations were prepared by serial dilution including 250, 125, 62.5 and 31.25 ppm and analyzed at 330 nm to give a linear calibration curve with a R^2=0.9996 value and y=0.4837x-1.8934 equation. The LOQ and LOD values were calculated as 0.75 and 0.22 mgL^{-1}, respectively. Extraction procedure for quantitative analysis was as follows: 4 g of well-grounded aerial parts of plant materials were extracted in 100 mL of methanol in ultrasonic bath for 30 min in ambient temperature. The solvent was removed by filtration and fresh 100 ml of methanol was added. The process was repeated triple. The solvents were evaporated to the dryness then a 20 mg/mL stock solution was prepared and filtrated using 0.22 µm syringe filter and directly injected to HPLC. The chromatograms of standard solutions were given in Figure 1.

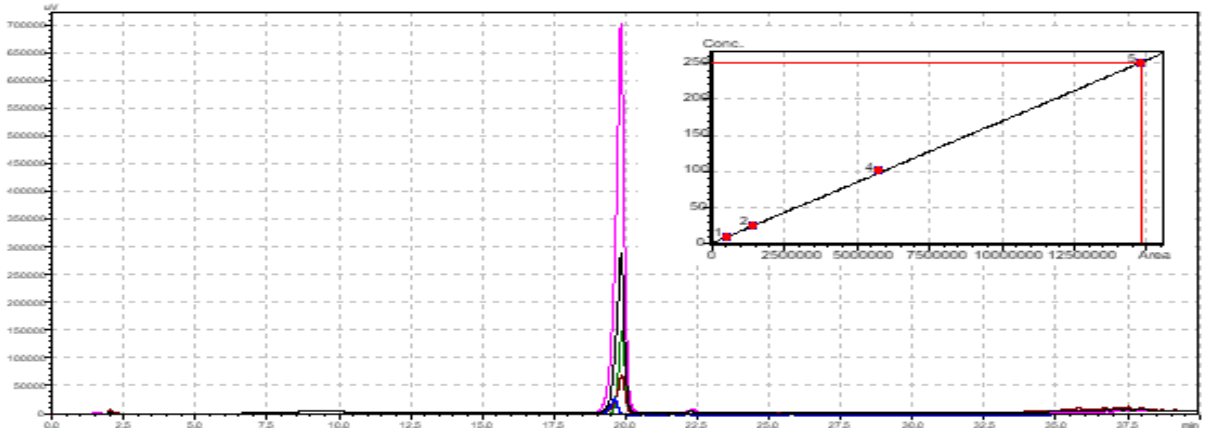

Figure 1. HPLC chromatograms of CA and calibration curve

RESULTS and DISCUSSION

Structural elucidation

Chlorogenic acid was isolated from methanolic extract of aerial parts of *Inula heterolepis* using various chromatographic techniques. The structure of CA was elucidated using NMR data. The ^{1}H and ^{13}C-NMR spectra were given in Figure 2 and Figure 3, respectively. ^{1}H-NMR (400 MHz, DMSO-d6); δ_H 7.04 (brs, H2), 6.78 (d, 8.2 Hz, H5), 6.98 (d, 8.2 Hz, H6), 7.42 (d, 15. 8 Hz, Hα), 6.15 (d, 15.8 Hz, Hβ), 5.08-5.04 (m, H1′), 1.78 (dd, 13.0, 7.6 Hz, H2′a), 2.03-1.98 (m, H2′b), 3.95-3.90 (m, H3′), 3.60-3.60 (m, H4′), 2.05-2.00 (m, H6′a), 1.98-1.96 (m, H6′b). ^{13}C-NMR (100 MHz, DMSO-d6) δ_C 126.0 (C1), 115.2 (C2), 145.4 (C3), 148.8 (C4), 115.1 (C5), 121.8 (Cα), 146.0 (Cβ), 166.2 (C7), 73.9 (C1′), 36.8 (C2′), 68.6 (C3′), 77.3 (C4′), 70.9 (C5′), 37.3 (C6′), 175.4 (C7′). Spectral data was fully agreement with literature (Tosovic and Markovic, 2016).

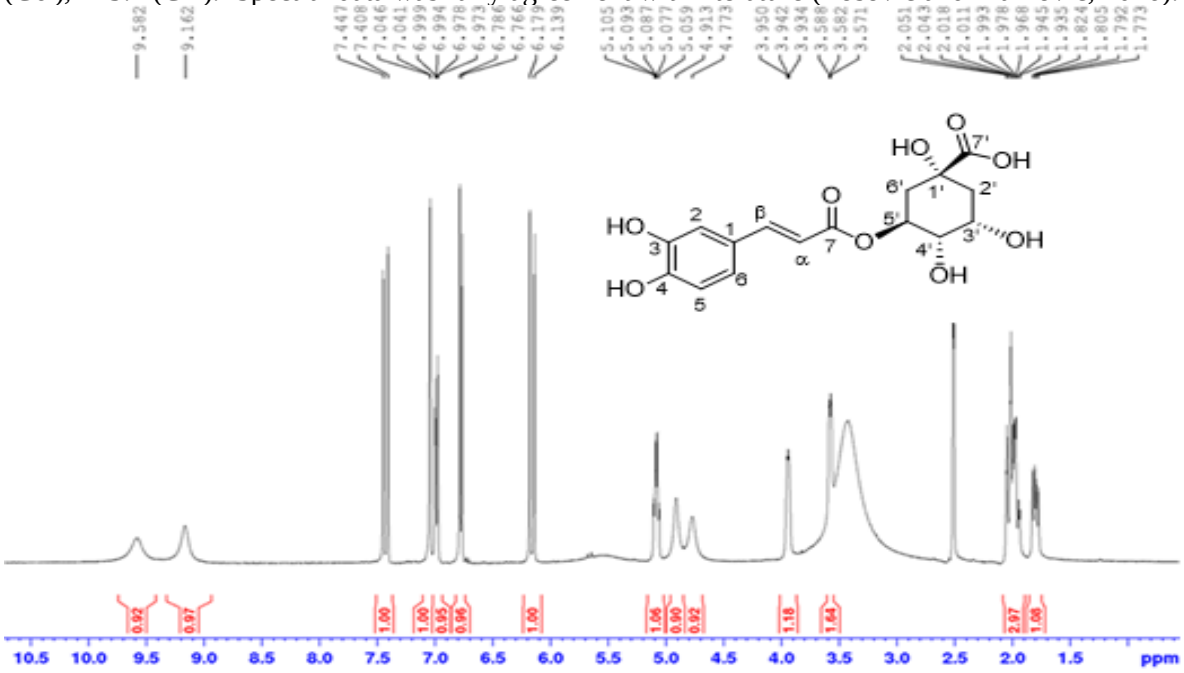

Figure 2. ^{1}H-NMR spectrum of CA in DMSO-d6

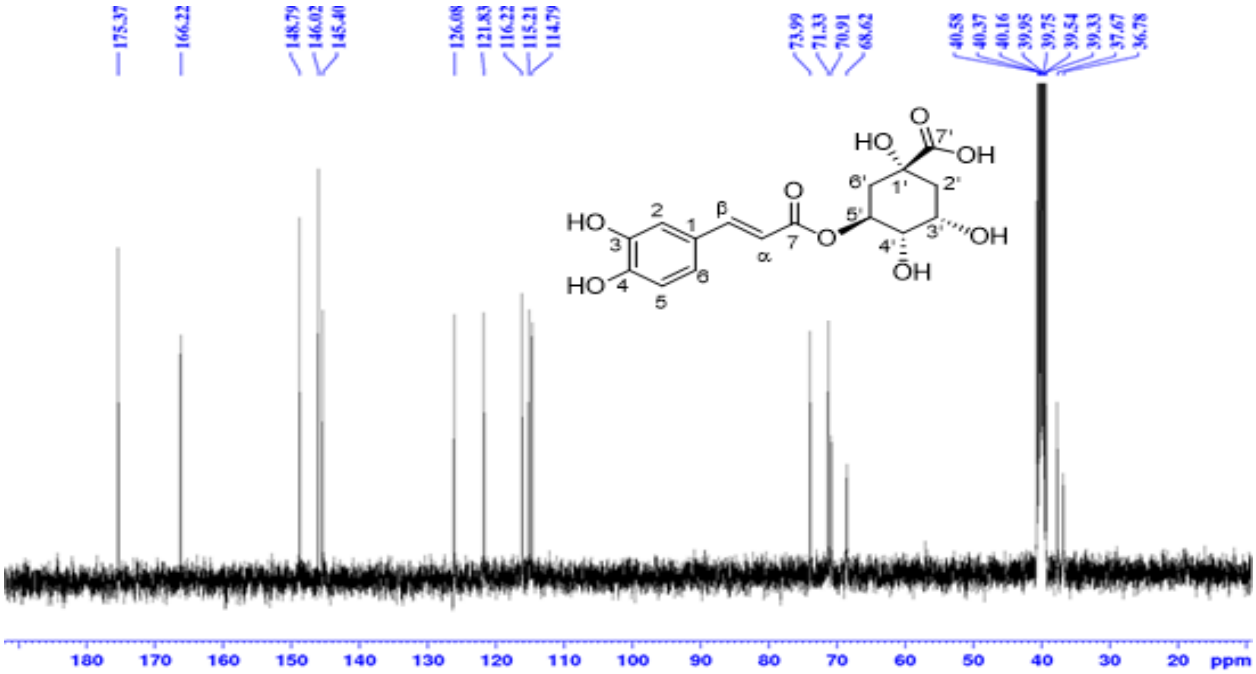

Figure 3. ^{13}C-NMR spectrum of CA in DMSO-d6

Quantative results

Quantitive analysis results were given in Table 1. According to the results, *Scorzonera aucherina* and *Tanacetum balsamita* were found to contain the highest level of CA by 46.22 and 37.17 mg/g among the analyzed plant materials. It was reported that CA is the major phenolic in methanolic extract of some *Scorzonera* species including *S. acuminata, S. cana, S. cinerea, S. eriophora, S. incisa, S. laciniata, S. parviflora,* and *S. sublanata* (Küpeli et al., 2012). The three *Helichrysum* species contains CA in different amount ranged from 11.07 to 23.27 mg/g extract. Some studies were reported the occurrence of CA in *Helichrysum* species (Babotă et al., 2018, Gradinaru et al., 2014). CA was not detected in 11 plants including *Onobrychis galegifolia, Onobrychis nitida, Ebenus macrophylla, Thymus convulutus, Chrysathesium stellerioides, Thymus pectinatus, Origanum acutidens, Rhinanthus angustifolius, Thymus vulgaris, Alchemilla erzincanensis,* and *Scrophularia libanotica.*

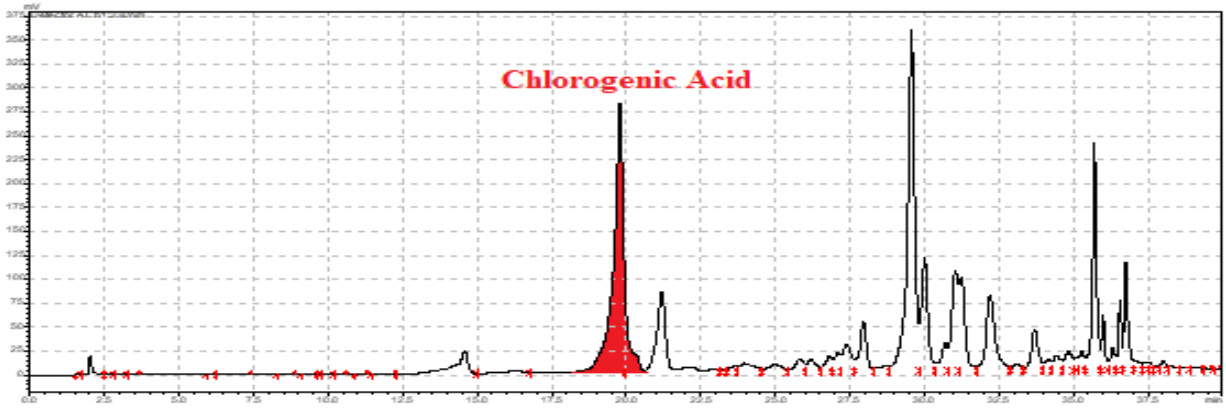

Figure 4. HPLC chromatogram of methanol extract of *I. heterolepis*

Table 1.Chlorogenic acid content of *Inula heterolepis* and the plants collected from Erzincan

Plant materials	CA content (mg/g extract)	Plant materials	CA content (mg/g extract)
Inula heterolepis	9,65	*Onobrychis nitida*	nd
Scorzonera aucherina	46,22	*Ebenus macrophylla*	nd
Trachomitum venetum	17,27	*Thymus convulutus*	nd
Achillea teretifolia	6,00	*Thymus pectinatus*	nd
Helichrysum plicatum	23,57	*Chrysathesium stellerioides*	nd
Helichyrsum arenarium subs. *erzincanicum*	13,13	*Origanum acutidens*	nd
Helichrysum arenarium	11,07	*Rhinanthus angustifolius*	nd
Tanacetum balsamita	37,17	*Thymus vulgaris*	nd
Vinca sonerii	13,04	*Alchemilla erzincanensis*	nd
Onobrychis galegifolia	nd*	*Scrophularia libanotica*	nd

***** nd: not detected

CONCLUSION

In this study the CA was isolated using various chromatographic techniques from *Inula heterolepis* for the first time and quantified in 20 plants collected from Erzincan, Turkey. According to the quantative analysis, in 9 of 20 plant including *Inula heterolepis, Scorzonera aucherina, Helichyrsum arenarium, Helichyrsum arenarium* subs. *erzincanicum, Trachomitum venetum, Achillea teretifolia, Helichrysum plicatum, Tanacetum balsamita,* and *Vinca sonerii*, chlorogenic acid content was found in various amount ranged from 9.60 to 48.92 mg/g extract. Chlorogenic acid was not detected in 11 plants including *Onobrychis galegifolia, Onobrychis nitida, Ebenus macrophylla, Thymus convulutus, Chrysathesium stellerioides, Thymus pectinatus, Origanum acutidens, Rhinanthus angustifolius, Thymus vulgaris, Alchemilla erzincanensis,* and *Scrophularia libanotica.*

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➢ *ORAL PRESENTATION*

Antioxidant capacity of various extracts of *Scorzenera aucheriana* collected from Erzincan

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Abstract

This present study was conducted to evaluate the antioxidative activity of hexane, ethyl acetate, and methanol extracts of aerial parts of *Scorzenera aucheriana* plant. The antioxidant activity was investigated using different assays namely DPPH, ABTS, and ferric reducing antioxidant power (FRAP) tests. Total phenolic (TPC) and total flavonoid contents (TFC) of extracts were also estimated spectrophotometrically. Results demonstrated that the methanol extract contain higher amount of total phenolic and total flavonoid when compared to other extracts. According to the results, IC_{50} values of the extracts in the DPPH scavenging assay were ranged from 6.14 to 207.12 mg/mL while Trolox has 9.12 mg/ml and the FRAP values were 0.41-10.25 µg Trolox/mg extract. Based on the results, the methanol extract of *S. aucheriana* have potential value as an antioxidant.

Keywords: *Scorzenera aucheriana*, antioxidant activity

INTRODUCTION

Antioxidants can inhibit or delay the oxidation process in living organism and food stuff. The intake of naturally occurring antioxidants such phenolics and vitamins can play important role to fighting free radicals. A great interest came out to finding new natural antioxidants from natural sources to replace synthetic ones due to restricted side effects. The greatest source of natural antioxidants is medicinal aromatic plants. Plants have been used for a large range of purposes including medicine, nutrition, flavorings, fragrance and industrial uses. *Scorzonera* species (Asteraceae) represented by 160 species around the world. In Turkey flora, 52 species were grown in Turkey, of which 31 species are endemic. *S. aucheriana* is one of these endemics. The genus *Scorzenera* contains many classes of secondary metabolites, including coumarins, stilbenes, lignans, phenolics, lactones, terpenes, and flavonoids (Milella et al., 2014, Sarı et al., 2019). This study was aimed to determine antioxidant capacity and estimation of total phenolic and flavonoid content of various extracts of *S. aucheriana*.

MATERIALS AND METHODS

Plant material

S. aucheriana plant samples were collected from Kemah-Erzincan road at June 2018. The samples were authenticated by Prof. Dr. Ali Kandemir. The samples were dried in shadow without exposure direct sunlight. After drying process, the plants were cut in to small pieces and deposited in our laboratory.

Extraction

100 g of *S. aucheriana* was first extracted with 100 ml of n-hexane in an ultrasonic bath for 15 minutes then stand overnight at room temperature. The solvents were filtered and evaporated. The above process was repeated sequentially for ethyl acetate and methanol to give 2.1 g of hexane, 5.7 g ethyl acetate, and 9.9 g of methanol extracts. The extracts were kept +4 °C till analysis.

Estimation of total phenolic and total flavonoid content

The total phenolic contents of *S. aucheriana* extracts was estimated using the Folin–Ciocalteu method (Elmastas et al., 2018). Briefly, 3 mL of extract solution (100 µg/mL) was mixed with 1 mL of Folin–Ciocalteu reagent and mixed well. After 10 min, 1 mL of Na_2CO_3 (10%) was added to the mixture and incubated at room temperature in dark for 60 min with. Afterwards, the absorbance of final mixture was measured at 765 nm against a blank without extract. The results were expressed as mg/g of gallic acid equivalents/gram dry extract (mg GAE/g).

Estimation of total flavonoid content

The total flavonoid contents of *S. aucheriana* extracts were estimated using the method previously (Karataş et al., 2018). An aliquot of 1 mL of extract solution (100 µg/mL) were added to 200 µL of 10% (w/v) $AlCl_3$ solution in water, 200 µL (1 M) potassium acetate and 5.6 mL deionized water. The mixture was incubated for 30 min at room temperature. The absorbance of final mixture was measured 415 nm against the blank. The data were expressed as mg/g of quercetin equivalents/g dry extract (mg QE/g).

Free radical scavenging ability

The free radical scavenging activity of extracts were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the spectrophotometric method. Briefly, 0.26 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml sample solution in methanol. The final concentration adjusted to 20, 40 80, and 160 µg/mL. The mixture was vortexed and allowed to stand at room temperature for 30 min in dark. Then the absorbance was measured at 517 nm in a spectrophotometer. The results were expressed in IC_{50} in µg/mL.

$ABTS^{+\bullet}$ radical Scavenging Ability

ABTS radical cation scavenging ability test is employed according the spectrophotometric method reported previously (Köksal and Gülçin, 2008). Firstly, the $ABTS^{+\bullet}$ cationic radical was generated by oxidation of ABTS with potassium persulfate. 1 ml of $ABTS^{+\bullet}$ solution was mixed with 3 ml extract solutions containing various concentration (20-160 µg/mL). After 20 minutes incubation in dark, absorbances of final mixture were measured at 734 nm. The results were expressed in IC_{50} in µg/mL.

Frap Activity

The reducing power of extract was determined according to the method of (Oyaizu, 1986) with slight modification. The phosphate buffer (0.2 M, pH 6.6) added to 100 µL of sample solution up to 1.25 mL and added 1,25 mL $[K_3Fe(CN)_6]$ (%1). The mixture was incubated at 50 °C in a water bath for 20 min allowed to cool to room temperature, and 1.25 mL TCA (%10) and 0,25 mL $FeCl_3$ (% 0.1) added. The absorbance of final mixture was measured at 700 nm. The absorbance of samples converted to mmol Trolox equivalent activity/g using calibration curve obtained various Trolox concentration (10-100 µmol/L). The results are expressed as µg Trolox equivalent activity/mg extract.

RESULTS and DISCUSSION

The phytochemical content of extracts was estimated by total phenolic content (TPC), total flavonoid content (TFC), and the antioxidant activity was evaluated by DPPH, ABTS, and FRAP methods. In all assays, antioxidant capacity of extracts was increased depending on the polarity of solvents. The methanol extract was found most antioxidant-active extract when compared to the other extracts. For methanol extract, the TPC was calculated 525.04 mg gallic acid equivalent/g extract, and TFC was calculated 213.33 mg quercetin equivalent/g extract. According to these findings, the methanolic extract of *S. aucherina* could be said to be quite rich in both phenolics and flavonoids. When compared DPPH and ABTS radical scavenging ability of extracts, DPPH scavenging ability was found 15-fold higher than ABTS. Especially in DPPH assay, methanolic extract was found more active than Trolox with 6.14 IC_{50} (µg/mL). The FRAP activity of extracts was found 0.41, 2.20, and 10.25 µg Trolox equivalent/mg extract for hexane, ethyl acetate, and methanol, respectively. As a result, methanol extract of *S. aucheriana* contains antioxidant-active molecules. Further studies such as chromatographic separation and structure determination are required to determine which molecules are responsible for the activity. The results were summarized in Table 1.

Table 1.Chlorogenic acid content of *Inula heterolepis* and the plants collected from Erzincan

Scorzenera aucheriana	TPC[1]	TFC[2]	ABTS[3]	DPPH[3]	FRAP[4]
Hexane extract	25,41	0,48	658,56	207,12	0,41
Ethyl acetate extract	43,56	35,24	718,25	181,48	2,20
methanol extract	525,04	213,33	92,5	6,14	10,25
Trolox	-	-	6,92	9,12	-

[1] (mg Gallic Acid Equivalent Phenolics/ g extract)
[2] (mg Quercetin Equivalent Flavonoids/g extract)
[3] (IC_{50} in µg/mL)
[4] (µg Trolox Equivalent/ mg Extract)

CONCLUSION

In conclusion, this study first on antioxidant activity of *S. aucheriana* along with total phenolic and total flavonoid contents. The results of the present study showed that the potency of the methanolic extract of *S. aucheriana* as potential source of natural antioxidants. Among all extracts, the methanol extract of plant contained the high level of phenolic and flavonoids, and showed the excellent and free radical scavenging activities. However, further research is needed to investigate chemical content of methanolic extract to purification and characterization of active components and develop some techniques for uses for pharmaceutical and nutraceuticals industries.

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➢ *ORAL PRESENTATION*

Analysis of domoic acid within *Nitzschia navis-varingia* and other three *Pseudo-nitzschia* species isolated from the northeastern Mediterranean Sea

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Abstract
Domoic acid (DA) is a water-soluble neurotoxin, known to be produced by the diatom genera *Pseudo-nitzschia* and *Nitzschia*. There are several records of potentially toxic *Pseudo-nitzschia* species along the Turkish seas. However, excluding a few studies performed in the eutrophic Golden Horn Estuary, İstanbul, almost no effort has been made to determine their toxicity in other areas of the Turkish seas. In this study, identifications of three potentially toxic *Pseudo-nitzschia* species have been performed with SEM. DA production of four nonaxenic diatom species, *Pseudo-nitzschia delicatissima/P. arenysensis*, *P. brasiliana*, *P. galaxiae* and *Nitzschia navis-varingica* isolated from the coastal waters of the northeastern Mediterranean Sea was investigated. In addition to SEM, *Nitzschia navis-varingica*, *Pseudo-nitzschia delicatissima/P. arenysensis*, and *P. galaxiae* were identified by comparing the 18S rDNA. Amplified sequences were used to construct a phylogenetic tree. Toxic strains of all four species are present in distinct regions of the World's oceans. Our results based on ELISA kits did not show any detectable levels of DA within any of the diatom species tested.
Keywords: Domoic acid, *Nitzschia navis-varingica*, *Pseudonitzschia delicatissima/P. arenysensis*, *Pseudo-nitzschia brasiliana*, *Pseudo-nitzschia galaxiae*, NE Mediterranean Sea.

INTRODUCTION

Neurotoxin domoic acid (DA) has been found within 26 *Pseudo-nitzschia* species out of 52 (Bates et al., 2019) and only within two *Nitzschia* species (*N. navis-varingica* and *N. bizetensis*) out of ~900 in the world's oceans so far (Lundholm and Moestrup, 2000). DA causes amnesic shellfish poisoning (ASP) in marine birds and mammals including humans upon consumption of marine organisms such as shellfish, anchovies, sardines and crustaceans which have bioaccumulated DA through the food chain. ASP leads to permanent short-term memory loss, brain damage, and death in severe cases (Bates et al., 1998). Potentially toxic *Pseudo-nitzschia* species have been reported along Turkish seas by many studies (Bargu et al., 2002; Lok et al., 2010; Tas and Yilmaz, 2015; Tas and Lundholm, 2017; Türkoğlu and Koray, 2002). However, based on our literature survey, there a few publications recording domoic acid levels (only in the Golden Horn Estuary, Halic, Sea of Marmara) along Turkish coasts (Dursun et al., 2016; Dursun et al., 2017; 2018; Tas et al., 2016).

Concentrations of domoic acid in cultures vary from trace levels (<1 ng ml^{-1}) to highly toxic levels (>100 ng ml^{-1}) (Fernandes et al., 2014). In addition, the EU guideline limit for shellfish consumption is reported as 20 μg DA g^{-1} (Bouchouicha-Smida et al. 2015). According to our literature survey, the highest reported cellular DA content is 117 pg DA $cell^{-1}$ in the Southern California Bight when cell abundance of *Pseudo-nitzschia* spp. was not very high (x10^4 cells L^{-1}) (Schnetzer et al. 2007). This amount was much higher than values reported in the Mediterranean Sea, 10^{-7}-0.6 pg DA $cell^{-1}$ (Sarno and Dahlman 2000, Cerino et al. 2005).

Although blooms of *Pseudo-nitzschia* species are observed in the Mediterranean Sea, domoic acid levels are found low in seawater (Arapov et al., 2016; Bosch-Orea et al., 2020; Cerino et al., 2005). Toxicity levels within shellfish samples are also under EU guideline limits for shellfish consumption (Hassoun et al., 2020; Leblad et al., 2013).

In addition to *Pseudo-nitzschia* species, one toxic *Nitzschia* species, *N. bizertensis* (2-7 x10^{-3} pg DA $cell^{-1}$) have been identified in the Bizerte Lagoon, the southwestern Mediterranean Sea previously (Bouchouicha-Smida et al. 2015). DA levels were under EU guideline limits within mussels and oysters in this region.

Toxicity levels in the Golden horn estuary, İstanbul were low (0.03-1 ng ml^{-1}) during 2013-2014 (Tas et al., 2016) while relatively high levels (maximum 21 ng ml^{-1}) were observed in May 2012 at a station in the same region when *Pseudo-nitzschia* abundance was over 10^5 cells L^{-1} (Dursun et al., 2017).

There are both toxic and non-toxic strains of *Nitzschia navis-varingica* in literature in the western Pacific region (Bates et al., 2018). However, there is no information about its toxicity in the Mediterranean Sea where first recorded in 2016 (Ayaz et al. 2018).

The aim of the present study was to identify three *Pseudo-nitzschia* species isolated from the northeastern Mediterranean Sea coast by SEM observation as well as with DNA sequence analysis and to determine if these species and *Nitzschia navis-varingica*, which was also isolated from NE Mediterranean, are toxic or not.

MATERIALS AND METHODS

Cultures of *Nitzschia navis-varingica* Lundholm & Moestrup, *Pseudo-nitzschia delicatissima* (Cleve) Heiden *P. arenysensis* Quijano-Scheggia, Garcés, Lundholm, *P. galaxiae* Lundholm & Moestrup and *P. brasiliana* Lundholm, Hasle & G.A.Fryxell were isolated from the surface waters of the northeastern Mediterranean Sea coast (36°36' N, 34°19' E and 36°47' N, 34°59' E) on 25 February 2016, 4 April 2018, 26 September 2018 and 26 September 2019, respectively.

F/20 Medium is used in cultures with a 12h:12h light-dark cycle under 20 ºC temperature and ~20 µmol s^{-1} light conditions. The salinity of seawater used for growing cultures is ~38.

For field emission scanning electron microscope (Zeiss Supra55) images, samples were first washed with 250 ml distilled water, filtered through 0.2 µm CA filters and resuspended with 5 ml distilled water. Following desalination, 10 ml of concentrated sulphuric acid and nitric acid was added onto cells and boiled within a water bath for 60 min. Cells were rewashed with 250 ml distilled water and filtered on 0.2 µm CA filters. Cells were coated with platinum by Quorum Q150R Sputter Coater and examined with SEM.

Domoic acid test kits were applied during the stationary phase of growth for both stored samples (on the19th day of growth at -20°C for 2.5 months) and for live cultures (on the 14th day of growth). The total domoic acid concentration was examined using Mercury Science Domoic Acid Screening Test Kit (Product # DAK-36). Before applying kits, cultures (cells and supernatant) were sonicated 30 min within 40KHz frequency Wisd 23 Model ultrasonic bath (WUC, Germany) by placing ice cubes into the water upon warming. Following sonication, samples were filtered through 0.2 (Minisart) syringe filters and DA was explored within the supernatant following manufacturer's instructions. Each sample was analysed with technical duplicates.

DNAs were extracted from algae samples using the commercially available kit (QIAamp DNA Minikit (Cat#51304, Qiagen, Valencia, CA) as per the manufactures' instructions. 18S rDNA genes were amplified using the sense strand primer 5' TACCGTCCTAGTCTCAACCATAA -3' (18S-F) and the anti-sense strand primer 5' CAGAAGTGAACCTTTTCTTC-3' (18S-R). PCR products were sequenced by Sentebiolab, Ankara, Turkey (https://sentebiolab.com.tr/).

RESULTS and DISCUSSION

Description of species based on SEM

Pseudo-nitzschia delicatissima (Cleve) Heiden /*P. arenysensis* Quijano-Scheggia, Garcés, Lundholm

Cells are lanceolate and symmetrical, 1.8-2 µm wide and 30-36 µm long (Fig. 2). Cells form stepped chains by the overlapping of the valve tips in girdle view (Fig. 2b). Two rows of poroids are present per stria (Fig. 2a). 10 poroids are located in 1 µm. The density of fibulae is less than that of striae. There are 22 fibulae and 39 striae in 10 µm. A large central interspace is located between the two central fibulae. Apices are long and tapering in valva view (Fig. 2c).

Distribution: Found in temperate marine waters (Amato et al., 2005; Quijano-Scheggia et al., 2009).

Toxicity: Both toxic (0.03-9.5 fg DA $cell^{-1}$) (Fernandes et al., 2014) and nontoxic strains of *P. delicatissima* are present (Bates et al., 2018; Prince et al., 2013).

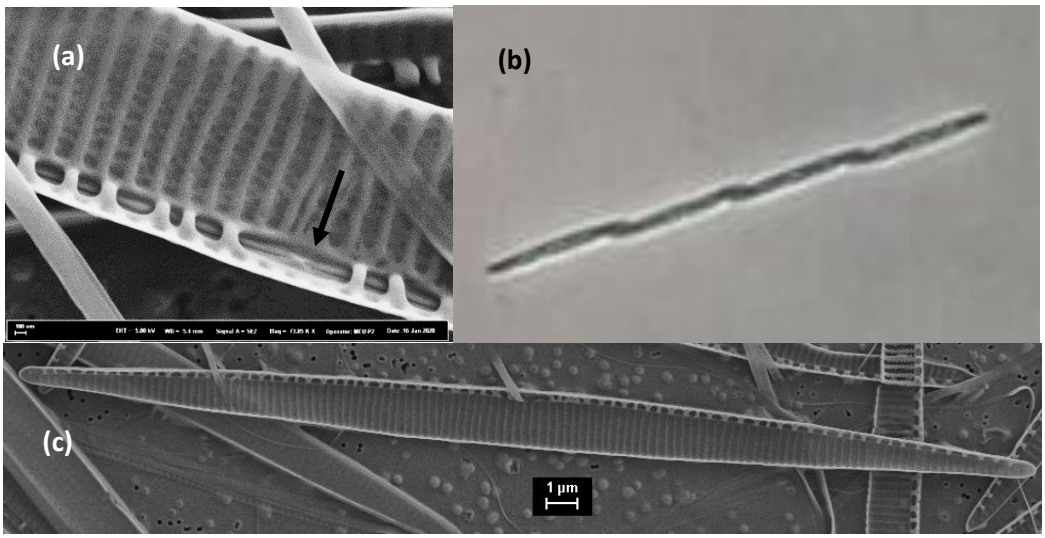

Figure 1. *Pseudo-nitzschia delicatissima/P. arenysensis* (a) biseriate striae and central interspace (arrow) (b) light microscope view (c) complete cell showing the tips of frustule

Pseudo-nitzschia brasiliana Lundholm, Hasle & G. A. Fryxell

The cells contain two yellow-brown chloroplasts (Fig. 2a). They are symmetrical and linear to lanceolate in shape. The transapical axis (width) is ~2.4 µm and the apical axis (length) 34-35 µm. There are two rows of poroids per stria (Fig. 2b). Central interspace is absent. Poroids do not contain hymen sectors. There are 8 poroids in 1 µm. The density of fibulae is equal to that of striae, which is 25 in 10 µm. Valva tips are broad and rounded (Fig. 2c).

Distribution: Brazil, South Korea, Gulf of Mexico, Gulf of California, Gulf of Panama, Vietnam, Thailand, Indonesia, Malaysia (Lundholm et al., 2002), Mediterranean (Sahraoui et al. 2011).

Toxicity: DA production of this species was first time reported in Bizerte Lagoon, Tunisia, southwestern Mediterranean Sea in cultures isolated from this region (8.9 fg DA cell^{-1}) (Sahraoui et al. 2011).

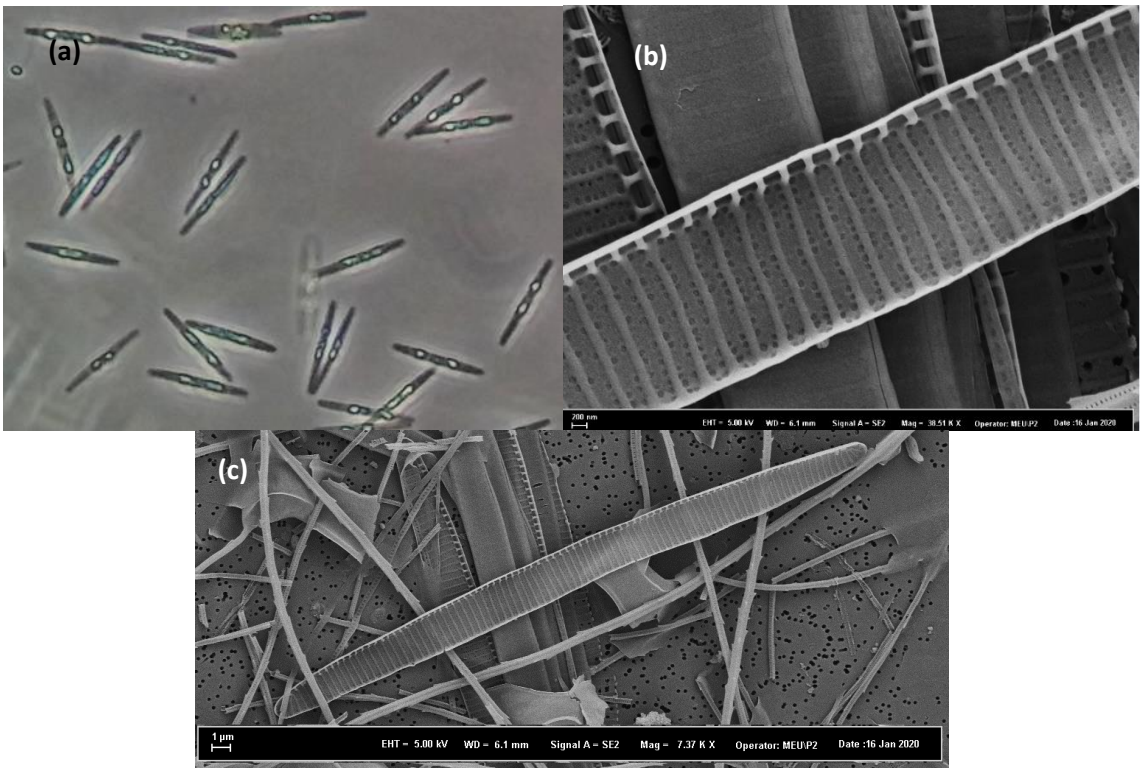

Figure 2. *Pseudo-nitzschia brasiliana* (light microscope image) (b) biseriate striae (c) complete cell showing the tips of cell frustule.

Pseudo-nitzschia galaxiae Lundholm & Moestrup

Cells are lanceolate and have rostrate apices (Fig. 3a, b). They are thin and weakly silicified. The apical axis is 25–58 µm long; the transapical axis is 1.9-2 µm. Two chloroplasts are present in the central region (Fig. 3b). The striae do not have poroids (Fig. 3c). Density of striae is much more than the density of fibulae. There are 56 striae and 24 fibulae in 10 µm (Fig. 3d). A large, central interspace is present. The valva is asymmetrical. Apices have pointed tips.

Distribution: Mexican and Australian waters (Lundholm and Moestrup, 2002) and the Mediterranean Sea (Cerino et al., 2005).

Toxicity: It was first found toxic in the western Mediterranean Sea and cellular concentrations of DA changed from 7.8 x 10^{-7} to 3.6 x10^{-4} pg cell^{-1} (Cerino et al., 2005).

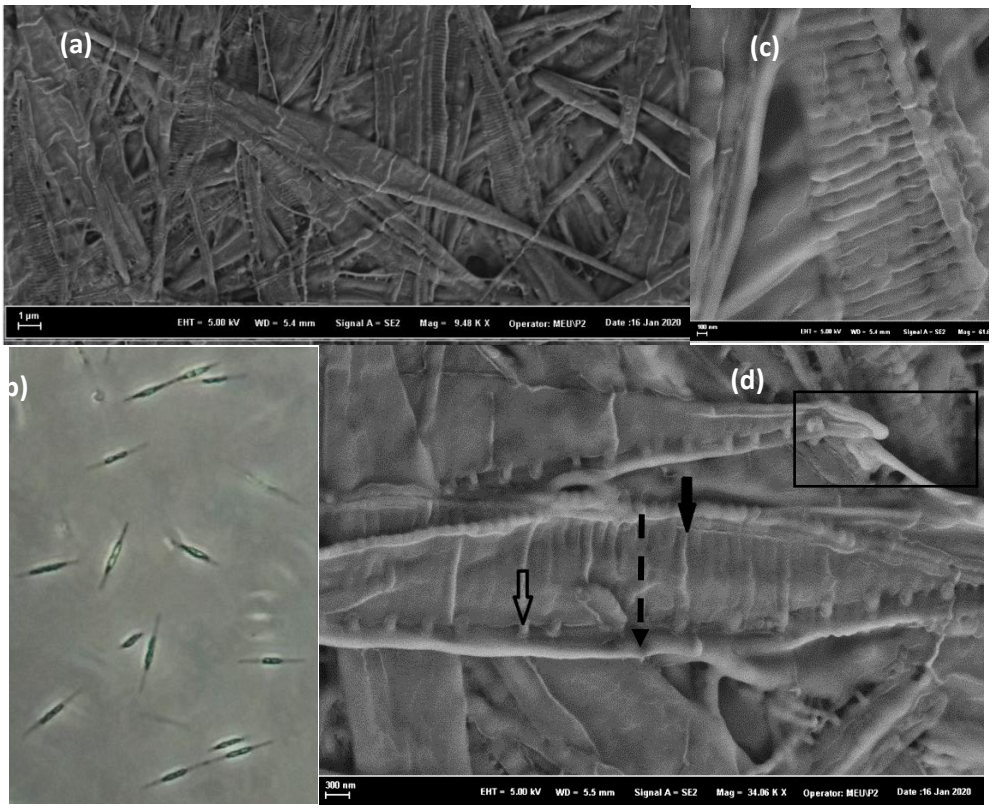

Figure 3. *Pseudo-nitzschia galaxiae* (a) a complete cell (b) light microscope view (c) striae (d) stria (filled arrow), fibula (empty arrow) and central interspace (dotted arrow), square shows the pointed tip.

Nitzschia navis-varingica Lundholm & Moestrup

Cells are singular with two chloroplasts and sometimes observed as doublets (Fig. 4a). They are 34-47 µm long, 4-6 µm wide, lanceolate and symmetrical in vava view and rectangular in girdle view with a slight depression in the central part. The pervalvar axis is broader than the transapical axis. There are 36 interstriae and 15 fibulae in 10 µm (Fig. 4b-d). Each stria is composed of one row of circular areolae (~5 areolae in 1 µm) (Fig. 4d). The raphe is eccentric, raised on a keel and divided by a central nodule (Fig. 4b).

Distribution: Vietnam, Malaysia, Australia, Indonesia, Philippines (Bates et al., 2018; Kotaki et al., 2004; Suriyanti and Usup, 2015)and recently in the Mediterranean Sea (Ayaz et al., 2018).

Toxicity: Both DA producing (0.04-15.3 pg DA cell^{-1}) (Kotaki et al., 2004, 2005) and not producing strains (Romero et al., 2012) have been reported.

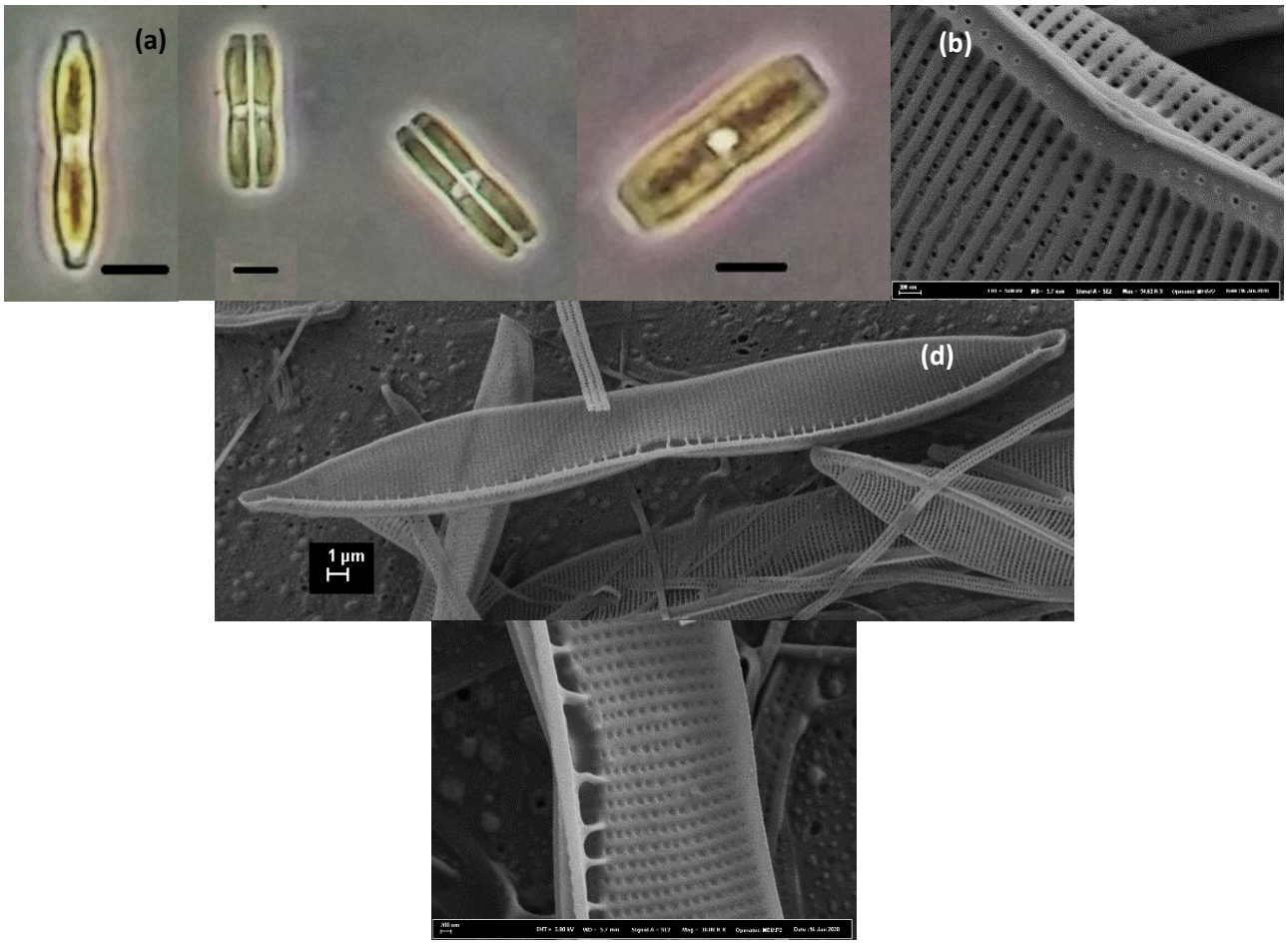

Figure 4. *Nitzschia navis-varingica* (a) light microscope images (scale is 10 µm) (b) outer view of the valve, central nodule (c) inner view of valve (d) circular areolae.

Cell concentrations and ELISA Screening for domoic acid

Cell concentrations of *Pseudo-nitzschia delicatissima*/ *P. arenysensis, P. galaxiae, P. brasiliana* and *Nitzschia navis-varingica* were 370 x 10^6 cells L^{-1}, 500 x 10^6 cells L^{-1}, 700 x 10^6 cells L^{-1} and 75 x 10^6 cells L^{-1}, respectively, on the 14th day of growth, when ELISA kit for domoic acid screening was applied. DA was not detected in any of the species.

Many *Pseudo-nitzschia* species and *N. Navis-varingica* have been observed to produce DA during the late-exponential phase and the highest cellular DA concentration has been detected during the early stationary phase in batch cultures (Bates et al., 2018; Kotaki et al., 2000). Our species were at the stationary phase of growth when DA screening test kits were applied. Initiation of the stationary phase was on the 9-10th day of growth.

According to EU regulatory decisions, DA analysis is performed systematically in France as soon as *Pseudo-nitzschia* spp. abundance exceeds 10^5 cells L^{-1} (Amzil et al., 2001). DA levels are also monitored in other European countries, USA, New Zeland and Australia (Jeffery et al., 2004).

High levels of DA were detected in shellfish tissue (240 µg DA g^{-1}) in the coastal waters of Ireland when *Pseudo-nitzschia* abundance was upto 230.000 cells L^{-1} in 2012 (ICES, 2013). In the present study, abundance values of species in batch cultures were far above than this concentration to be able to detect sufficient DA.

Molecular Analysis of isolated algae

The 18S sequences of the isolates MED_01, MED_02, and MED_03 were submitted to GenBank under accession numbers MW315998, MW315990, and MW316000, respectively. The 18S rDNA sequences of isolated algae were compared to the 18S rDNA sequencing identified in the GenBank database and BLAST. The results of the 18S rDNA analysis showed that the 1 isolate was *N. navis-varingica* and the others two were *Pseudo-nitzschia galaxiae* and *Pseudo-nitzschia arenysensis*, respectively.

CONCLUSION

Even though no toxin was detected in any of the species in the present study, increasing levels of environmental pollution and global warming may cause a change in the dominance of toxic strains in the future. These factors have recently been shown as a reason for harmful algal blooms (Kudela, 2019).

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➢ *ORAL PRESENTATION*

Kekik (*Thymus serpyllum*), adaçayı (*Salvia officinalis*) ve ceviz (*Juglans regia*) iç yaprağı ekstraktlarında total antioksidan-oksidan durum

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Özet

Tıbbi ve aromatik bitkiler geleneksel ve modern tıpta çok uzun zamandan beri kullanılmaktadır. Gerek hastalıkların önlenmesi, sağlığın sürdürülmesinde; gerekse hastalıkların iyileştirilmesinde kullanılan bitkiler çok sayıda çalışmaya konu olmuştur. Bitkilerin bu etkileri, yapılarında bulunan antioksidan bileşenlerle ilişkilendirilmektedir.

Bitkisel kaynaklı birçok gıdanın yapısında bulunan antioksidan içerik, gıdaları bozulmalara karşı korumakla beraber, tüketilmeleri sonucunda vücuda antioksidan madde kaynağı olmakta ve oksidatif hasara karşı vücut savunmasını güçlendirmektedir.

Antioksidan özellik gösteren birçok bitki ve baharat labiatae familyasına aittir. Bu familyaya ait türler; flavonoidler, terpenik bileşikler ve fenolik asitler içermesi nedeniyle önemli fizyolojik aktivitelere (antioksidan ve antimikrobiyel) sahiptir. Adaçayı (*Salvia officinalis*) ve kekik (*Thymus vulgaris*) bunlardan sadece ikisidir. Besin kalitesi çok yüksek olan ceviz (*Juglans regia L.*) meyve olarak tüketilmekte, bunun yanısıra yeşil kabuğu ve yaprakları ilaç/kozmetik sanayinde ve boyar madde olarak tekstil sanayinde kullanılmakta; farklı kısımlarının anti-kanserojenik, anti-mikrobiyal, antioksidan özellikleri nedeniyle geleneksel tıpta dikkat çekmektedir. Bu çalışmada; soksilet cihazı ile elde edilen kekik ve adaçayı ekstraktı ile atık madde kabul edilen ceviz iç yaprağı (cevizin ihmal edilen parçası; testa) ekstraktının total antioksidan/oksidan kapasitesi tayin edilmiş; ceviz iç yaprağının potansiyel durumu adaçayı ve kekik gibi iyi birer antioksidan olduğu bildirilen bitkilerle kıyaslanmıştır.

Canlı sistemlerde antioksidanlar etkileşim halindedirler ve genel olarak sinerjist çalışmaktadırlar. Bir antioksidandaki azalma diğerlerindeki artış ile dengelenmektedir. Total antioksidanların ölçümü, antioksidanların tek tek ölçümünden bazen daha değerli bilgiler verebilmektedir. Bu çalışmada, total antioksidan-oksidan kapasite (TAK-TOK) ölçümü pahalı olmayan, hassas, güvenilir ve hızlı sonuç veren bir metot olarak kabul edilen, Rel Assay Diagnostics (RL010/RL009; Türkiye) marka kit ile yapılmıştır.

Çalışmada; en yüksek antioksidan kapasite adaçayı ekstraktında tesbit edilmiş; ceviz iç yaprağı ekstraktının ise kekik ve adaçayına yakın antioksidan kapasiteye sahip olduğu belirlenmiştir. Diğer taraftan total oksidan değerlere bakıldığında, en az oksidan potansiyel adaçayı ekstraktında görülürken, kekik ve ceviz iç yaprağı ekstraktlarında ciddi farkla yüksek oksidan kapasite olduğu belirlenmiştir.

Anahtar Kelimeler: Kekik (*Thymus serpyllum*), Adaçayı (*Salvia officinalis*), Ceviz (*Juglans regia*) iç yaprağı, Antioksidan-Oksidan Kapasite

Total antioxidant-oxidant status in extracts of thyme (*Thymus serpyllum*), sage (*Salvia officinalis*) and internal septum of walnut (*Juglans regia*) kernel

Abstract

Medicinal and aromatic plants have been used in traditional and modern medicine for a long time. Plants used in the treatment of diseases have been the subject of many studies. These effects of plants are associated with the antioxidant components found in their structure.

Many herbs and spices with antioxidant properties belong to the labiatae family. The species belonging to this family have important physiological activities (antioxidant and antimicrobial) due to its content of flavonoids, terpenic compounds and phenolic acids. Sage (Salvia officinalis) and thyme (Thymus vulgaris) are just two of them. Walnut (Juglans regia L.), which has a very high nutritional value, is consumed as fruit. Besides, its green shell and leaves are used in the pharmaceutical/cosmetic industry and as a dye in the textile industry; it draws attention in traditional medicine due to the anti-carcinogenic, anti-microbial and antioxidant properties of its different parts. In this study; the total antioxidant/oxidant capacity of the thyme, sage and walnut internal

septum (a neglected part of walnut kernel; considered as a waste material) extract obtained by the soxilet device. Potential status of walnut internal septum was compared to herbs such as sage and thyme, which are reported to be good antioxidants.
Measurement of total antioxidants can sometimes provide more valuable information than measuring antioxidants one by one. In this study, TOS (RL009) and TAS (RL010) were evaluated photometrically in thyme, sage and walnut internal septum extract with available commercial assay kits (Rel Assay Diagnostics, Turkey).
In the study; the highest antioxidant capacity was determined in sage extract; walnut internal septum extract has an antioxidant capacity close to thyme and sage. When the total oxidant values were examined, the least oxidant potential was seen in sage extract, while there was a significantly higher oxidant capacity in thyme and walnut internal septum extract.
Keywords: Thyme (*Thymus serpyllum*), Sage (*Salvia officinalis*), Walnut (*Juglans regia*) internal septum, Antioxidant-Oxidant Capacity

GİRİŞ

Tıbbi ve aromatik bitkiler geleneksel ve modern tıpta çok uzun zamandan beri kullanılmaktadır. Gerek hastalıkların önlenmesi, sağlığın sürdürülmesinde; gerekse hastalıkların iyileştirilmesinde bir araç olarak kullanılan bitkiler çok sayıda çalışmaya konu olmuştur (Dağdelen ve ark., 2014). Son yıllarda hastalık etkenlerinin ve çeşitlerinin artmasıyla beraber, sentetik yapılı ilaçların yetersiz kalması ve yan etkilerinin saptanması doğal ürünlerin kullanımına olan meyli artırmıştır. Bu nedenle bitkilerin mikrobiyolojik ve farmakolojik özellikleri çok yönlü olarak araştırılmakta ve ilgi çekmektedir (Çoban ve Patır, 2010). Çeşitli bitki ekstraktları ve uçucu yağların bazı bakteri ve mantar türleri üzerine etkili olduğu bilinmekte; ancak daha kapsamlı araştırmalara ihtiyaç duyulmaktadır (Dağcı ve Dığrak, 2005).
Tıbbi bitkiler, günümüzde birçok hastalığa karşı kullanılabilen bileşimlerin doğal kaynağıdır. Birçok bitki, insanlar üzerinde önemli biyolojik etkisi olan geniş çeşitliliğe sahip kimyasal maddeler içerir. İlaç elde edilen bitkilere olan talep; düşük maliyetli olması, yan etkilerinin olmaması ve toksik etkilerinin az olması dolayısıyla hem gelişmiş hem de gelişmekte olan ülkelerde artış göstermektedir (Faydaoğlu ve Sürücüoğlu, 2013).
Antioksidan özellik gösteren birçok bitki ve baharat labiatae familyasına aittir. Labiatae familyasına ait cinsler özellikle terpenik bileşikleri (mono-, di-, triterpenler), flavonoid, fenolik asitleri içermesi nedeniyle önemli fizyolojik aktivitelere (antioksidan ve antimikrobiyel) sahip bitkileri içermektedir. Adaçayı (*Salvia officinalis*) ve kekik (*Thymus vulgaris*) bunlardan sadece ikisidir (Öğüt, 2014). Bitkinin yaprak, çiçek ve odunsu kısımlarında bulunan bu bileşikler; lipitlerin, karbonhidratların ve proteinlerin serbest radikallerce okside olmalarını engellemek amacıyla aromatik halkalarındaki hidroksil grubunda bulunan hidrojeni verebilmektedirler (Çoban ve Patır 2010; Öğüt 2014).
Adaçayı; diş otu veya Meryemiye olarak da bilinir. Ballıbabagiller (Lamiaceae) familyasından Salvia cinsini oluşturan kokulu bitkilere verilen addır. Ülkemizde de bol miktarda yetişir. Yapraklarının kurusu çay olarak tüketildiği gibi, et yemeklerine koku ve lezzet vermek için de kullanılır. Antioksidan etkiye sahip olan adaçayının yapısındaki en önemli etken madde fenolik bileşenlerdir (Çoban ve Patır, 2010). Ballıbabagiller (Lamiaceae) familyasından Thymus cinsine tâbi olan kekik bitkisi, toprak sıcaklığının fazla olduğu kayalık ve dağlık bölgelerde yetişir. Ülkemizde yaygın olarak kullanılan kekiğe de antioksidan özellik kazandıran fenolik bileşiklerdir. Bu maddeler, kekiğe kendine özgü kokusunu da verir (Çoban ve Patır 2010; Köksal ve ark., 2010). Ülkemiz için ekonomik açıdan önemli bir ürün olan Ceviz (Juglans regia L.) ise Juglandaceae ailesine aittir. Ceviz doğrudan/kurutularak tüketilebildiği gibi, reçel, pestil, ezme şekline getirilip kullanılabilen ve besin kalitesi çok yüksek olan bir üründür. Ayrıca, ceviz meyvesini saran yeşil kabuğunun ve yaprak kısımlarının ilaç/kozmetik sanayinde ve boyar madde olarak tekstil sanayinde kullanıldığı; yine anti-kanserojenik özelliği nedeniyle geleneksel tıpta kullanıldığı bildirilmektedir. Özellikle genç yeşil yapraklarda fazla miktarda bulunan juglon maddesinin çok güçlü antioksidan ve antimikrobiyal özelliğe sahip olduğu bildirilmektedir (Köksal ve ark., 2010; Yaman, 2012).
Hücrenin normal aktiviteleri ve/veya UV, sigara gibi ekzojen faktörler sonucunda oluşan serbest radikaller, canlı hücrelerinde antioksidan enzim sistemleri tarafından etkisiz hale getirilmektedir. Ancak vücudun mevcut antioksidan sistemi yetersiz kaldığında bu serbest radikaller hücre membranını, genetik materyalleri ve/veya enzimatik olayları etkileyerek hücre hasarına neden olabilmektedir. Oluşan bu oksidatif hasar kardiyovasküler hastalıklara, inflamasyona ve kanser oluşumuna kadar giden çeşitli hastalıklara yol açabilmektedir. Bu bakımdan dışarıdan yapılacak antioksidan desteğinin, oksidatif stresle baş etmede yardımcı olabileceği bildirilmektedir (Selvaraju ve ark., 2012). Dejeneretif hastalıklara yakalanma riskinin, antioksidanlarca zengin

gıdaların tüketilmesiyle azalacağına olan inanış, antioksidanlara yönelen ilgiyi artırmıştır. Antioksidanların yaşlılığı geciktirici; pek çok hastalığa karşı iyileştirici, önleyici ve tedavi edici rolleri olduğu çeşitli çalışmalarla ortaya konmuştur. Bu nedenle, gıdalarda ve biyolojik sistemlerde doğal olarak bulunan birçok molekülün antioksidan kapasitesinin çalışılması önem kazanmıştır (Okan ve ark., 2013).
Günümüzde oksidan ve antioksidanların ölçümünde kullanılan metodlar kalorimetrik, floresans ya da kemiluminens esaslı yöntemlerdir. Bu ölçümler zaman ve emek isteyen, pahalı, rutin kullanımda zorlayıcı ve kısıtlayıcı yöntemlerdir (Korcan 2013; Erel 2005; Erel 2004). Bitkisel kaynaklı antioksidanlar, serbest radikal gidericisi, peroksit parçalayıcısı, enzim inhibitörleri ve sinerjistler olarak fonksiyon görürler (Nizamlıoğlu ve Nas 2010). Bazen total antioksidanların ölçümü, antioksidanların tek tek ölçümünden daha değerli bilgiler verebilmektedir. Diğerlerinden farklı olarak tam otomatik kitlerle çalışılan TAK/TOK tayin yöntemi ucuz ve güvenilir bir şekilde ölçüm yapmayı mümkün kılmaktadır (Erel 2005; Erel 2004).
Türkiye, bitki örtüsünün zenginliği, değişik iklim ve coğrafi koşullara sahip olması dolayısıyla pek çok medeniyete ev sahipliği etmiştir. Bu durum ülkemizin zengin bir halk ilacı kültürüne sahip olmasını sağlamıştır. Nesiller boyu aktarılarak günümüze kadar gelen folklorik bilgilerin kaybolmaması ve halk ilacı olarak kullanılan bitkilerin bilimsel olarak değerlendirilmesi gerekmektedir (Ezer ve Avcı 2004).
Bu çalışmada; ceviz iç yaprağının antioksidan durumu, total antioksidan/oksidan kapasite tayini üzerinden araştırılmış, adaçayı ve kekik gibi iyi birer antioksidan olduğu bildirilen bitkilerle kıyaslanmıştır.

MATERYAL VE METOD

Materyal:

Kırşehir'e ait yerli ve taze mahsül olan cevizler Ağustos-Ekim 2017 tarihlerinde toplanıp kurutulmuştur. Bir aylık depolama sonrası, tüm cevizlerin kabuğu çıkartılıp ceviz iç yaprakları meyveden ayrılmıştır. Araştırmaya konu olan ceviz iç yaprakları oda şartlarında güneş görmeyecek şekilde ince bir tabaka halinde serilerek kurutulmuştur. Kurutma sonrası < 4 ^{0}C de hava geçirgen özel bir ambalajda saklanmıştır. Ceviz iç yaprakları ve piyasadan temin edilen yine 2017 üretim tarihli kekik ve adaçayı; önce havanda sonra elektrikli öğütücü kullanılarak toz haline getirilmiştir.

Kekik, Adaçayı ve Ceviz İç Yaprağının Ekstraksiyon Prosedürü:

Örnek özütlerinin eldesinde Şengün 2018' de yer alan ekstraksiyon metodu modifiye edilerek kullanılmıştır. Toz haline getirilmiş materyaller 20'şer gramlık kartuşlara ayrılmış ve etanol (%95, v/v, Merck) ile soksilet ekstraktör (BUCHI Ekstraksiyon Sistemi Model B811) kullanılarak 50^{0}C'de yaklaşık 6 saat ekstrakte edilmiştir. Etanol ekstraktları önce kaba filtre, sonra da Whatman 4 filtre kâğıdından geçirilerek katı partiküllerinden ayrılmıştır. Satrifüj işlemi sonrası süpernatantlar alınarak rotary evaporatörde (BUCHI Rotavapor Model R-144) 40^{0}C'de ve basınç altında yoğunlaştırılmıştır. Etanol uzaklaşana kadar bekletilen ekstraktlar kullanılıncaya kadar +4^{0}C'de, ışık ve hava almayacak şekilde saklanmıştır. Her bir numune çift çalışılarak ortalama değer alınmıştır.

Total Antioksidan/Oksidan Kapasite (TAK/TOK) tayini:

Total Oksidan Kapasite (TOK):

Adaçayı, kekik ve ceviz iç yaprağı ekstratlarının TOK değeri, Rel Assay Diagnostics (RL009; Gaziantep-Türkiye) marka kit ile ölçülmüştür.
Ölçümün prensibi, örnekte bulunan oksidanların ferröz iyon-orto-dianisidin kompleksini ferrik iyona oksitlemesi ve ferrik iyonun asidik ortamda kromojen ile mavi-yeşil renkli kompleks oluşturması esasına dayanmaktadır (Erel 2005). Örnekte bulunan toplam oksidan miktarı ile orantılı olan kompleksin renk şiddeti, spektrofotometrik olarak 530 nm dalga boyunda ölçülmektedir.
Yöntemin kalibrasyonunda hidrojen peroksit çözeltisi, standart olarak kullanılmaktadır.

Çözeltiler:

- Çalışma tamponu (Reaktif 1)
- Prokromojen çözeltisi (Reaktif 2)
- Stok standart (800 mM H_2O_2 ekivalanı/L) çözeltisi
- Çalışma standart (20.0 µmol H_2O_2 ekivalanı/L) çözeltisi

Çalışma:

	Örnek	**Standart**
Çalışma standardı	-	75 µL
Örnek	75 µL	-
Çalışma tamponu	500 µL	500 µL
• Tüplerin OD_1 değerleri, 530 nm dalga boyunda okundu.		
Prokromojen çözeltisi	25 µL	25 µL

Standart ve numune tüpünün OD_2 değerleri, 37°C'de 5 dk inkübasyonu takiben 530 nm dalga boyunda okunmuştur.

Formül:

$$\mu mol\ H_2O_2\ ekivalanı/\ L = \frac{\Delta OD\ örnek\ x\ 10}{\Delta OD\ standart} \qquad (1)$$

Adaçayı, kekik ve ceviz iç yaprağı ekstratlarının TOK değerleri (1) formülü ile hesaplanmış; litre başına mikromol hidrojen peroksit ekivalanı olarak verilmiştir (µmol H_2O_2 ekivalanı/L).

Total Antioksidan Kapasite (TAK):

Adaçayı, kekik ve ceviz iç yaprağı ekstratlarının TAK ölçümü, Rel Assay Diagnostics (RL010; Gaziantep-Türkiye) marka kit kullanılarak yapılmıştır.

Ölçümün prensibi, örnekte bulunan antioksidanlar tarafından, koyu mavi-yeşil renkli 2,2'-azinobis 3-etil benzotiazolin-6-sülfonat radikalinin renksiz ABTS formuna redüklenmesi esasına dayanmaktadır (Erel 2004). Antioksidanların miktarlarına ve kapasitelerine bağlı olan ABTS redüksiyonunun derecesi, spektrofotometrede 660 nm dalga boyunda renk farkından kaynaklanan absorbans değişikliği ile tayin edilmektedir.

Yöntem, "Trolox ekivalanı" olarak adlandırılan ve vitamin E analoğu olan stabil bir antioksidan standart çözelti ile kalibre edilmektedir.

Çözeltiler:

- Çalışma tamponu (Reaktif 1)
- Renkli ABTS radikal solüsyonu (Reaktif 2)
- Standart 1 (0.0 mmol Trolox Ekivalanı/L)
- Standart 2 (1.0 mmol Trolox Ekivalanı/L)

Çalışma:

	Örnek	**Standart 1**	**Standart 2**
Çalışma tamponu	500 µL	500 µL	500 µL
Standart 1	-	30 µL	-
Standart 2	-	-	30 µL
Numune	30 µL	-	-
• Tüplerin OD_1 değerleri, 660 nm dalga boyunda okundu.			
ABTS radikal solüsyonu	75 µL	75 µL	75 µL

Standart ve örnek tüplerinin OD_2 değerleri, 37°C'de 5 dk inkübasyonu takiben, 660 nm dalga boyunda okunmuştur.

Adaçayı, kekik ve ceviz iç yaprağı ekstratlarının TAK değerleri (2) formülü ile hesaplanmış; TAK değeri, litre başına milimol Trolox ekivalanı olarak verilmiştir (mmol Trolox ekivalanı/L).

Formül:

$$mmol\ Trolox\ Ekivalanı/L = \frac{\Delta OD\ standart\ 1 - \Delta OD\ örnek}{\Delta OD\ standart\ 1 - \Delta OD\ standart\ 2} x\ Standart\ 2\ konsantrasyonu \qquad (2)$$

BULGULAR ve TARTIŞMA

Ceviz sadece yüksek besin değeri nedeniyle değil, aynı zamanda sağlığa olan birçok faydası nedeniyle de her zaman büyük ilgi görmüştür. Çeşitli klinik çalışmalar, ceviz meyvesinin yüksek antioksidan aktivite (Haddad ve ark 2014), antiinflamatuvar potansiyel (Hayes ve ark 2016, Acquaviva ve ark 2019), glikoz ve lipidi düşürme etkisi (Bamberger ve ark 2017), antimikrobiyal ve anti-aterojenik etkiler (Acquaviva ve ark 2019) ve antidepresan etkiler (Arab ve ark 2019) ortaya çıkardığını göstermiştir.

Ceviz ağacı uzun bir tıbbi kullanım geçmişine sahip olduğundan, yeşil ceviz, çiçek, ağaç kabuğu, kabuk ve yapraklar gibi diğer kısımları farklı geleneksel tıp sistemlerinde kullanılmıştır (Ebrahimi ve ark 2019). Bazı araştırmalar ceviz parçalarının biyolojik aktivitelerinin belirli yönlerini araştırmıştır. Örneğin, ceviz çiçeğinin metanolik ekstresi kayda değer antihipoksik, antiinflamatuvar, antioksidan ve antidepresan aktiviteler göstermiştir (Nabavi ve ark 2011). Farklı çözücüler kullanılarak hazırlanmış ceviz ağacının kabuk ekstresi çeşitli bakterilere karşı antimikrobiyal aktivite göstermiştir (Bakht ve ark 2017; Zakavi ve ark 2013). Ayrıca ceviz kabukları, antioksidan, antimikrobiyal ve bitki büyümesini uyarıcı özelliklere sahip piroligneöz asit için önemli bir biyo-kaynak olarak kabul edilmiştir (Jahanban-Esfahlan ve Amarowicz 2018). Çeşitli deneysel ve klinik çalışmalar, diyabetik hayvanlarda veya hastalarda ceviz yapraklarını değerlendirmiştir (Asgary ve ark 2008; Hosseini 2014). Ceviz yapraklarının seçici olarak Gram pozitif bakterilerin büyümesini engellediğini gösteren kanıtlar vardır (Pereira ve ark 2007).

Ceviz ağacının farklı kısımlarının bileşimi ve biyolojik aktiviteleri birçok çalışmada araştırılmış olsa da, cevizin iç yaprağı daha az değerlendirilmiştir. Yapılan bazı çalışmalar ceviz iç yaprağının fitokimyasal ve farmakolojik yönlerini araştırmış; elde edilen bulgular ceviz iç yaprağının işe yaramaz bir atıl ürün değil, değerli özelliklere sahip doğal bir bitkisel materyal olabileceğini ortaya koymuştur. Bir çalışmada ceviz iç yaprağının kimyasal bileşimi değerlendirilerek; lipitler, düşük moleküler ağırlıklı fenolik karboksilik asitler, fenolik aldehitler, kateşinler, proantosiyanidinler, oligomerik ve polimerik fenolik maddeler gibi çeşitli bileşikleri içeren zengin bir hammadde olduğu bildirilmiştir (Bezhuashivili ve Kurashvili 1998). Son zamanlarda ceviz iç yaprağının fenolik bileşimi ve besin içeriğinin araştırıldığı bir makale yayınlanmıştır (Hu ve ark 2019). Bahsi geçen araştırmaların bulguları, ceviz iç yaprağının fitokimyasal profili hakkında ciddi düzeyde bilgi vermekte; olası farmakolojik aktivitelerinin derinlemesine incelenmesini gerekli kılmaktadır. Son yıllara ait deneysel bir çalışmada ceviz iç yaprağından izole edilen suda çözünür polisakkarit fraksiyonunun, iki Gram-negatif (*Escherichia coli* ve *Pseudomonas aeruginosa*) ve ayrıca iki Gram-pozitif suşa (*Staphylococcus aureus* ve *Listeria monocytogenes*) karşı doza bağlı (0.2-1.2 mg/mL) önemli bir antibakteriyel aktivite sergilediği bulunmuştur (Meng ve ark 2017). Yine yakın tarihli bir çalışmada, ceviz iç yaprağından elde edilen polisakkaritlerin anti-proliferatif etkisi MTT testi ile, insan hepatosellüler karsinoma hücre hattı (HepG-2) ve insan mide karsinoma hücre hattı (BGC-823) üzerinde araştırılmıştır. Bu araştırmaya göre polisakkaritler, HepG-2 ve BGC-823 hücre hatlarının çoğalmasını doza bağlı bir şekilde (8-500 µg / mL) belirgin olarak bastırmış ve maksimum konsantrasyonda antitümör aktivitesi HepG-2 ve BGC-823 hücre hatlarında sırasıyla % 67.39 ve % 61.25 olarak bulunmuştur (Meng ve ark 2018). Tıbbi bitkilerin, en çok araştırılan özelliklerinden biri de hipoglisemik potansiyelidir. Deneysel bir çalışmada, diyabetik farelere 4 hafta oral uygulanan dört farklı doz ceviz iç yaprağı su ekstresi (200-800 mg / kg) glikoz seviyelerini sadece maksimum dozda düşürebilmiştir (Dehghani ve ark 2012). Başka bir çalışmada ceviz iç yaprağı su ekstresinin (200-400 mg/kg) 15 günlük oral uygulamasının kan glikoz seviyelerini iyileştirebileceği ve ayrıca diyabetik farelerde hepatik hasarın ilerlemesini inhibe edebileceği bulunmuştur (Zangeneh ve ark 2018). Ceviz iç yaprağı etanol ekstresinin daha önemli hipoglisemik potansiyel sergilediğini bildiren çalışmalar da mevcuttur (Hajikhani ve Solati 2010).

Pek çok endojen ve eksojen süreç serbest radikaller üretir. Normal koşullarda, reaktif oksijen/nitrojen türleri antioksidan savunma sistemleri tarafından nötralize edilir. Radikal oluşumu ile antioksidan savunma sistemi arasındaki denge oksidanlar lehine bozulduğunda oksidatif hasar meydana gelir. Oksidatif hasar, kardiyovasküler hastalıklar, çeşitli kanser türleri gibi birçok kronik hastalığın patogenezinde rol oynar (Liguori ve ark 2018). Antioksidan maddelerin en önemli kaynağı bitkisel gıdalardır. Bundan dolayı diyetle alınan antioksidanlar genellikle fitokimyasal antioksidanlar olarak adlandırılmaktadır (Güleşci ve Aygül 2016). Antioksidan bakımından zengin gıda veya takviyelerin hastalıkların önlenmesinde yararlı etkileri olup olmadığı konusunda tartışmalar vardır, ancak çok sayıda deneysel çalışma, antioksidan aktivite yoluyla birçok doğal ürünün birçok hastalığın ilerlemesini ve komplikasyonlarını inhibe edebileceğini göstermiştir (Kiani ve ark 2018; Fard ve ark 2015). Bitki çaylarının birçok akut ve kronik hastalıkların gelişme riskini azaltmaya yardımcı olabileceği bildirilmekte; çayların bu etkileri yapılarında bulunan antioksidan bileşenlerle ilişkilendirilmektedir (Alok ve ark 2014). Bir çalışma kekik ve adaçayı gibi tıbbi ve aromatik bitkilerin antimikrobiyal ve antioksidan aktiviteleri hakkında derlenmiş bilgiler vermektedir (Faydaoğlu ve Sürücüoğlu 2013).

Bir araştırmada, ABST (2,2′-azino-bis-3-etilbenztiazolin-6-sülfonik asit), DPPH ve FRAP yöntemleri kullanılarak ceviz iç yaprağı ekstresinin antioksidan aktivitesi değerlendirilmiş; antioksidan aktivite ABST, DPPH ve FRAP yöntemlerinde sırasıyla, 174,28 ± 9,68 mg troloks eşdeğeri (TE)/g-ceviz iç yaprağı kuru ağırlığı, 255,89 mg TE/ g-ceviz iç yaprağı kuru ağırlığı ve 400,97 mg TE/ g-ceviz iç yaprağı kuru ağırlığı şeklinde bildirilmiştir (Rusu ve ark 2018). Başka bir çalışmada ceviz iç yaprağından elde edilen polisakkaritlerdeki radikal temizleme aktivitesinin; DPPH, ABST, hidroksil radikali süpürme ve FRAP yöntemlerinde doza bağlı bir artış gösterdiği bildirilmiştir (Meng ve ark 2017). Ceviz iç yaprağının nitrik oksit üretimini inhibe ederek anti-inflamatuvar aktivite oluşturduğu da elde edilen bulgular arasındadır (Wang ve ark 2017).

Antioksidan bileşiklerin uygulamalarından biri de gıda üretimi ve depolama sürecinde meydana gelen oksidatif hasara karşı koruyucu rolüdür. Bu bağlamda sentetik antioksidanların kullanılması gıda endüstrilerinde yaygındır. Ancak günümüzde toksisiteleri nedeniyle kullanımları sınırlı kalmış, dolayısıyla doğal antioksidan arayışı artmıştır (Nadeem ve ark 2015). Bir çalışmada ceviz iç yaprağı etanol ekstresinin geleneksel tereyağının raf ömrü üzerine antimikrobiyal ve antioksidan etkileri değerlendirilmiş; ceviz iç yaprağının tereyağında kullanılabilecek mükemmel bir doğal antimikrobiyal ve antioksidan ajan olabileceği sonucuna varılmıştır (Mehdizadeh ve ark 2019). Antioksidan aktiviteyi ifade etmenin farklı yolları ve farklı yöntemlerinin uygulanması, diğer taraftan farklı ekstraksiyon yöntemleriyle hazırlanan farklı ekstrelerin kullanılıyor olmasından dolayı bu değerleri karşılaştırmak imkansızdır. Ancak genel olarak hepsi ceviz iç yaprağının yüksek antioksidan aktiviteye sahip olduğunu göstermektedir.

Bu çalışmada adaçayı, kekik ve ceviz iç yaprağı ekstraktlarının TOK/TAK ölçümü, güvenilir ve hassas bir yöntem olan Rel Assay Diagnostics marka kit kullanılarak yapılmıştır. TAK; örneklerdeki vitaminleri, enzimatik radikal yakalayıcı sistemleri, bilinmeyen antioksidanları ve her çeşit antioksidan etkileşimlerini kapsayan, bütün antioksidanların ölçümüdür. Farklı antioksidanların ayrı ayrı ölçülmesi olası aditif etkileri göstermede yetersiz kalabilmekte; zaman, emek ve maliyet gerektirebilmektedir. Bu durumlarda total antioksidanların ölçümü, antioksidanların tek tek ölçümünden daha değerli bilgiler verebilmektedir (Saril ve ark 2012). Literatürde bu yöntemle, anne sütünde (Saril ve ark 2012), kan ve doku örneklerinde (Akcılar ve ark 2015) total antioksidan/oksidan kapasite tayin eden çalışmalar mevcuttur. 2014 yılına ait bir çalışmada, Türkiye'de ticari olarak satılan 9 farklı karışık veya saf bitki çayı örneği incelenmiş, bal ilavesinin antioksidan aktiviteyi önemli ölçüde artırdığı tespit edilmiştir (Özdatlı ve ark 2014). Bu çalışmadaki ölçümler sonucunda, adaçayı, kekik ve ceviz iç yaprağı etanol ekstrelerinin TOK değerleri sırasıyla 1,80±3,21 ; 5,48±2,87 ; 7,64±1,69 μmol H_2O_2 ekivalanı/L olarak; TAK değerleri ise yine aynı sırayla 4,54±2,17 ; 4,44±3,16 ; 3,88±1,77 mmol Trolox ekivalanı/L olarak belirlenmiştir.

SONUÇ

Bu çalışmada; en yüksek antioksidan kapasite adaçayı ekstraktında tesbit edilmiş; ceviz iç yaprağı ekstraktının ise kekik ve adaçayına yakın antioksidan kapasiteye sahip olduğu belirlenmiştir. Diğer taraftan total oksidan değerlere bakıldığında, en az oksidan potansiyel adaçayı ekstraktında görülürken, kekik ve ceviz iç yaprağı ekstraktlarında ciddi farkla yüksek oksidan kapasite olduğu belirlenmiştir.

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➢ *ORAL PRESENTATION*

Purification of Biobased Ally Acrylates Via Reactive Extraction

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Abstract
Allyl acrylates are polymerizable monomers that contain two double bonds with different reactivities. They are versatile materials and can be used for wide variety of applications. Synthesis, characterization and purification of soybean oil based ally acrylate is demonstrated in this study. Soybean oil was dried and brominated at allylic positions at the first step of the synthesis. The reaction of allylicaly brominated soybean oil (ABSO) with acrylic acid in polar solvent gives acrylate ester of soybean oil at high yields. The main problem after the synthesis was the removal of the acrylic acid from the medium. For this purpose, crude product was extracted with deionized water. When considering the environmental impact, unreacted acrylic acid should be recovered even from this aqueous broths. Reactive extraction appears to be one of the best way acrylic acid recovery. For this purpose methyl tertiary butyl ether can be used as an industrially acceptable and cheap extractant.

Keywords: Allyl esters, Allyl bromides, Plant oil triglycerides, Bio-based materials.

INTRODUCTION

Allylic acrylates are versatile compounds. They include two types of carbon-carbon double bond. Because of the different reactivity rates (Rodrigo 2007). Allyl acrylate monomers can be used as photosensitive materials. The presence of a photo or thermo reactive groups in polymers has given new insights to polymer science. When counting of conservation of natural resources, energy, environmental preservation, and productivity, photopolymerization techniques have considerable advantages over thermal curing techniques. Therefore, photosensitive polymerization techniques can find applications in many fields, such as photo curable coatings, photo recorders, photolithography, microelectronics, energy exchange materials, liquid crystalline display, integrated circuit and printing technologies (Gissot 2005 D'aleio 1967, Coessens 2001 and Gaylord 1956.).

Allyl acrylate and allyl methacrylate are two of the most widely used polymerizable allyl esters. Alternative to these petroleum based monomers, one can also obtain more green ones from plant oil triglycerides. Plant oil triglycerides are desirable materials due to their complex nature, purity and renewability. There are 4 reactive centers may be presented in a plant oil triglycerides. These are allylic positions, α- methylene groups to carbonyl, double bonds, and ester groups (Cavusoglu 2015, Sahin 2016 and Cayli 2010a 2010b, and 2011).

In this study, modification of the plant oil triglycerides and their methyl esters at allylic positions was introduced. By using of NBS (N-bromo succinimide), allylic positions are converted to reactive bromides. When these allylic bromides are reacted with anions of acrylic acid, corresponding allyl esters are obtained (Cayli 2008 and Winkler 2014)

MATERIALS AND METHODS

Soybean oil was food grade and it was dried in vacuum oven before use. NBS (N-bromo succinimide), KOH, acrylic acid, tributylamine, tertiary butyl methyl ether, NaOH and phenolphthalein purchased from Merck and they were used as received. IR characterization of compounds was performed by Perkin-Elmer FT-IR 1600 series spectrometer using KBr windows. The ^{1}H NMR were recorded on a Varian 400-MHz NMR instrument (Varian Associates, Palo Alto, CA) operating at a frequency of 399.986 MHz for proton. The spectra were recorded as ppm (δ) with $CDCl_3$ as a solvent.

Synthesis of Acrylated Soybean Oil: Acrylic acid (20 g, 0.277 mol) was dissolved in 50 ml distilled water. KOH (3 g, 0.0535 mol) was dissolved in 50 ml of water. Then this solution was added to acrylic acid solution. The resulted solution contained 0.0535 mol K acrylate. To this clear solution, 15 g of allylicaly brominated soybean oil (ABSO) was added. The mixture was stirred 24 hours at 60 °C. The crude product was washed with water several times then washed with $NaHCO_3$ until no bubbling was observed. The crude product was used without any purification. Approximately 95 % yield was obtained.

Extraction of Acrylic Acid: The extractions of acrylic acid from aqueous solutions were performed in a closed 100 mL erlenmeyer flask. Both organic and aqueous phases were introduced in flasks. The concentrations of tributylamine in organic phase was changed form 0 molal to 0,8 molal in each trial. After the introduction of both phases, the erlenmeyer flasks were stirred over a magnetic stirrer equipped with a constant temperature bath at 25 ˚C for 4 hours to ensure equilibrium. After stirring, the erlenmeyer flasks were transferred into trays, and a settling time for definite phase separation. After settling, samples of the aqueous phase were taken. The concentration of acrylic acid in the aqueous phase was determined by 0.1 molal sodium hydroxide solution. Phenolphthalein was used as an indicator.

Table 12. The Results of The Acrylic Acid Extraction with Tributyl Amine in TBME

Diluent	Concentration of TBA in TBME (mol/kg)	Concentration of Acid in Aqueous Phase (mol/kg)	Concentration of Acid in TBME Phase (mol/kg)	Extraction Efficiency (%)
TBME	0,2	0,3	1,2	80
	0,4	0,12	1,38	92
	0,6	0,1	1,4	93,33
	0,8	0,07	1,43	95,33
	1,0	0,06	1,46	96

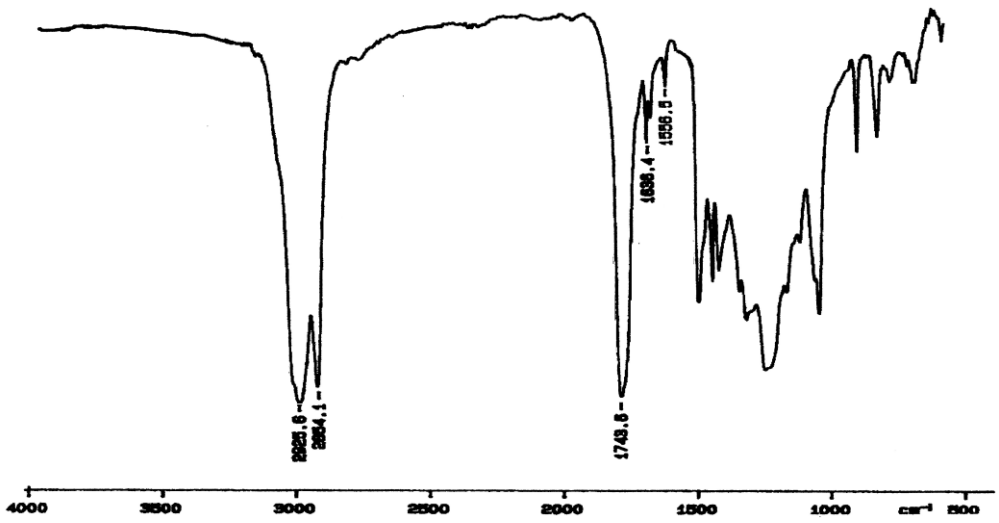

Figure 1. IR Spectrum of Acrylated Soybean Oil

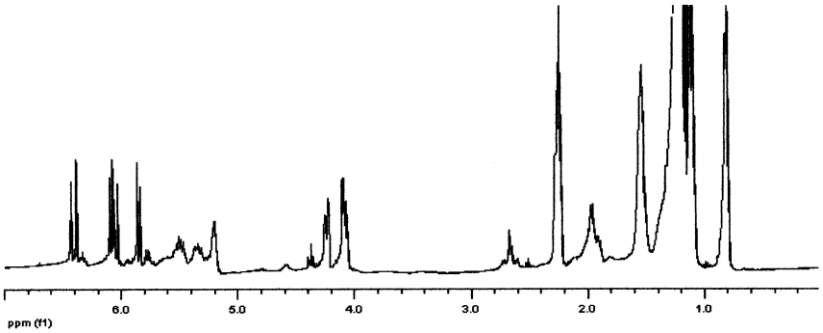

Figure 2. ^{1}H NMR Spectrum of Acrylated Soybean Oil

RESULTS and DISCUSSION

Characterization of the monomers: The product was characterized by IR and ^{1}H NMR techniques. In the IR spectrum acrylated soybean oil, peak at 1725 cm^{-1} was observed as a shoulder near 1743 cm^{-1} peak due to the presence of acrylate esters. Peaks at 1660-1630are getting stronger due to C=C stretching vibration of acrylate groups. A new peak was also observed at 1092 cm^{-1} due to the C-O stretching new ester groups (Figure 1).

In the ^{1}HNMR spectrum, the unique peaks of the acrylate groups was observed at 6.2, 6.0, 5.8 ppm. Moreover new peaks were observed at 5.2 and 5.4 ppm due to the single protons of allylic ester (Figure 2). The yield was calculated just measuring the ratio between one of the acrylate protons and protons of alpha methylene group. This measurement revealed that one triglyceride molecule can contain 2.5 acrylate groups.

Extraction of Acrylic Acid: Acrylic acid is a valuable material for polymer industry. It was synthesized via oxidation of propene. Due to the environmental concern, studies on the acrylic acid synthesis via fermentation process still continues. After polymerization reaction or fermentation, separation of acrylic acid from the medium is important. Reactive extraction is one of the most promising techniques. Extraction is a separation technique that used for removing of a substance from a matrix. In a reactive extraction, a reactive compound is added to extractant liquid by this way almost all of the substance can be collected from medium.

In this work, TBME was used an efficient and readily accessible extractant. Even at 0.2 molal tributylamine contained TBME could remove 80 % acrylic acid from the solution. The highest removal was observed at 1 molal tributylamine concentration as 96%. The optimal concentration of tributylamine was determined as 0.8 molal (Table 1)

CONCLUSION

In this study, an easy and efficient method for the synthesizing of plant oil triglyceride based allyl acrylate was demonstrated. It can be used as a co monomer with many conventional monomer. After synthesis, excess acrylic acid was successfully removed by using tributylamine and TBME system. The low boiling point of TBME is also beneficial for the separation of acrylic acid from organic layer. That process could be useable for industry and this subject is under consideration.

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➢ *ORAL PRESENTATION*

Synthesis and Characterization of Diisothiocyanate Derivative of Isobutylene

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Abstract
Isobutylene is a four carbon branched alkene. The polymers of isobutylene is widely used for many applications. Butyl rubber is a type of isobutylene polymer. Especially, applications of this polymer in the Biomedical Industry is getting attentions. Synthetic heart valves and veins can be made of butyl rubber. These materials are durable and inert to body fluids. In this work, modification of isobutylene was shown. For this purpose, 3-chloro-2-chloro methyl-1-propen was reacted with ammonium thiocyanate. Isothiocyanate and thiocyanate mixture was obtained first. After 24 hour reflux, all thiocyanates turned to isothiocyanate derivatives via 1,3 sigmatropic shift. FTIR, ^{1}H NMR method was used to characterize the synthesized material.
Keywords: isobutylene, allylic dichloride, rodanide, sigmatropic rearrangement, diisothiocyanate of isobutylene.

INTRODUCTION

Isobutylene is an unsaturated, odorless and colorless hydrocarbon. It is the main component of butyl rubber. Butyl rubber is a rubbery material and it was the first rubber to be synthesized. Butyl rubber is produced commercially by combining isobutylene and isoprene. It has good shock absorption characteristics and low moisture and gas permeability and is used in many commercial applications. Butyl rubber also used for some bio medical applications such as heart valves and veins (Formela 2014).

If high biocompatible isobutylene rubber is synthesized, modification of the isobutylene monomer or polymer is necessary. Unfortunately inertness of the monomer and polymer makes that task not easy. In this work a simple and versatile method is demonstrated for the synthesis of diisothiocyanate derivative of isobutylene. For that purpose a special compound first modified to thiocyanate derivative and then by refluxing of that compound, 1,3 sigmatropic shift occurred (Cayli, 2008, DeWolfe 1956, Emergon 1971, Krueger 1941) (Figure 1) and diisothiocyanate derivative synthesized. According to the best of our knowledge, that work was the first report of the synthesis of 3-isothiocyanato-2-isothiocyanatomethyl-1-propene

MATERIALS AND METHODS

Acetone, ammonium thiocyanate (NH_4SCN), dichloromethane (CH_2Cl_2), 3-chloro-2-chloromethyl-1-propene, and tetrahydrofurane (THF) were purchased from Merck and they were used as received. IR characterization of compounds was performed by Perkin-Elmer FT-IR 1600 series spectrometer using KBr windows. The ^{1}H NMR were recorded on a Varian 400-MHz NMR instrument (Varian Associates, Palo Alto, CA) operating at a frequency of 399.986 MHz for proton. The spectra were recorded as ppm (δ) with $CDCl_3$ as a solvent.

Synthesis of 3-thiocyanato-2-thiocyanatomethyl-1-propene: In a 100 ml round bottom flask, 1.52 g of NH_4SCN was dissolved in 50 ml of THF. After all ammonium salt was dissolved, a solution of 1.24 g of 3-chloro-2-chloromethyl-1-propene in 25 ml of THF was added. The blurred mixture was stirred for 6 hour. After filtration, this mixture was refluxed for 24 hours. The solvent of the mixture then was removed by rotary evaporator. The crude product is only composed of 3-thiocyanato-2-thiocyanatomethyl-1-propene.

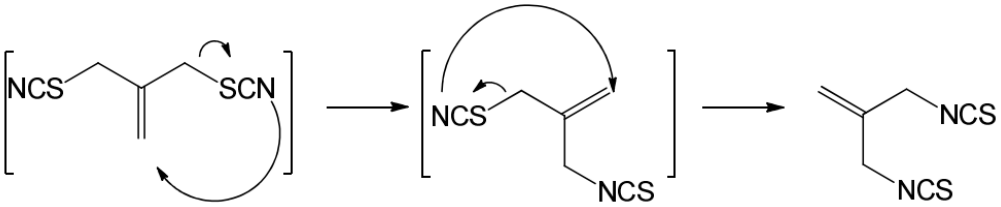

Figure 14. 1,3 sigmatropic shift of 3-thiocyanato-2-thiocyanatomethyl-1-propene

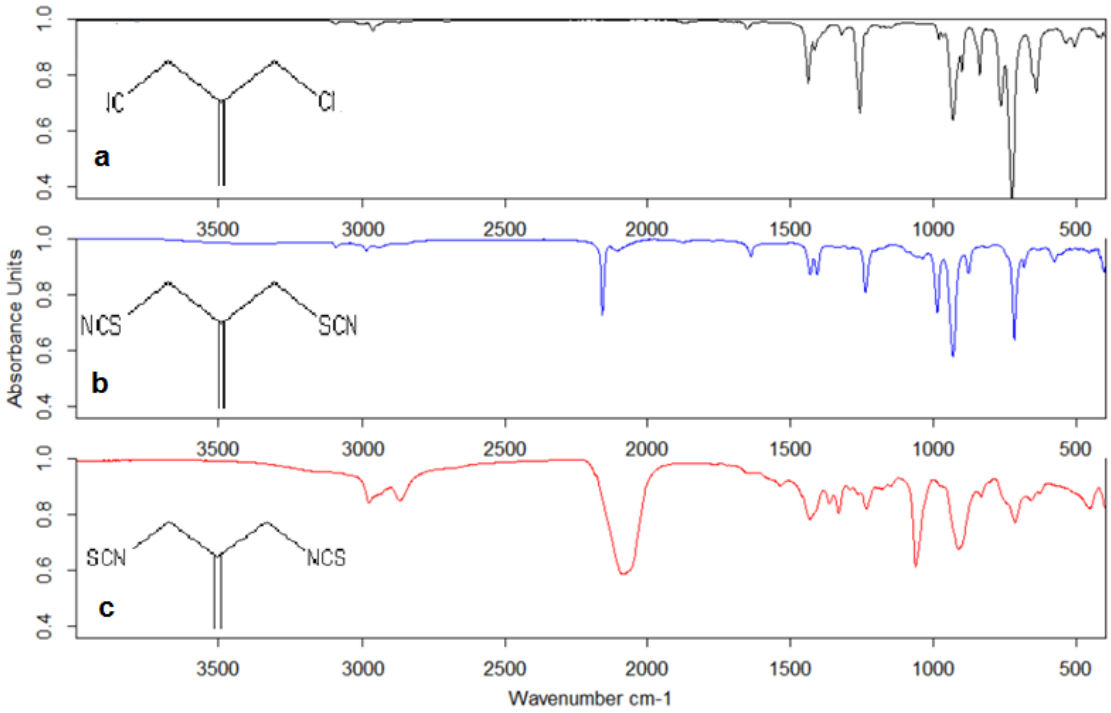

Figure 2. FTIR Spectrum of **a**- 3-chloro-2-chloromethyl-1-propene, **b**- 3-thiocyanato-2-thiocyanatomethyl-1-propene and **c**- 3-isothiocyanato-2-isothiocyanatomethyl-1-propene

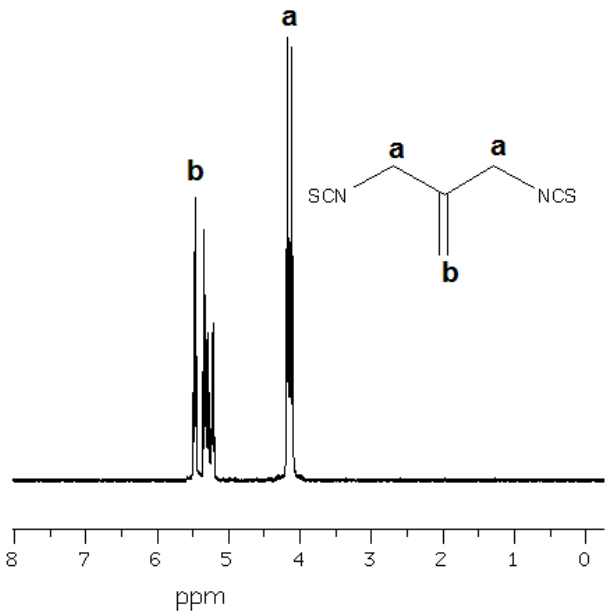

Figure 3. [1]H NMR spectrum of 3-isothiocyanato-2-isothiocyanatomethyl-1-propene

RESULTS and DISCUSSION

Characterization of 3-isothiocyanato-2-isothiocyanatomethyl-1-propene: In this study, three type of solvent was used for the synthesis. Among them the best results was obtained when THF was used. During the reaction, equimolar precipitate of NH_4Cl was observed when THF was used. Probably due to the solubility of the NH_4Cl, good results was not observed in acetone or dichloromethane.

When the precipitation completed. The reaction mixture was filtered. When the solvent of the filtrate was removed an oily material was obtained. After refluxing of the oily material in THF for 24 hours a yellowish compound was observed.

Characterization of the material synthesized, were performed by IR and ^1H NMR techniques. In the IR spectrum of 3-chloro-2-chloromethyl-1-propene, a peak at 770 cm^{-1} was observed because of the C-Cl bond. A peak at 1460 cm^{-1} indicated the presence of double bond. When thiocyanate derivative was synthesized a 2 new peak was determined at 2084 and 2154 cm^{-1} due to the presence of –SCN and -NCS group. The intensity of the peak at 2084 so small and this also showed that the amount of thiocyanate group is minor. When that compound was refluxed the situation changed and the peak at 2084 cm-1 was the only broad peak and that also proved that the only derivative was of 3-isothiocyanato-2-isothiocyanatomethyl-1-propene (Figure 2).

In the ^{1}HNMR spectrum of the final product, two peaks was observed. The peak at 4.0 ppm indicate the presence of α methylene hydrogens to –NCS groups. The hydrogens of the double bond give a multiple between 5.0 and 5.5 ppm (Figure 3).

CONCLUSION

By this work an easy and safe method for the synthesis of a derivative of isobutylene was demonstrated. At the first step of the synthesis, 3-chloro-2-chloromethyl-1-propene was reacted with ammonium thiocyanate to give thiocyanated derivative. When this derivative was refluxed thiocyanate derivative of isobutylene turned to 3-isothiocyanato-2-isothiocyanatomethyl-1-propene via 1,3 sigmatropic shift. Homo and co-polymerization reactions of this compound would be possible and at the end of this reactions a polymer with reactive pendant groups would be obtained. This subject is under investigation.

ACKNOWLEDGEMENTS

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➢ ***ORAL PRESENTATION***

Immunohistochemical investigation of cannabinoid receptor 1 and 2 expression in placentas with intrauterine growth retardation, placenta previa and preeclampsia

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Abstract
Aim: To investigate the effect expression of endocannabinoid receptors (CBR) on placental development that stimulate activation of intracellular signaling pathways in normotensive, preeclampsia, placenta previa and IUGR placentas.
Materials and Methods: A total of 40 placentas(10 preeclampsia, 10 placenta previa, 10 IUGR and 10 normal placentas)were subjected to routine histological tissue protocol. Hematoxylin-Eosin and cannabinoid receptor 1/2immunohistochemical staining were performed. Preparations were evaluated and micrographed under light microscopy.
Results: Fibrinoid tissue, vascular dilatation and congestion, hyalinization, degeneration, and vacuolar structures were observed in tertiary villi structures of preeclampsia, placenta previa and IUGR placentas. Apoptotic appearance, pyknosis and hypertrophy were observed in cytotrophoblasts and syncytiotrophoblasts. In preeclampsia, CB1 reaction was observed in decidual nuclei and cytotrophoblasts of some tertiary villi. CB2 expression was moderately positive in decidual cytoplasm, but negative in cytotrophoblasts of root villi. Mild CB1 expression was observed in cytotrophoblasts of stem villi and tertiary villi in placenta previa sections, but mainly negative in cytotrophoblasts. CB1 expression was positive in some vascular endothelial cells and syncytial nodes. CB2 was positively expressed in the nucleus and cytoplasm of decidual and endothelial cells of some small vessels. Mild CB1 expression was detected in some syncytial nodes in maternal root villi in IUGR placenta and in mononuclear cells especially around hemorrhagic areas. CB1 expression was generally negative in syncytiotrophoblast cells.
Conclusion: Development of decidual cells in preeclampsia, placenta previa and IUGR caused trophoblastic invasion, increased syncytial nodes and apoptosis and degenerative process. Therefore, this situation depends on CB1 and CB2 receptors presence.
Keywords: Placenta, preeclampsia, previa, cannabinoid receptor, intrauterine growth retardation

INTRODUCTION

The placenta is a temporary organ constituting of maternal and fetal-derived structures and plays role in the circulation between the mother and fetus, fetal nutrition, many metabolic activities, and hormones production necessary for the continuation of pregnancy. Function of placenta terminated at the end of the pregnancy (Handwerger and Freemark, 2000; Yetter, 1998) As embryo develops, substances exchange between fetal and maternal blood circulation towards the end of pregnancy decreases due to some abnormal structural changes such as thickening of the basement membrane of foetal capillaries, increased fibrous tissue in the villous stroma and fibrinoid accumulation in chorionic plate and on root villi in the junction may be observed (Handwerger and Freemark, 2000; Sadler and Langman, 2012).
Intrauterine growth retardation (IUGR) is a fetal growth retardation disorder in which the expected fetus weight for gestational age is below the 10% percentile. Most of the IUGR cases that adversely affect the development of the fetus due to maternal, fetal, and utero-placental causes are caused by primary or secondary utero-placental circulatory insufficiency (Koestenbauer, 2006; Maryland, 2000). Preeclampsia is characterized by hypertension and proteinuria developing after the 20th week of pregnancy. It is one of the most common causes of maternal, perinatal morbidity and mortality even in developed countries. Despite extensive research, the etiology and pathogenesis of preeclampsia are not fully understood (Lugo and Cassady, 1971; Simon et al., 1990). Placenta previa occurs when the placenta attaches to the inside of the uterus, near or above the cervical opening. It affects about 0.5% of pregnancies. Risk factors include pregnancy and smoking in old age, previous cesarean section, labor induction, or termination of pregnancy. Incidence of placenta previa is 1 in 300 deliveries (Christianson, 1976; Gilbert et al., 2003).
Cannabinoid receptors (CBR) are cell membrane receptors and a part of endocannabinoid system involved in various physiological processes such as appetite, pain sensation, mood, and memory (Crane et al., 1999). Cannabinoid receptors belong to a class of G protein-linked super receptor family (Martin et al., 2002). The two known subtypes of named cannabinoid receptors are CBR1 and CBR2. CB1R receptors play an important role in the pathophysiology of many diseases due to their regulatory roles in peripheral organs and central nervous system. Studies showed CB1R has important role in the hypotensive effect of cannabinoids (Cinar and Gunduz Cinar, 2011). These studies show that the endocannabinoid system can be used as a target in the treatment of hypertension on the cardiovascular system. Fatty acid amide hydrolase inhibitors have also been found to have antihypertensive effects through CB1 receptors in recent studies (Bonz et al., 2003). For this reason, association of CB1R with hypertension, one of the most important indicators of preeclampsia, may elucidate preeclampsia mechanism. CB2R is mainly found in the immune system and hematopoietic cells but recent studies have shown its presence in brain regions (Cinar and Gunduz Cinar, 2011). CB2R is encoded by the human gene CNR2 (Bonz et al., 2003). The main endogenous ligand for the CB2 receptor is 2-Arachidonoylglycerol (2-AG) (Costa et al., 2015).
The aim of this study is to investigate the expression of endocannabinoid receptors (CBR) in placental development that stimulate activation of intracellular signaling pathways in normotensive, preeclampsia, placenta previa and IUGR placentas.

MATERIALS AND METHODS

Ethical approval was taken from Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee with record of number 2020/68. In our study, placentas from 10 preeclampsia, 10 placenta previa, 10 IUGR and 10 normotensive pregnant women (regardless of age) were obtained from Dicle University Medical Faculty Hospital Gynecology and Obstetrics Clinics. All patients signed informed patient consent form. Placentas were taken to Dicle University Faculty of Medicine Histology and Embryology Department Laboratory for routine paraffine waxing tissue protocol.

Histological tissue processing

Tissues were fixed with zinc-Formalin solution (catalog no: Z2902, Sigma-Aldrich, St. Louis, MO, US) and washed under tap water by 5 minutes. Tissues were passed through ascending alcohol series for about 24 hours. Tissues were washed with xylene 2x30 minutes and incubated within paraffin

wax. 5 µm sections were cut with microtome (catalog no: Leica RM2265, Wetzlar, Germany). Deparaffinized within xylene for 2X30 minutes, sections were brought to distilled water. Some of the sections were stained with routine Hematoxylin and Eosin, the rest were kept for immunohistochemical staining.

CB receptor I and CB receptor II immunostaining

Hydrogen peroxide solution (catalog no: TA-015-HP, ThermoFischer, Fremont, CA, USA) were dropped on sections for 20 minutes. After washing in PBS for 3X5 minutes, ultra V Block (catalog no: TA-015-UB, ThermoFischer, Fremont, CA, USA) was applied to sections for 8 minutes. Sections were incubated with primary antibodies anti-cannabinoid receptor I (catalog no: ab23703, Abcam, Cambridge, USA) and anti- cannabinoid receptor II (catalog no: ab3561, Abcam, Cambridge, USA) at +4ºC overnight. Sections were allowed to warm at room temperature for 30-60 minutes. Sections were washed with biotinylated secondary antibody (catalog no:TP-015-BN, ThermoFischer, Fremont, CA, USA) for 14 minutes. Streptavidin-peroxidase (catalog no:TS-015-HR, ThermoFischer, Fremont, CA, USA) was dropped onto sections for 15 minutes. Clearing with PBS, DAB (catalog no: TA-001-HCX, ThermoFischer, Fremont, CA, US) was used as chromogen. Sections were counter stained with Harris hematoxylin and mounted with entellan (catalog no:107961, Sigma-Aldrich, St. Louis, MO, United States). Slides were analyzed with Zeiss Imager A2 Zen 3.0 software (Germany) and photomicrographed.

RESULTS

Hematoxylin – Eosin Staining

No pathology was observed in the placental sections of the normotensive. Vascular structures were normal and cytotrophoblasts and syncytiotrophoblasts were located regularly. No inflammation was observed in the villous stromal areas and interstitial areas (Figure 1a). Increased number of syncytial bridge and nodes, dilation and congestions in the vascular structures were observed in sections of preeclamptic placentas. Compared to the normotensive placentas, common fibrinoid areas, intense hemorrhage, and mononuclear cell infiltration in the intervillous area were seen. Hyalinization, degeneration, and vacuolar structures were recorded in tertiary villus structures. Cytotrophoblasts and syncytiotrophoblasts showed apoptotic nuclei with pyknosis and hypertrophy (Figure 1b). Similar pathology of preeclampsia was observed in sections of placenta previa. Fibrinoid tissue and vascular dilatation and congestion were more common than preeclampsia. Hyperplasic cytotrophoblasts and increased syncytial nodes were recorded (Figure 1c). Villous structures histologically preserved their structure in IUGR placental sections. Hyalinization in villous stroma, congestion, and dilatation in some vascular structures and fibrinoid areas were observed (Figure 1d).

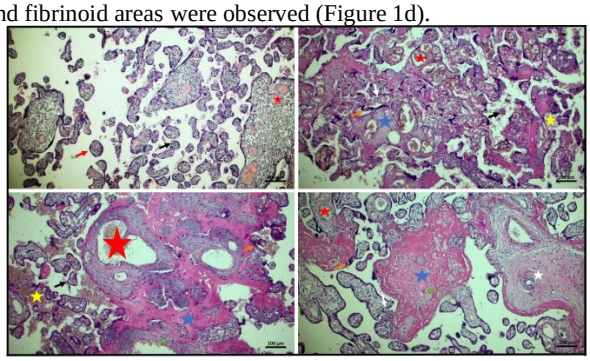

Figure 1: Hematoxylin Eosin staining sections**. a)** In the normotensive placentas, normal tertiary villi (red arrow), regularly organized cytotrophoblasts and syncytiotrophoblast (black arrow), normal blood vessels (red star); **b)** In preeclamptic placentas, increase in syncytial nodes/bridges (orange arrow), fibrinoid (blue star), dense intervillous hemorrhage (yellow star), congestion/dilatation/thrombosis (red star), cell infiltration (white arrow), degenerated cytotrophoblasts and syncytiotrophoblasts nuclei (black arrow) **c)** In placenta previa, increased fibrinoid in the root villi (blue star), dilatation/congestion (red star), intervillous congestion (yellow star), hyperplasic cytotrophoblast cells (black arrow), increased syncytial node (orange arrow), apoptotic decidual nuclei (green arrow); **d)** In IUGR placenta, increased syncytial nodes (orange arrow) and fibrinoid areas (blue star), vascular congestion/dilatation (red star), villous stromal hyalinization (white star) and cell infiltration (white arrow).

Cannabinoid 1 receptor (CB1R) immunostaining

In normotensive placentas, CB1R expression was weakly observed in some decidual nuclei and cytoplasm (Figure 2a). In the placental sections of the preeclampsia placentas, only decidual nuclei showed CB1R expression, not their cytoplasm. Moderate CB1R expression was observed in cytotrophoblasts of some tertiary villi. (Figure 2b). In sections of placenta previa, although CB1R expression was mostly negative in cytotrophoblasts, some cytotrophoblasts in root and tertiary villi showed mild expression. The expression was positive in some vascular endothelial cells and syncytial node areas (Figure 2c). In the placental sections of IUGR, CB1R expression was observed in some syncytial nodes of maternal part and mononuclear cells around hemorrhage. Syncytiotrophoblasts mainly showed negative CB1R expression (Figure 2d).

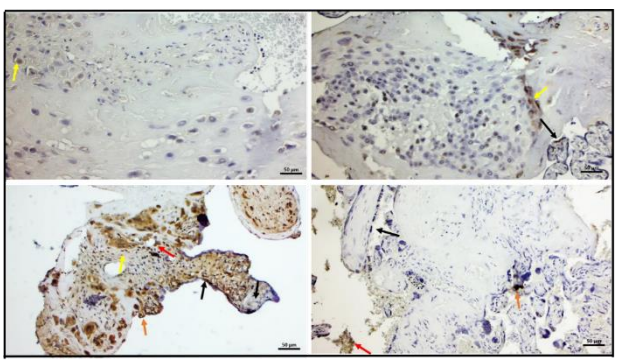

Figure 2: CB1R immunohistochemical staining. **a)** In normotensive placentas, CB1 expression in decidual nuclei and cytoplasm (yellow arrow); **b)** In preeclamptic placentas, positive CB1R expression in decidual nuclei (yellow arrow) and some floating villi (black arrow) but negative in decidual cytoplasm **c)** In sections of placenta previa placenta, Positive CB1R expression was in syncytial nodes (orange arrow), vascular endothelial cells (red arrow) and cytotrophoblast cells of stem and floating villi (black arrow) **d)** In IUGR placentas, slight CB1R expression in some syncytial nodes (orange arrow), positive CB1R expression in mononuclear infiltrated cells (red arrow), negative CB1 expression in syncytiotrophoblast cells (black arrow).

Cannabinoid 2 receptor (CB2R) immunostaining

CB2R expression was mostly negative in the normotensive placentas but positive in some decidual cells and trophoblast cells of root villi (Figure 3a). In the placental sections of the preeclampsia, CB2R expression was positive in the decidual cytoplasm, but negative in the Hofbauer cells and cytotrophoblast cells of the root villi (Figure 3b). Positive CB2R expression was observed in the decidual nuclei and cytoplasm and vascular endothelial cells in the sections of placenta previa. Trophoblast cells of chorionic villi mainly showed negative CB2R expression (Figure 3c). In the sections of IUGR placenta, positive CB2R expression was observed in some fibroblast cells, blood cells and some leukocytes located around fibrous plaques of root villi (Figure 3d).

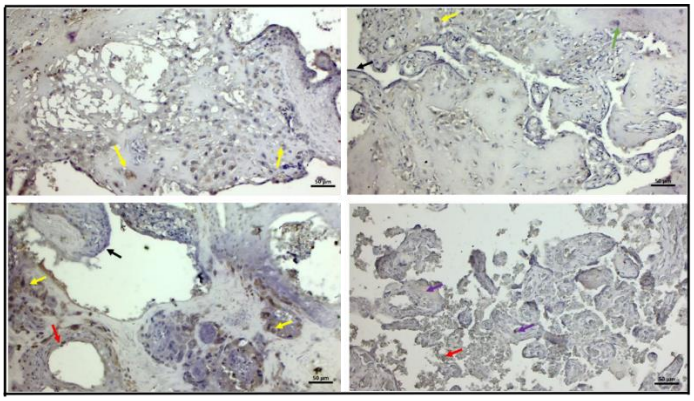

Figure 3: CB1R immunohistochemical staining. **a)** Mild CB2R expression in some decidual nuclei and stromal area (yellow arrows) in normotensive placentas; **b)** Positive CB2R expression in the decidual cytoplasm (yellow arrow), negative CB2 expression in cytotrophoblast cells (black arrow) of stem villi, stromal cells and Hoffbauer cells (green arrow) in preeclamptic placentas; **c)** Positive CB2R expression decidual nucleus and cytoplasm (yellow arrows) and vascular endothelium (red arrow), negative CB2 expression in cytotrophoblasts of chorionic villi (black arrow) in placenta previa sections; **d)** Positive CB2 expression in intervillous blood cells (red arrow) and some leukocyte cells (purple arrows). in IUGR placentas.

DISCUSSION

Preeclampsia is a common disease among pregnancy complications, mainly associated with maternal and perinatal mortality and morbidity (Aplin et al., 2020). Placental vascular lesions in preeclampsia is a condition that affects the development of villus and is associated with trophoblastic invasion and causes significant histopathological damage. Studies showed that trophoblast invasion causes syncytial degeneration in villi, increase in syncytial nodes and bridges, and congestion in blood vessels depending on the severity of preeclampsia (Ridder et al., 2019). In our study, congestion, dilatation, and thrombosis in vascular structures, increase in syncytial nodes and bridges, degenerated cytotrophoblasts were observed in the preeclampsia placentas. Cell infiltration was evident in the intervillous space (Figure 1b). Placenta previa is defined as abnormal placentation in the lower segment of the uterus in the inter-placental region. During this formation, increased villous infarction and fibrinoid formation and congestion in the vessels were reported. This is due to insufficient vascular perfusion (Silver, 2015). Histological sections of placenta previa showed increased fibrinoid in the inter-villous area and vascular congestion (Figure 1c). This situation caused a significant change in the structure of decidual cells by affecting trophoblastic invasion in the maternal region due to vascular insufficiency. IUGR is affected from endogenous, genetic, and environmental factors during pregnancy, causing both placental and fetal abnormal development. In a study, hyperplasic cytotrophoblasts, thickening of vascular basement membrane, increased fibrinoid accumulation were observed in placentas of IUGR (Khajuria and Sharma, 2019). In our study, common fibrinoid areas in some villi and syncytial nodes were observed. Local hyalinization areas due to insufficient blood flow were observed as a result of thickening in the basement membrane of the blood vessels (Figure 1d).

Endocannabinoids and both cannabinoid receptors (CB1R and CB2R) were reported to play a role in the regulation of blastocyst maturation, oviductal transport, implantation and maintenance of pregnancy throughout the embryonic development. Plasmatic levels of the endocannabinoid were fluctuated during normal pregnancy and induced decidual cell death and impaired normal placental development. The effect of endocannabinoid signaling molecules was shown during trophoblast cell differentiation. Change in CB1R expression level has been shown to inhibit trophoblast cell proliferation, differentiation and invasiveness resulting in abnormal placentation and fetal development (Fonseca et al., 2013).

In a study, CB1R and CB2R expression was reported in healthy and preeclamptic women. Especially, CB1R expression was intensively observed in the placenta. Both syncytiotrophoblasts and decidual cells showed CB1R and CB2R expression. They also stated that although CB1R localization appears mostly in vascular endothelial cells and smooth muscle cells, CB2Rs are less common and this decrease may affect trophoblastic activity. By findings, it is thought that the endocannabinoid system may play a role in the development and implantation of the placenta. It has also been shown that this system may play a role in the formation of the maternal decidualization, trophoblastic cell differentiation and invasion. Consequently, high CB1R expression may lead to a weak placentation through insufficient trophoblast invasion and may cause preeclampsia (Fügedi et al., 2014). In the immunohistochemical examination of our study, mild CB1R expression was observed in some decidual cells and trophoblast cells in normal placentas (Figure 2a). Preeclampsia placentas showed CB1R expression in only decidual nuclei and cytotrophoblasts of some tertiary villi (Figure 2b). CB1R was expressed in syncytiotrophoblasts, vascular endothelial cells and syncytial nodes in placenta previa (Figure 2c). In placentas of IUGR, mononuclear cells showed a positive CB1R reaction, while in decidua and cytotrophoblasts the expression was negative (Figure 2d).

CB2R was reported to activate DNA methylation in the immune system cells, brain and sperm of humans and animals. While the effects of CB2 receptors on the placenta are not fully visible, it was stated that their expression is genetical in terms of localization in placental villi which affecting placental structure (Innocenzi et al., 2019).

In a study, it was reported that CB2R caused a decrease in alkaline phosphatase activity, chorionic gonadotropin secretion and leptin m-RNA level in the placenta. Therefore, CB2R affects the expression of metabolic enzymes and may interfere with the morphological structure of trophoblasts (Costa et al., 2015). In our study, positive CB2R expression was observed in decidual cells and negative expression in cytotrophoblast cells, syncytial nodes, and stromal cells in the preeclamptic placentas (Figure 3b). Given that trophoblastic invasion increases in placenta previa (Duzyj et al., 2018), CB2 expression increased in decidual cells, vascular endothelium, and some trophoblasts in placenta previa (Figure 3c) and this was thought to be due to insufficient utero-placental blood flow. In IUGR placental sections, CB2R expression showed a positive reaction in the stromal fibroblast and intervillous leukocyte cells while negative expression was observed in decidua cells (Figure 3d). Accordingly, it was thought that the genetic character

of the CB2R impairs the embryological development and placental development and disrupts the developmental structure of these cells in the placenta showing intrauterine growth retardation.

CONCLUSION

It is concluded that endocannabinoid receptors are important regulators affecting decidual development and trophoblast differentiation in preeclampsia and placenta previa and IUGR anomalies during the placental development period. In preeclamptic placentas, high CB1R expression in decidual cells may cause increased apoptosis level. Changes in CB1R expression may disrupt trophoblastic invasion and may play a role in the pathogenesis of preeclampsia. CB1R expression is marked in the syncytial nodes and vascular endothelial cells in placenta previa suggesting that CB1R induces abnormal placental development in the placenta with angiogenic effect and affecting cell attachment complexes. In IUGR placentas, CB1R expression was positive especially in the inflammation areas of villi, which may provoke abnormal development.
Since CB2R in the preeclamptic placenta is more expressed by decidual cells, this situation will lead to an increase in apoptosis and may affect trophoblast differentiation and invasion in case of severe preeclampsia. In the placenta previa placentas, increased CB2R expression both in decidual nuclei and cytoplasm and in the vascular endothelium located in the root villi may induce cell apoptotic development and cause abnormal vascular development. CB2R expression in some leukocyte cells and fibroblasts in the stromal region of the villi suggest that it may alter the development of villi and adversely affect the development of decidua and the trophoblastic invasion .

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3. POSTER PRESENTATIONS
3.1. ABSTRACTS

➢ *POSTER PRESENTATION*

Methylene Blue removal by graphene oxide/alginate/hydroxyapatite hydrogel Beads

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Abstract

In this study, hydroxyapatite (HAP), graphene oxide (GO) and sodium alginate (SA) were used as friendly adsorbent to remove methylene blue (MB) dye from aqueous solution. This nanocomposite was successfully synthesized by introducing HAP and GO into alginate gel by an ionic gelation technique to form beads. The SA/HAP/GO nanocomposite hydrogel beads were characterized using Fourier-transform infrared spectroscopy (FTIR) and point of zero charge (pHpzc). The influence of many experimental factors such as contact time (30-300 min), initial dye concentration (20-200 ppm), adsorbent dosage (0.095-3,18g/L), temperature (22-40°C) and pH of dye solution (2-11) on the MB adsorption capacity (mg/g) and removal efficiency (%) was studied. MB removal efficiency was 93.47 % at optimum conditions (contact time: 210 min, initial MB concentration: 50 ppm, SA/HAP/GO dose: 1.6g/L, temperature: 22°C, pH: 8.83). The kinetic adsorption study of SA/HAP/GO followed the pseudo-second-order kinetics. Adsorption isotherms were simulated by Langmuir, Freundlich and Dubinin-Radushkevich models. The adsorption equilibrium is described by the Freundlich isotherm demonstrating that physisorption is the rate controlling mechanism. The maximum adsorption capacity from the Langmuir isotherm equation attains 333.33 mg/g. The thermodynamic investigations showed that the adsorption of MB on SA/HAP/GO beads were spontaneous ($\Delta G<0$, -50.11 kJ/mol) and exothermic ($\Delta H<0$, -57.94 kJ/mol). In addition, five successive adsorption and desorption (0.1 mol/L HCL) cycles were carried out. The developed absorbent were showed a 6.6% mass loss after the last cycle in the adsorption process. This finding suggested that SA/HAP/GO hydrogel beads are a promising and recyclable adsorbent for the removal of MB from aqueous solution.

Keywords: graphene oxide, alginate, hydroxyapatite, methylene blue, adsorption.

➢ *POSTER PRESENTATION*

Effects of propolis extract medium on *in vitro* sperms activation of infertile asthenozoospermic men

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Abstract

Background: the disability to have a child baby is a big problem for millions of couples throughout the world. Medical herbal plants have been used in many countries to overcome such problems and many of these plants treated male infertility *in vivo* and *in vitro*. Therefore; this study has investigated the bioactive effect of propolis extract medium on certain sperm function parameters *in vitro* of infertile men.
Aim of study: This study has evaluated the beneficial pharmacological effects of propolis extract on certain sperm function characters of asthenozoospermic men.
Materials and methods: Twenty-five of semen samples from patients complaining from asthenozoospermia were analysed and diagnosed. The layering procedure was used for *in vitro* sperm preparation and activation. Two concentrations of propolis extract (0.5 mg and 1mg / 1 ml of phosphate buffer solution) medium were used and compared with Hams-F 12 medium (control) and phosphate buffer solution (PBS). Certain sperm function parameters were recorded.
Results: The results have shown that there was a significant ($P<0.05$) effect on active sperm motility grade A when using 1mg/ml propolis medium compared to 0. 5mg/ml, Hams-F12 (control) and PBS media. A significant ($P<0.05$) improvement in sperm motility and morphologically normal sperm (MNS) percentage was observed in a media containing propolis extracts compared to before activation and after activation by using PBS and HamsF-12 media.
Conclusion: This study has found that the using of propolis extract has a significant implication on *in vitro* enhancement of sperm motility and normal morphology percentage.

Keywords: propolis extract, in vitro activation, asthenozoospermia

➢ *POSTER PRESENTATION*

Rheological characterization of the oleogels and emulgels prepared

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Abstract

Various oleogels and emulgels based on single (saturated triglycerides, waxes, polyglycerides, fatty acids, amphiphiles, etc.) or multi-component gelators (triglyceride+cholesterol, pectin+calcium chloride, carbohydrate polymers+amphiphiles, saponins+amphiphiles, food proteins+amphiphiles, etc.) with different techniques (direct gelation, filled emulsions, multi-layer emulsions, aqueous phase-gelled emulsions, etc.) were prepared and evaluated in this project (TÜBİTAK 217O094). The oleogels/emulgels were defined as soft materials with viscoelastic flow behaviour. Measurement of viscosity, flow parameters, storage, and temperature stability is essential to determine the food applications of the gels. Oscillatory rheological tests were used to analyze the samples for flow behaviour and dynamic mechanical/thermal stability. Amplitude sweep tests were performed to determine the non-destructive deformation range (linear viscoelastic region), the storage (G') and loss (G'') modulus within this range. Similarly, frequency sweep tests were performed to describe the time-dependent behaviour at the LVR. This test with high and low frequencies stimulate the fast and slow-motion on time scales. Hence, storage stabilities were determined. Further, samples were sheared under constant dynamic-mechanical conditions with temperature ramp to observe how to gel structure changes with external heating/cooling. Results were quite informative for predicting the thermal food process stability of the samples. Similarly, time sweep tests were completed to observe the structural recovery ability of the gels after applying and ceasing very high shear stresses to the samples resting at the LVR. This test simulates the effects of mechanical food processes (pomping, mixing, whipping, agitation,etc). Creep test was used to evaluate the friction behaviour of cocolin samples successfully. Lastly, the effects of different component ratios for the same oleogel or emulgel were efficiently analyzed by the rheological tests. It was observed that rheological analyses were more informative and realistic for gel flow behaviour, mechanical/thermal/storage stability, and simulates much better for the sensory properties than those of the gravimetric analyses, centrifuge tests, and calorimetric analyses.

Keywords:Oleogel, emulgel, multi-component, stability, rheology, property.

➢ *POSTER PRESENTATION*

Food applications of some oleogels and emulgels prepared

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Abstract

Various oleogels and emulgels were prepared and characterized within the TÜBİTAK 217O094 project. Some selected samples were tested for food applications, as well. Sunflower and beeswax oleogels of flaxseed oil were replaced tallow fat in Turkish sucuk production. The new sucuks were very low in saturated fatty acids and contained significant amounts of PUFA. The textural properties of the sucuks with oleogels were not good enough,and the structure could not fully be developed. The sensory qualities of the sucuks were similar to the control sample. Future research is needed to improve texture and slicing properties. Flaxseed oleogels prepared with sunflower and whale spermaceti waxes were used to prepare mayonnaise with 50% fat, without any hydrocolloid or starch additions. The low-fat mayonnaises were very successful, and the consumers liked them more than the control sample. The commercialization of this product is foreseen. Lastly, sunflower wax and polyglycerol stearate oleogels were used to prepare cocolin samples. The cocolins are imitation chocolate products with no chocolate fat, but with chocolate powder and other ingredients. It was observed that the shape and thermal stabilities of the cocolins were much higher than the control chocolate prepared in the laboratory. Although the cocolins starts melting at lower temperatures, they stay longer at higher temperatures; hence, they could be considered summer season chocolates. The sensory properties were similar to the control sample, and the consumer accepts abilities were high enough. The findings of this study indicate that proper oleogels could have applications in various food products to serve the low saturated, zero *trans*-fatty acid plastic consistency fats with ease of application. Further studies to improve some properties of each food product are also determined. Especially mixing oleogels with main solid fat with various ratios could provide both healthy fatty acids and textural properties for the product produced.

Keywords: oleogel, sucuk, mayonnaise, cocolin, sensory, quality

➢ *POSTER PRESENTATION*

C677T and A1298C MTHFR gene polymorphisms are related to ischemic stroke in Ukrainian population

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Abstract

Endothelial dysfunction is strongly associated with vascular damage and the occurrence of thrombotic complications. Hyperhomocysteinemia is one of the common causes of endothelial dysfunction. Homocysteine metabolism depends on the activity of the methylenetetrahydrofolate reductase enzyme (MTHFR). There is a direct correlation between MTHFR activity and the plasma level of homocysteine. Therefore, the aim of our work was to check the possible link between C677T and A1298C polymorphisms of the *MTHFR* gene and ischemic atherothrombotic stroke development in the Ukrainian population.
Material and methods. The whole venous blood from 170 patients with ischemic stroke (IS) and 124 control subjects was used. The polymorphism C677T (rs1801133) and A1298C (rs1801131) of the *MTHFR* gene were genotyped using the PCR-RFLP method (polymerase chain reaction followed by the analysis of the restriction fragments length). SPSS software (version 17.0) was used for statistical data processing.
Results. The distribution of *MTHFR* C677T-genotypes in control group was: CC – 46.0%, CT – 48.4%, TT – 5.6%. In stroke patients: CC – 52.3; CT – 35.9, TT – 11.8% (P = 0.044). Regression analysis under recessive model of inheritance revealed that the risk of IS in TT-genotype carriers is 2.3 times higher (CI = 1,111-5,449, P = 0,049), than in main C-allele carriers. The distribution of MTHFR A1298C-genotypes in control subjects was: AA – 46.0%, AC – 44.3%, CC – 9.7%. In stroke group: AA – 42.3%, AC – 37.1%, CC – 20.6% (P = 0.039). Analysis under the recessive model of inheritance showed that CC-homozygotes has a 2.3-fold higher risk of stroke occurrence compared to A-allele carriers (CI = 1,323-3,449; P = 0.027).
Conclusions. Thus, the C677T and A1298C polymorphisms of the *MTHFR* gene are related to ischemic atherothrombotic stroke in the Ukrainian population. The risk of stroke in carriers of TT-genotype (C677T-polymorphism) and CC-genotype (A1298C-polymorphism) is higher compared to wild-type allele carriers.

Keywords: gene polymorphism, MTHFR, ischemic stroke.

➢ *POSTER PRESENTATION*

Integrative analysis of multi-cellular genome-scale metabolic networks with cell type specific transcriptome data predicted by deconvolution algorithms: application to Parkinson's disease

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Abstract

Loss of structure or function of brain cells causes different types of neurodegenerative diseases such as Parkinson's Disease (PD). The Genome scale metabolic network (GMN) models, when integrated with omics data, allow systematic investigation of the effect of diseases on metabolism by constraint based computational methods such as Flux Balance Analysis (FBA). Since bulk gene expression data is convolution of different cell types available in a certain tissue, the analysis of GMN models by the integration of cell type specific gene expression data can give better results than integration using bulk gene expression data. Different deconvolution algorithms were developed to overcome this issue such as MIND. Bulk transcriptome data belonging to PD patients and control subjects were obtained from the public transcriptome database, Gene Expression Omnibus (Dataset series number: GEO: 20295). The bulk transcriptome data were deconvolved by MIND algorithm for both control and PD groups separately. The brain specific two-cell GMN (neurons and astrocytes) model, iBrain671, was used, which included 994 reactions controlled by 671 genes. The differential expression analysis was performed for both astrocyte and neuron separately. Gene ontology (GO) analysis was performed using the results of differential expression analysis. The results of the GO analysis were highly related to PD. FBA was performed using condition specific GMN that with gene expression data obtained from deconvolution processes. Integrative analysis by constraint based modelling approach using cell type specific gene expression data shows promising results in terms of the understanding the effect of PD on the brain.

Keywords: Genome scale metabolic network, Parkinson's Disease, Flux Balance Analysis, Omics data, Deconvolution

➢ *POSTER PRESENTATION*

Development of Spicy Spreadable Olive Oil Products by Oleogelation Technology

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Abstract

Oleogels are edible lipid gels structurally resembling soft-solid materials at room temperature. Oleogels could be produced with any edible liquid oil for various hardness and melting properties. Their major advantage is being free from *trans* fatty acids and very low in saturated fatty acids. Some molecules called organogelators are added into the liquid oil to prepare the oleogels. In this master thesis study, virgin olive oil (VOO) oleogels were prepared with sunflower wax (SW) and whale spermaceti wax (WW) to prepare spreadable spicy fats. First, the wax oleogels of VOO with 5, 7, and 10% addition levels were prepared. Amplitude sweep rheological measurements of the prepared oleogels and a commercial breakfast margarine were measured to select the most suitable organogelator addition levels. After that oleogels with added powder spices (thyme, flaked red pepper, cumin, and turmeric) were prepared. Finally, prepared spicy and spreadable VOO oleogels were analyzed for rheological properties, common physico-chemical properties, sensory descriptions and consumer acceptances. Results indicated that they were fairly stable solid fats very similar to breakfast margarine in terms of rheological properties. The oleogels were very stable at room temperature and even at centrifugal conditions. The oleogels had various color tones depending on the added spice. Their free fatty acidity and peroxide values were low and in acceptable ranges. X-ray diffraction analyses indicated the presence of □′ type crystal polymorphs. Sensory descriptive analyses showed that their hardness, spreadability, liquefaction properties were very similar to the breakfast margarine. Further, there was very little or none waxy aroma and rancidity in the oleogels. On the other hand, some grassy aroma and spice specific aromas were dominantly present. Consumers usually liked these products. Overall, the spicy spreadable VOO oleogels could be a new soft-solid product for direct edible consumption to expand olive oil health benefits to the consumers.

Keywords: oleogel, virgin olive oil, spicy, rheology, sensory, consumer

➢ *POSTER PRESENTATION*

An electrochemical sensor platform based on graphene oxide modified pencil graphite electrode for the voltammetric determination of food colorant sunset yellow

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Abstract

Food additives are substances used to prevent, protect or correct unwanted changes in the taste, odor, appearance, structure and other qualities of foods. Color is one of the most important properties of foods. Sunset Yellow (E110) (SY) is a water-soluble synthetic azo dye and is an orange-red powder. It can be found in many foods and products for example candies, beverages, bakery products, cosmetics and medicines. Excess intake of sunset yellow can cause serious health problems like vomiting, bronchitis, asthm, hyperactivity, learning problems, genotoxicity and cancer [1]. Considering the literature, many analytical methods have been used for the determination of sunset yellow [2,3]. In this study, electrochemically prepared graphene oxide modified pencil graphite electrode (EGO-PGE) was made by one-step chronoamperometry method for the determination of sunset yellow [4]. EGO-PGE stands out with its features such as short analysis time, simplicity, low cost, sensitivity and selectivity. Cyclic voltammetry, electrochemical impedance spectroscopy, scanning electron microscopy were used for the characterization of the EGO-PGE. The developed simple platform was successfully applied for orange juice sample.

Keywords: Food colorant; sunset yellow; three electrode system; graphene oxide; pencil graphite electrode; differential pulse voltammetry.

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➢ *POSTER PRESENTATION*

Electrochemical determination of food colorant allura red using pretreated pencil graphite electrode by differential pulse voltammetry

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Abstract

The use of food additives is becoming widespread in the world daily. Many foodstuffs lose their color after going through various industrial processes. That's why, food dyes which is a class of food additives are used. Synthetic food dyes have advantages such as high stability, good water solubility, color uniformity and low cost [1,2]. Allura red (E129) is a synthetic food azo dye in dark red powder form. In addition to all these advantages of food dyes, exposure to high amounts of allura red causes important health problems such as carcinogenicity, chromosomal aberration, genotoxicity, hyperactivity, mutagenicity and neurotoxicity. It is added to alcohol, beverages, cotton candy, frozen foods and various cosmetic products [3,4]. Therefore, the amount of allura red in food and beverages should be determined. In this work, it is aimed to rapid and simple electrochemical determination of allura red. For this purpose, the pretreated pencil graphite electrode was developed under the optimum conditions. Using the conventional three-electrode system, measurements were performed with cyclic voltammetry and differential pulse voltammetry methods and their applicability to real samples was tested.

Keywords: Food azo dye; allura red; pretreated pencil graphite electrode; cyclic voltammetry; differential pulse voltammetry.

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➢ *POSTER PRESENTATION*

Electrochemical determination of riboflavin based on functionalized multi-walled carbon nanotube/gold nanoparticle/pencil graphite electrode

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Abstract

Riboflavin (Vitamin B2) is described as a group B vitamin that is the central component of two coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) [1]. In vitamin B2 deficiency, painful red tongue, sore throat, chapped lips (cheilosis), and inflammation of the corners of the mouth occur. RF should be taken with dietary supplements such as eggs, green vegetables, milk, and other dairy products, meat, mushrooms, and almonds because it cannot be synthesized in the body [2]. For these reasons, the accurate and reliable determination of riboflavin is significant.
Plenty of analytical methods have been reported for riboflavin determination, such as spectrophotometric, HPLC, electrophoresis, and chemiluminescence. However, most of these methods limit their applications as they are expensive, time-consuming, complexity and require pre-treatment processes. As an alternative to these methods, electrochemical methods have become very interesting for riboflavin determination thanks to their advantages such as accuracy, simplicity, sensitivity, and low cost [3]. In this study, a new sensor was created using gold nanoparticle and functionalized multi-walled carbon nanotube and used for riboflavin determination. The characterization processes of the prepared modified electrode were carried out using cyclic voltammetry, electrochemical impedance spectroscopy and scanning electron microscopy techniques.

Keywords: Riboflavin, Nanoparticle, Nanotube, Cyclic voltammetry, Differential pulse voltammetry

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➢ *POSTER PRESENTATION*

Supramoleküler etkileşimlerle yıldız tipi amfifilik kopolimerlerin oluşturulması ve karakterizasyonu

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Özet

Kendiliğinden birleşme ile misel yapılarını oluşturan amfifilik kopolimerlerin ilaç taşıyıcı sistemlerde uygulanması son yıllarda dikkat çekmektedir. Amfifilik kopolimerlerden oluşan polimerik miseller hidrofobik bir çekirdeğe ve hidrofilik bir kabuk yapıya sahip nano boyutlu, küresel, supramoleküler kolloidal partiküllerdir. "Çekirdek-kabuk" yapısı sayesinde polimerik miseller ilaçların çözünürlüğünü, stabilitesini, salımını ve biyoyararlanımını arttırmak için hidrofobik ilaçları kapsülleyebilir. Yıldız şekilli polimerler aynı moleküler ağırlık ve bileşimdeki lineer polimerlerle karşılaştırıldığında daha küçük hidrodinamik yarıçap, daha düşük çözelti viskozitesi, daha küçük difüzyon katsayısı ve daha düşük kritik misel konsantrasyonuna sahiptir. Güçlü kovalent etkileşimlerle karşılaştırıldığında konak-konuk etkileşimi gibi kovalent olmayan etkileşimlerle bağlanmış supramoleküler amfifilik polimerden oluşturulan miseller son zamanlarda dikkat çekmektedir**.** Siklodekstrinler (CD) çeşitli molekülleri iç boşluklarına hapsedebildikleri için supramoleküler kompleksleri oluşturmada konak molekülü olarak yaygın şekilde kullanılmaktadır. Biyouyumlu ve düşük toksisite gösteren CD'ler suda çözünür hidrofilik dış yüzeye ve hidrofobik iç boşluğa sahiptir. Bu benzersiz yapı CD'lerin ilaçlar, sürfaktanlar ve polimerler gibi çeşitli konuklarla inklüzyon kompleksi oluşturmasına ve ilaç taşıyıcı sistemlerde kullanılmasına olanak sağlamaktadır. CD'lerle etkileşime girebilen çeşitli organik gruplar arasında adamanten (AD) 10^3-10^5 M^{-1}'lik yüksek bir bağlanma sabiti ile CD'nin iç boşluğuyla sıkı bir kompleks oluşturmaktadır. CD ve AD arasında oluşan konak-konuk kompleksinden yararlanılarak ilaç ve gen taşınımı için çeşitli CD temelli kendiliğinden birleşmiş sistemler geliştirilebilir.
Bu çalışmada CD'ler ile fonksiyonlandırılmış yıldız polimer sentezlenip, CD'lerin inklüzyon kompleks oluşturma özelliğinden faydalanılarak supramoleküler amfifilik yıldız kopolimerlerden miseller oluşturulup karakterizasyonu yapılmıştır. Elde edilen misellerin potansiyel ilaç taşıyıcı sistem olarak kullanılması incelenmiştir.

Anahtar Kelimeler: Siklodekstrin, supramoleküler etkileşim, yıldız polimer, misel, ilaç taşıyıcı sistemler.

➢ *POSTER PRESENTATION*

Polimerizasyonla indüklenmiş kendiliğinden birleşme (PISA) yöntemi ile folik asit onksiyonlandırılmış polimerik misellerin hazırlanması ve karakterizasyonu

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Özet

İlaç taşıyıcı sistem olarak polimerik miseller küçük partikül büyüklükleri, yapısal stabiliteleri, yüksek ilaç yükleme kapasiteleri, suda çözünürlük, düşük toksisite, etkin madde salımının kontrollü olması gibi özelliklere sahiptir. Bu özellikleri sayesinde ideal bir taşıyıcı sistem oluşturdukları düşünülmektedir. Çeşitli monomerler ile kopolimerler sentezlenerek elde edilen polimerik miseller farklı tasarım imkanları sayesinde yaygın olarak ilaç taşıyıcı sistemler ve diğer pek çok biyolojik uygulamalar için avantaj sağlar. Amfifilik blok kopolimerlerle misel elde edilmesi blok kopolimerlerin statik etkiyle kendiliğinden birleşmesiyle sağlanır. Bloklardan biri için seçici olan çözücü içerisinde kendiliğinden birleşme özelliğine sahiptirler. Kopolimerin blok hacim oranlarına göre küreler, solucanlar, silindirler ve veziküller dahil olmak üzere çok çeşitli polimerik morfolojiler oluşturulabilir. Polimerlerin alışılmış yöntemlerle kendiliğinden birleşmesi, oluşacak polimerik morfolojileri genellikle sınırlayan seyreltik çözelti (<%1) içinde gerçekleştirilir. Güncel bir yöntem olarak polimerizasyonla indüklenmiş kendiliğinden birleşme (PISA) ise bu sınırlamayı ortadan kaldırır. Kontrollü/yaşayan radikal polimerizasyona dayanarak, PISA metodu ile amfifilik blok kopolimerlerin sentezi, kürelerden veziküllere gelişen morfolojiler oluşturmak için ortaya çıkan etkili bir yaklaşımdır. Bu yöntemde polimerizasyon, başlatıcı polimer için seçici bir çözücü içerisinde çözünmeyen bloğun polimerize edilmesi sonucu farklı yapıların oluşum sürecine dayanır. Zincir uzaması başlayan çözünmeyen bloğun polimerizasyon derecesi kendiliğinden birleşme sürecini kontrol eder. Bu çalışmada, tersinir katılma-ayrılma zincir transfer (RAFT) polimerizasyonu ile HEMA ve GMA monomerleri kullanılarak fonksiyonlandırılabilir GMA üniteleri içeren hidrofilik bir makrobaşlatıcı elde edilmiştir. GMA ünitelerinin folik asit ile fonksiyonlandırılması sağlandıktan sonra yine RAFT polimerizasyonu ile MMA monomerlerinin polimerizasyonu gerçekleştirilmiş, PISA yöntemiyle miseller ve diğer morfolojiler oluşturulması amaçlanmıştır. Bu yolla elde edilen fonksiyonel misellerin ilaç taşıyıcı olma potansiyelleri araştırılmıştır.

Anahtar Kelimeler: Amfifilik kopolimer, tersinir katılma-ayrılma zincir transfer (RAFT) polimerizasyonu, polimerizasyonla indüklenmiş kendiliğinden birleşme (PISA), folik asit.

➢ *POSTER PRESENTATION*

The effects of Tartrazine and its usage areas

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Abstract

Tartrazine (T) is a powder dye with an orange-yellow color. It is easily soluble in water. Its molecular weight is 534.37 g/mol and melting point is 300 °C. It is commonly used in cosmetics industry in soaps, nail polishes, shampoos, and other hair products, as well as in vitamins, medicines, and drug capsules in pharmaceutical industry. It is also used in ice creams, candies, cotton candy, pudding, gelatin, jams, pastry mixes, pastries, custard powder, biscuits, crackers, sodas and alcoholic drinks, energy and sports drinks, powdered drink mixes, fruit-based drinks, chewing gums, pastas, corn chips, popcorn, potato chips, and several processed foods. The main metabolism of T includes sulfanilic acid and aminopyrazolone. Metabolites of T could generate reactive oxygen species (ROS), lead to oxidative stress and have an impact on hepatic and renal structures and biochemical profiles. Oxidative stress results in cellular damage by altering the antioxidant-oxidant balance in favor of the oxidants and via the production of free radicals/reactive oxygen species that attack physical macromolecules (i.e., DNA, lipids, proteins). These damages often cause diseases such as senescence and cardiovascular diseases, cancer, renal diseases, neurological diseases, muscle and liver diseases.

Keywords: Tartrazine, usage areas, health.

➢ *POSTER PRESENTATION*

Synthesis, characterization and investigation of some properties of carboxylic acid substituted Ni(II) phthalocyanine

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Abstract

Phthalocyanines has been studied in many fields and subjects since the first time it appeared as a by-product during the synthesis of o-cyanobenzamide from acetic acid and phthalimide in 1907. The cavity in its center is large enough to make complex with many metal atoms and the macrocyclic structure consisting of iminoizoindolin units containing four nitrogen atoms is symmetrical and pyrole units are directly involved in complexation. The longevity of phthalocyanines is due to their being photophysically and photochemically stable macrocyclic structure. Thanks to the conjugated 18-π electron system of this cyclic structure, there is a strong emission and absorption characteristics in the visible region. In addition, the functional and photochemical properties of the ring structure can be adjusted precisely by attaching functional groups with various numbers and features to the peripherally and non-peripherally positions of the annular structure, and also by replacing the central metal atom. The unsubstituted phthalocyanine molecule has low solubility in organic solvents, but with the presence of substituted groups, the phthalocyanine compounds acquire the ability to dissolve. However, the physical, chemical and electronic properties of phthalocyanines may be improved according to the properties, number and location of the substituted groups attached to their peripherally and non-peripherally positions. The hydrogen atoms in the center of the phthalocyanine molecule are replaced by metals to form metallo phthalocyanine derivatives. The nature of the central metal also affects the properties of the phthalocyanine molecule [1-4].
In this study, novel non-peripherally tetra substituted nickel(II) phthalocyanine containing carboxylic acid groups were synthesized. The prepared compound was characterized by ^{1}H NMR, UV-VIS, IR, MS and elemental analysis techniques. It has been observed that the synthesized compound is highly soluble in common organic solvents such as DMSO, DMF, THF and toluen. The solubility problems in common solvents that are one of main problems of phthalocyanine compounds have been overcome by means of carboxylic acid group which is bonded to the structure. The aggregation behaviors of this phthalocyanines were studied depend on solvents and concentrations. Aggregation studies showed that the compound does not tend to aggregate in solvents. The performed spectroscopic studies showed that the compound was obtained successfully. Thus, it can be used for different application areas.

Keywords: Phthalocyanine, Nickel(II), Carboxylic acid, Aggregation

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➢ *POSTER PRESENTATION*

The Effect of Drought Stress on Germination of Einkorn Wheat (*Triticim monococcum)* Seeds

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Abstract

Wheat is one of the most produced grain products in Turkey as well as in the world and has a significant proportion in food sector. Similar to other food raw materials, wheat production should be increased to meet the food demand of the increasing world population. Development of new cultivars which are tolerant to biotic and abiotic stress factors is one of the first approaches and wild plant populations neglected in the past are the most important genetic resources to meet such requirements. Diploid einkorn wheat (*Triticum monococcum*) which is also known as Kaplıca wheat in Turkey is one of the most important genetic resources to improve tolerance levels of cultivated wheat varieties against to some of specific stress factors. This study was carried out to determine the drought tolerance level of einkorn wheat at germination stage. Local einkorn wheat seeds were germinated in the presence of various PEG-6000 concentrations (0%, 3%, 6%, 9%; 10%, 15%, 20%, 25% and 30%). Completely randomized block design with four replications of 30 seeds *were placed on two layers of filter paper moistened with 3 ml of deionized water in covered diameter Petri dishes* (80x15mm) and were germinated at 20 ± 0.5 ° C in the dark. The seeds showed the radicle exceeded 2-3mm from paleas were counted as germinated seeds and were daily recorded. The germination percentage, germination rate and homogeneity of the seeds were calculated and were analyzed with the SAS package program. The differences between the means were determined at the 5% significance level using the least significant difference (LSD) method. The results of the study showed that ≥ 25% PEG concentrations significantly reduced germination percentage of einkorn seeds while low PEG concentrations promoted germination compared to control seeds.

Keywords: Einkorn wheat, germination, drought, polyethylene glycol

➢ *POSTER PRESENTATION*

Çanakkale Boğazı'nda bulunan Akdeniz midyesi'nin (*Mytilus gallopravincialis* Lamarck, 1819) et kalitesinin aylık değişimi

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Özet

Son yıllarda molluska filumu altında yer alan çift kabuklu (bivalvia) ve gastropod türlerinin üretimine olan ilgi sahip oldukları besin içeriğinin kalitesinden dolayı karasal canlılara göre artmıştır. Bu yüzden çift kabuklu türleri dünyadaki su ürünleri üretimi miktarına bakıldığında önemli bir potansiyele sahiptir. Akdeniz midyesi (*Mytilus galloprovincialis* L.,*)* sadece Türkiye'de değil tüm dünyada tanınan ve sevilerek tüketilen bir su ürünüdür. Bu nedenle iç piyasada tüketilmesi ve ihracatta etkin rol oynaması günümüzde olduğu gibi gelecekte de ülkemiz ekonomisine katkıda bulunmaya devam edecektir. Bu çalışmada Akdeniz midyesi bireyleri Mart 2019-Şubat 2020 tarihleri arasında Çanakkale'nin Gelibolu ilçesindeki Ilgardere köyündeki yetiştiricilik alanından(N 40º16'41 "ve E 26º29'50") toplanmıştır. Her bir örnekleme için zamanında çevresel parametreler kaydedildi. Toplanan bireyler laboratuvara nakledildi ve örneklerin toplam ağırlığı, yaş et ağırlığı, et kuru ağırlığı ve kabuk kuru ağırlığı ölçüldü. Ardından, örneklenen tüm bireyler için aylık et verimi ve kondisyon indeksi değerleri hesaplandı. Bu çalışmada *M.galloprovincialis*'in et verimi , kondisyon indeksi, ham kül, ham yağ ve kabuk bileşen miktarları hesaplanmıştır. Çalışmada kullanılan midyelerin et verimi , kondisyon indeksi, ham kül,ham yağ ve kabuk bileşen ortalama değerleri sırasıyla; 23,17 , 11,57 , 11,18 , 14,58 ve 56,28 olarak hesaplanmıştır.

Anahtar Kelimeler: *Mytilus galloprovincialis,*Akdeniz midyesi,Çanakkale Boğazı,Kondisyon indeksi,Ham yağ

➢ ***POSTER PRESENTATION***

Highly soluble peripherally tetra-substituted zinc(II) phthalocyanine bearing piperidine moieties for photodynamic therapy

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Abstract

Cancer is a pathological condition in which the balance between cell proliferation and cell death is destroyed. Although many methods are used in the treatment of cancer, alternative methods are still in search. In treatment methods, it is desirable to minimize side effects and to target tumor cells without damaging healthy cells. Today, new cancer treatments are needed to develop because of the disadvantages of conventional cancer treatments that used in the clinical applications. In this context, one of the alternative methods of interest is photodynamic therapy (PDT). PDT enables reactive oxygen species in the presence of a photosensitizer molecule activated by light of a certain wavelength, and thus cell death. The basis of the cancer treatment by PDT depends on light irradiation. The other necessary requirements for this treatment are photosensitizers which can be activated by light and oxygen. Singlet, together with oxygen production, has played an important role in PDT studies. It is extremely important for PDT to develop photosensitizers which can absorb light at long wavelength region show. The interaction of the produced singlet oxygen with the cancerous cell reveals how important the phthalocyanines are in the health field. Phthalocyanines with high chemical and thermal stability are extremely valuable because of their unique physical and chemical properties. The phthalocyanines in various structures offer an opportunity to expand their application areas such as electrophotography, optical data storage, electrochromic imaging devices, gas sensor, liquid crystal, and use as a colorant for laser technology. The solubility of phthalocyanine derivatives has an important role for using phthalocyanines in many technological applications. To increase the solubility of phthalocyanines, studies are carried out to synthesize phthalocyanine derivatives which contain various substituents in peripherally/non-peripheral positions and metals in ring of phthalocyanine [1-4].

In this study, zinc(II) phthalocyanines bearing piperidine substituents were synthesized, purified and characterized. After that, the photophysical and photochemical properties of these phthalocyanines were investigated to see their capabilities for using them as photosensitizers. The characterization of this phthalocyanine is clarified with elemental analysis, IR, ^{1}H-NMR, UV-Vis and MS techniques. The photophysical and photochemical [fluorescence spectra, fluorescence quantum yields (Φ_F), singlet oxygen quantum yields (Φ_Δ) and photodegradation quantum yields (Φ_d)] and aggregation properties of this phthalocyanine were investigated in DMSO solvent for determination of their photosensitizing abilities in photocatalytic applications such as photodynamic therapy. The result were evaluated and in accordance with the literature. According to our data, we believe that the photosensitizers we use may be a potential drug candidate for PDT studies.

Keywords: Phthalocyanine, Soluble, Photodynamic therapy.

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➢ *POSTER PRESENTATION*

Untargeted urinary metabolomic profiling in individuals with different levels of kidney function

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Abstract

Chronic kidney disease (CKD) is a growing public health concern that burdens people and healthcare. Discovering novel biomarkers contributes to an understanding of CKD at the molecular level and characterizes possible new drug targets. Metabolomics ensures a direct snapshot of the physiological state of an organism and reveals a metabolome change in the biological system (biological fluid, tissue, cell, organ, or organism). The purpose of this study is to elicit the metabolome profiling of urine in all CKD stages and investigate whether urine metabolite profiles are significantly different. Samples from 100 participants with CKD, 20 in stages 1, 20 in stage 2, 20 in stage 3, 20 in stage 4, and 20 in stage 5 were collected and stored at -80 C for analysis. Development and optimization of a metabolomic method was performed in order to increase the coverage of the metabolome and identify the metabolic profiling of urine at a different stage. The efficiency of sample preparation on the coverage of metabolome, and reproducibility of the system was investigated. Pretreatment with water and methanol was performed and analyzed by UPLC-ESI-Q-TOF-MS/MS. The raw data set obtained from the UPLC-ESI-Q-TOF-MS/MS instrument was processed by commercial Progenesis QI 2.0 software to perform peak picking. Following multivariate statistical analysis such as Principal component analysis (PCA), metabolome identification was performed against National Institute of Standards and Technology (NIST) databases. A sample run from 100 participants with CKD have yet to be performed. Pre-treatment of methanol extraction increased the losses. Good sample preparation recovery was obtained by diluting the water. In our sample preparation protocol, methanol extraction pretreatment was identified as the main cause of metabolite losses or increased data variability during metabolomics analysis by UPLC-ESI-Q-TOF-MS/MS. The most suitable method that could be used in the clinic was developed.

Keywords: Chronic kidney disease, UPLC-ESI-Q-TOF-MS/MS, metabolomic profiling analysis

➢ *POSTER PRESENTATION*

ISO kalite standartları ve iyi imalat uygulamaları (GMP) kılavuzunun karşılaştırılmalı olarak değerlendirilmesi

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Özet

Kalite, temelde müşteri ihtiyaçlarının karşılanmasıdır. ISO standartlarındaki müşteri odaklılığın aksine, ilacın müşterisi hasta kişidir. Hasta kişi kendi başına ilacın kalitesini değerlendiremez. Bu çalışmada, bir kuruluşun kalite yönetim sisteminin temelini oluşturan ISO standartları ile ilaç endüstrisinde kullanılan ve temel kalite gereksinimlerini belirten GMP kılavuzunun benzerlik ve farklılıkları incelenmiştir. İlacın kalitesi çok önemlidir ve günümüzdeki ilaç kalite anlayışı, daha önce yaşanan kötü deneyimler sonucunda ortaya çıkmıştır. Bu nedenle, ilaç üretiminde GMP Kılavuzlarına uygunluk bir zorunluluktur ancak ISO standartlarına uyum ve bunu belgelendirme pazarda daha güvenilir olmak, müşteri memnuniyetini artırmak gibi amaçlarla firmaların kendi talebidir. ISO standartları ile GMP Kılavuzu arasındaki diğer bir fark, ISO standartlarının tek bir sektöre özgü olmaması ve tüm ürün veya hizmet sektörlerine uygulanabilmesi, GMP Kılavuzunun ise ilaç üretimine özgü olmasıdır. Kalite sisteminin kurulması, uygulanması ve katılımı üst yönetimin sorumluluğunda olduğundan, ISO standartlarında üst yönetim oldukça önemlidir. GMP Kılavuzunda kalite sistemi, en alt seviyeden başlayarak yapılacak işlemler açıkça belirtildiği için aşağıdan yukarıya doğru çalışır. Sonuç olarak, GMP kılavuzu ve ISO standartları benzer olmakla birlikte, GMP kılavuzunun daha kapsamlı, ne yapılması gerektiği konusunda daha öğretici ve daha spesifik olduğu görülmüştür.

Anahtar Kelimeler: ISO Standartları, GMP Kılavuzu, İlaç Endüstirisi

➢ *POSTER PRESENTATION*

Bioinformatics Analysis of *DREB1A* and *DRS1* Genes Related to Drought Stress in Selected Plants

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Abstract

Plants are exposed to different biotic and abiotic stress conditions as a result of not meeting certain conditions or if existing conditions are not sufficient. Although the long- and short-term responses of plants to stress are different, these reactions produce different results in each organ of the plant. Drought stress, which is one of the abiotic stress factors, is an important factor restricting vegetative production in our country and in the world. Drought is defined as a period without precipitation that lasts long enough to cause a noticeable decrease in the water content of the soil and plant growth and directly affects the growth and development and yield potential of plants. In order to understand drought tolerance mechanisms at the molecular level, determining the expression levels of drought related genes is of great importance. Drought-induced transcription factors and gene products such as LEA (late-embryogenesis abundant) and DREB (Dehydration response element binding) play an important role in the response to water deficiency; They activate signal transduction pathways, protect cellular structures and provide drought tolerance. In this study, *Arabidopsis Thaliana*, *Beta Vulgaris* (Beet), *Cucumis Sativus* (Cucumber), *Gossypium raimondii* (Cotton), *Solanum lycopersicum* (Tomato), *Solanum tuberosum* (Potato), *Vitis vinifera* (Grape vine)) plants were selected and we performed Bioinformatics analysis of *DREB1A* and *DRS1* (*DROUGHT SENSITIVE 1*) genes, which are known to have an important function in drought related studies. In *Arabidopsis*, homologous sequences of *DREB1A* and *DRS1* genes and their orthologs were found in Ensemble Plants database. Molecular structures of genes in selected plants were revealed (Exon-Intron). The amino acid sequences of the genes were aligned in MacVector, and a phylogenetic tree was created using the alignment data. Similarities of DREB1A and DRS1 sequences with each other were determined and their three-dimensional structures were created in the SWISS-MODEL database using sequence information.

Keywords: Drought stress, Abiotic stress, *DREB1A*, *DRS1*, Bioinformatics

➢ *POSTER PRESENTATION*

Voltammetric determination of pyridoxine using electrochemically pretreated electrode

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Abstract

Pyridoxine is a B group vitamin related to the metabolism of proteins, lipids, and carbohydrates. Pyridoxal phosphate, which is a type of pyridoxine, is used in enzymes as a co-factor or prosthetic group for the metabolism of amino acids [1]. In the amino acid decarboxylase reaction leading to the formation of monoamine neurotransmitters, vitamin B6 is closely related to the function of the nervous system. It is also reported to have an important role in the immune and endocrine systems and has beneficial effects in the treatment of HIV-1 and cancer. The most prominent symptoms caused by pyridoxine deficiency are related to the nervous system. In its deficiency, hyperacusis, extreme irritability, impaired alertness, abnormal health movements appear in animals and humans. Considering the importance of vitamins for human health, sensitive and selective methods should be developed to determine fast in different matrices such as food, medicine, and biological fluids [2].

Many analytical methods have been reported in the literature for determining vitamin B6, including spectrophotometry, liquid chromatography, and electrochemical methods. However, most of these methods limit their application because they are expensive, time-consuming, complex, and require pre-treatment processes. As an alternative to these methods, electrochemical methods have become very interesting for pyridoxine determination thanks to their advantages such as accuracy, simplicity, sensitivity, and low cost [3].

In this study, a sensor was created using H_3PO_4 supporting electrolyte solution in order to constitute a new perspective for the pyridoxine determination. The characterization processes of the modified electrode prepared were carried out using cyclic voltammetry, electrochemical impedance spectroscopy, and scanning electron microscopy techniques.

Keywords: Pyridoxine, Pretreated electrode, Sensor, Cyclic voltammetry, Differential pulse voltammetry.

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➢ *POSTER PRESENTATION*

Biyoaktif bir bileşik olan resveratrolün gıdalarda bulunma düzeyi ve fonksiyonel özellikleri

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Özet

Resveratrol (trans-3,4',5-trihydroksistilben) bir fitoaleksindir. Fitoaleksinler, bitkilerde mikrobiyal enfeksiyon, yaralanma ve ultraviyole ışınlara tepki olarak sentezlenmektedir. Resveratrolün en önemli kaynağının üzüm olduğu ayrıca zambak, okaliptüs, dut, yabanmersini ve yerfıstığı da dahil olmak üzere 70'in üzerinde bitki türünde doğal olarak bulunduğu saptanmıştır. Polifenolik yapıda olan resveratrol antioksidan aktiviteye sahiptir ve lipid peroksidasyonunu ve buna bağlı hücre ölümünü önlediği bilinmektedir. Koroner kalp hastalıklarını, kanseri ve nörodejeneratif hastalıkları önleyici, yaşlanmayı geciktirici, çeşitli fungus ve virüs gelişimini durdurucu, pıhtı önleyici etkilerinin olduğu bilinmektedir. Resveratrolün sağlık üzerine birçok olumlu etkisinin olduğunun belirlenmesi ile son yıllarda yapılan bilimsel araştırmalar bu bileşik üzerine yoğunlaşmaktadır. Resveratrolün doğal olarak kaynağı olan gıdaların sağlıklı beslenme açısından öneminin anlaşılması ve buna bağlı olarak da tüketiminin artması öngörülmektedir. Bu derlemede resveratrolün gıdalarda doğal olarak bulunma düzeyi ve resveratrol içeren gıdaların tüketiminin insan sağlığı üzerine olumlu etkileri hakkında bilgi verilmeye çalışılmıştır.

Anahtar Kelimeler: Resveratrol, biyoaktif, fitoaleksin, fenolik bileşik, antioksidan

➢ *POSTER PRESENTATION*

Preparation and characterization of poly(acrylicacid)/boron mineral (PAA/BM) composite material which may be used for energy conversion and storage systems

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Abstract

Acrylic acid is a colorless and pungent substance and polymerizes when exposed to light, heat or catalyst. Acrylic acid is a substance used in many areas in modern industry, including in the manufacture of products such as plate, paint, orlon, fiber, yarn, glass and plexiglass [1]. Boron is a semi-metal and has several allotropes where amorphous boron is a dark brown powder and crystalline boron is black extremely hard solid of low conductivity at room temperature. Elemental boron may be used as a dopant in the semiconductor industry [2]. The advantage of composite materials is that they combine the best properties of the components [3].
The goal of this study was to prepare and characterize poly(acrylic acid)/boron mineral (PAA/BM) composites for use in energy conversion and storage. Thus for this purpose, PAA/BM composites were prepared by *ex-situ* solution mixing method with the respective characterizations carried out. The process of crushing the BM particles was carried out using the ultrasonication method. It is known that polymeric materials containing boron minerals have high thermal stabilities. In this context, besides the thermal properties of the composite materials prepared, the solution properties were also examined. Pure PAA and PAA/BM composite films were produced from these materials for use in optical devices or devices in various industries. Both chemical and physical properties of these films were studied. By using different spectroscopic and microscopic methods, interactions between PAA and BMs were investigated, electrical conductivities of polymer and composite films were measured and their morphologies were examined. As a result of this study, the invaluable boron mineral was utilized for new composite material preparation with PAA for potential in the preparation of energy conversion and storage systems and devices. Additionally, the composite materials prepared with semiconductor boron element can be investigated as semiconductor materials in the electronics industry.

Keywords: Poly(Acrylic Acid), Boron Mineral, Composite Material, Energy Conversion and Storage

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➢ *POSTER PRESENTATION*

Design and investigation of some vitamin K3 compounds

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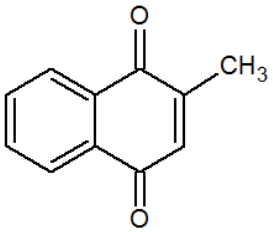
Abstract

Heterocyclic organic compounds as quinones show antitumor effect. The main interest in these compunds especially nitrogen-containing ones based on their potential to produce tumor-selective toxicity. This selectivity happens by the oxygen tension between normal and tumor cells and amount of the activated enzymes. So these heterocyclic quinonoid compunds which has been using in the prodrugs is a promising development in cancer treatment [1]. Menadione (K3) is a synthetic analogue which act as a provitamin. The antitumor effect of vitamin K has been investigated since 1947. Menadione has discovered as being useful as radiosensitizing agent and helps to the cancer patients. Vitamin K3 has indicated antitumor effect both in vitro and in vivo studies when combined with other conventional chemotherapeutic agents. For instance, it has been observed that vitamin K3 combined with vitamin C inhibits the growth of tumor cells [2].
Therefore, in the light of these scientific informations, vitamin K3 has a great pharmacological importance. In this study, a novel series of hetero group substituted methylquinones were synthesized according to Michael addition mechanism. The structures of the novel quinone products were purificated by using column chromatography. Their structures were characterized by microanalysis, Fourier transform infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR), ^{13}C nuclear magnetic resonance (^{13}C NMR) and mass spectrometry (MS).



Vitamin K3 (Menadione)

Keywords: Quinones; Menadione; Antitumor activity

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➢ *POSTER PRESENTATION*

Synthesis and biological properties of N-substituted naphthoquinone molecules

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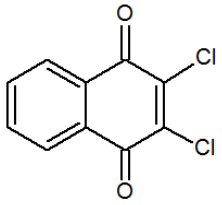
Abstract

The quinone molecules are common in numerous natural products. They are widely known with some important biological activities such as antitumor, antibacterial, antimalarial and antifungal. The most importantly, several researches and reports have been published during years which related to anticancer activities of quinone molecules [1]. In the search for new potential anticancer drugs, quinone compounds especially including pyridine moieities in the ring have been synthesized involving one-pot-step. The new N-heterocyclic quinone products has shown the best antitumor effect [2]. Therefore it can easily be said that the heteroatom-substituted naphtoquinones indicate more biological activity. Especially quinonoid compounds with –thio, -amino and -chloro molecules have more effective antifungal activity. The structure- activity relationship has proved number and position of nitrogen(N) atoms in heterocyclic ring is also significant [1]. The aim of this study, heteroatom substituted naphthoquinones were synthesized according to Michael addition mechanism. The structures of these quinone products were purificated by using column chromatography. Their structures were characterized by microanalysis, Fourier transform infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}H NMR), ^{13}C nuclear magnetic resonance (^{13}C NMR) and mass spectrometry (MS).



2,3-Dichloro-1,4-napthoquinone

Keywords: Naphthoquinones; Heterocyclic; Biological activities.

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➢ *POSTER PRESENTATION*

CDH2 and TP53 gene expression in metastatic breast cancer patients

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Abstract

Although there are treatment developments in early breast cancer patients, the fact that metastasized patients cannot be treated yet and the survival in these patients is low causes breast cancer to be the most serious cause of death among women. Beside metastatic problem, it is also difficult for the patient to undergo biopsy procedures that require surgery for diagnostic purposes. Therefore, the detection of new clinically important biomarkers with non-invasive early screening techniques that do not cause any complications to the patient are of great importance. In this respect, two known genes that are demonstrated to be involved in cancer progression will be analyzed in fresh blood samples of breast cancer patients with metastasis. In this study, RT-qPCR technique was performed to analyze the expression level of target genes (CDH2 and TP53) in metastatic breast cancer patients (n=25) and healthy donors (n=25). To evaluate the results SPSS program was used and $p<0.05$ was considered statistical significant. The study was approved by the Ethics Committee (No: 09.2019.204) and supported by Marmara University Scientific Research Projects Coordination Unit under grant number SAG-C-DRP-120619-0222. CDH2 expression level was found significantly up-regulated in metastatic breast cancer patients compared to the expression level of healthy controls (fold change: 1.6; $p= 0.003$). On the other hand, the expression level of TP53 was found significantly down-regulated in case group compared to control group (fold change: 0.85; $p= 0.02$). Consequently, up-regulated expression of CDH2 and down-regulated expression of TP53 may be an important clinical diagnostic biomarkers for detection of metastasis in fresh blood samples of breast cancer patients. However, further studies in large scale are needed to understand the exact role of these genes together with other interactive partners in breast cancer metastasis detection.

Keywords: Metastatic Breast Cancer, CDH2, TP53

➢ *POSTER PRESENTATION*

The role of synthesis methodology on cytotoxicity properties of dextran-coated nanoceria against cancer cells

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Abstract

Cancer is one of the most difficult diseases of the age to cure. Many conventional methods such as chemotherapy, radiotherapy, and surgery have been used for cancer treatment. Among them, chemotherapy is a well-known and preferable treatment in clinics with limitations such as toxicity, drug retention and accumulation at the tumor side. Last decade, nanotherapeutics for cancer therapy is an emerging field in medicine, which can overcome the limitations of conventional chemotherapy. They offer possibilities with high drug loading efficiency with low drug leakage, constant drug delivery directly to the cancer cells. Due to the promises of nanoparticles to increase in drug efficiency at the tumor site, they lack the selectivity among healthy and cancer cells. Cerium oxide nanoparticles have enormous potential as antioxidant and radioprotective agents for cancer applications. They show inhibitory effects on cancer progression while being toxic to tumor cells and non-toxic to stromal cells. This remarkable feature of cerium oxide nanoparticles related to coexistence of Ce^{+3} and Ce^{+4} ions and ability of oxygen vacancies formation on their surface, which enables them to interact with and modulate free radicals. The oxidation states (+3 or +4) allow nanoceria act as ion scavenger, which make it toxic to tumor cells and non-toxic to stromal cells. The oxygen vacancy formation and oxidation state, which define cytotoxic properties of nanoceria, is highly dependent synthesis methodology. Therefore, we aimed to show the relation in between the synthesis type and cytotoxicity properties against cancer cells and healthy counterparts. Dextran coated nanoceria were synthesized with three different synthesis methodologies. Following; detail material characterization, newly synthesized dextran-coated nanoceria were compared in terms of dose and time dependent cytotoxic and genotoxic behavior. This study was supported by International Centre for Genetic Engineering and Biotechnology (ICGEB) under CRP/TUR 18-03 project number.

Keywords: Nanoceria, Cancer treatment, Nanoparticle-based therapy

➢ *POSTER PRESENTATION*

Purification and Characterization of Sugar Ester Producing Lipase from *Cryptococcus diffluens* D44

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Abstract

Microbial lipases are widely used in various industrial areas due to their regio-, chemo- and enantioselectivity characteristics to different substrates. Lipases are also used to produce sugar esters that are the most popular nonionic and nontoxic biosurfactant. Since they are biodegradable, non-toxic, non-irritant to skin and no-odour properties, they are used in the food, pharmaceutical and cosmetic industries. In the present work, *Cryptococcus diffluens* D44 lipase which has an ability to synthesize fructose mono octanoate was purified and characterized. Lipase produced from *Cryptococcus diffluens* D44, which are the yeast strains isolated from petroleum sludge, was first precipitated by using acetone and then two sequential chromatographic steps as ion exchange and size-exclusion were applied. DEAE sepharose anion exchange chromatography resulted in two different peaks including lipase activity (Lip1 and Lip4). Further purification of Lip1 and Lip4 by Sephadex G-100 gel filtration resulted in three types of lipases as Lip1-1, Lip1-2 and Lip4-1. The optimum temperature of Lip1-1, Lip1-2 and Lip4-1 were determined as 60ºC, 65ºC and 65ºC, respectively. Optimum pH of Lip1-1 and Lip1-2 were determined as pH 9.0 while Lip 4-1 showed highest activity at pH 5.0. Moreover, purified lipases showed the highest activity in the presence of methanol. This work was supported by Marmara University BAPKO, Research Fund-the project number FEN-C-YLP-141118-0594 and TUBITAK-KAMAG Project-115G079.

Keywords: lipase, sugar esters, pharmaceuticals, biosurfactants

➢ *POSTER PRESENTATION*

Green synthesis of carbon quantum dots from *Momordica charantia*

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Abstract

Fluorescent carbon quantum dots (CQDs) are an emerging class of nanomaterials in the carbon family. There are various inexpensive and renewable resources that can be used to synthesize green CQDs, which have received immense attention from researchers because of their improved aqueous solubility, high biocompatibility, and eco-friendly nature compared with chemically derived CDs. Additional surface passivation is not required, as heteroatoms are present on the surface of green CDs in the form of amine, hydroxyl, carboxyl, or thiol functional groups, which can improve their physicochemical properties, quantum yield, and the probability of visible light absorption. Green CQDs have potential applications in the fields of bioimaging, drug/gene delivery systems, catalysis, and sensing. In this study, it was aimed to synthesize CQDs from *Momordica charantia* for the first time.

In the study, it was planned to obtain CQD by green synthesis in the water environment with the microwave method, which is an environmentally friendly, effective, easily applicable and economical method. Powdered plant debris in water was irradiated in microwave oven (800 W, 30 min), centrifuged, dialysed against water and finally freeze-dried. Water/substance ratio, applied voltage and contact time were studied for optimization of fluorescence properties of CQDs. Fluorescence, UV-vis, FT-IR, TEM and quantum efficiency analysis of CQDs; characterization of structural, morphological, fluorescence, surface chemical properties were done.

The study demonstrated that CQDs could be easily synthesized from *Momordica charantia* without using any organic solvents. The CQDs showed excellent fluorescence properties, indicating possible use for bioimaging, drug/gene delivery systems, catalysis, and sensing.

Keywords: Carbon Quantum Dot, *Momordica charantia*, Fluorescence Properties

➢ *POSTER PRESENTATION*

Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration?

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Abstract

Osteoarthritis (OA) primarily affects hyaline cartilage in load-bearing joints such as knee, hip and shoulder resulting in narrowing of the joint space, subchondrial bone thickening, osteophyte formation, joint swelling, pain, inflammation and severe degeneration in the relevant joints especially cartilage. OA is one of the most common causes of disability with increasing prevalence with age, affecting quality of life and productivity. Although OA was previously seen as a disease of wear and tear, increasing knowledge on articular cartilage physiology has shown that the mechanism is not only mediated by biomechanical forces but also inflammatory, biochemical and immunological factors. Therefore, characterization of OA is important for preventing the disease and establishing a treatment protocol. It is known that many factors play a role in the development of OA, which is one of the joint diseases frequently encountered in the clinics. The changes observed in the metabolic activities of chondrocytes forming the basic structure of joint cartilage, are considered among the factors contributing to the OA development. In normal cartilage, although metabolic activity is slow, it is known that the metabolic activities of chondrocytes that form the cartilage structure increase, although the mechanism of activation is not fully understood in case of cartilage damage. With the increasing metabolic activities of chondrocytes, matrix metalloproteinases and aggrecanase enzymes are produced that actively break down the cartilage. In this phase, cartilage can't be repaired causing more cartilage loss, creating an important step in OA development. As a result, decrease in the anabolic and proliferative responses of chondrocytes and progressive cartilage loss occur. Since the pathological changes seen in the joints are common to all OA types, understanding this pathology is important in terms of developing treatment strategies for OA. Therefore, in our study, we aimed to investigate the degenerative processes playing role in OA pathophysiology.

Keywords: osteoarthritis, chondrocyte, cartilage, cartilage degeneration, joint.

➢ *POSTER PRESENTATION*

Novel immunomechanisms in hypertension: the role of neoantigens

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Abstract

Hypertension is a multifactorial disease involving the interaction of several genes with environmental factors. It is one of the strongest risk factors for almost all different cardiovascular diseases including coronary disease, left ventricular hypertrophy, valvular heart diseases, cardiac arrhythmias, cerebral stroke and renal failure. Increasing evidence suggests that inflammation contributes to hypertension, and if efforts are taken to block inflammation, the end-organ damage and severity of blood pressure elevation can be reduced. Furthermore, T cells also seem to be involved in hypertension, indicating that the adaptive immune system might contribute to this disease. This is an emerging area of investigation, and the exact manner by which T cells and other inflammatory cells are activated and contribute to hypertension is not understood. It is interesting to speculate that an important mechanism underlying T cell activation in hypertension is formation of neoantigens; antigens that are not identified as self, which could be produced in response to hypertensive stimuli, such as AII or salt. These neoantigens are processed and presented by dendritic cells to promote T cell activation. It is conceivable that low levels of blood pressure elevation could induce these changes, perhaps via mechanical trauma to peripheral tissues. Therefore, in this study, it is aimed to summarize the role of neoantigens in the pathogenesis of hypertension.

Keywords: Hypertension, immune system, inflammation, neoantigens, T cells

➢ *POSTER PRESENTATION*

Analytical Method Development and Validation of Mucopolysaccharide Polysulfate in Cream Formulation by Size Exclusion Chromatography

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Abstract

In this study, a new size exclusion chromatographic method has been developed and validated for the analysis of Mucopolysaccharide polysulphate (MPS) used as anti-inflammatory and antithrombotic agent in cream formulation. MPS was measured analyzed in the Repromer OH-4000 (10 µm, 8.0×300 mm) - Repromer OH-5000 (10 µm, 8.0×300 mm) columns by 0.05M sodium sulfate isocratic elution mobile phase system at 40°C with a flow rate of 1 mL/min and detected by using refractive index detection. The method was validated for limit of quantification, limit of detection, linearity, robustness, recovery, precision and accuracy using to Bioanalytical Method Validation Guidance (EMEA/CHMP/EWP/192217/ 2009). The calibration curve showed a linearity at 0.45-1.35 mg/mL range. The limits of detection and quantification were found to be 45.0 and 90 µg/mL, respectively. Assay recovery of MPS from cream formulation at 0.45, 0.90 and 1.350 mg/mL concentrations were evaluated. Intra-day and inter-day relative standard deviation values were calculated to be less than 2.193 %. The mean recovery was calculated as 97.37%. The validated method was applied successfully to the determination of MPS in cream formulation.

Keywords: Mucopolysaccharide polysulfate, size exclusion chromatography, cream formulation, RID detector

➢ *POSTER PRESENTATION*

Amino fonksiyonel nanokompozitlerin sentezi ve karakterizasyonu

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Özet

Bu çalışmada, güçlü manyetik ve yarı iletken özelliklerinden dolayı magnetit (Fe_3O_4), katalitik ve biyolojik uygulamalar dahil olmak üzere çeşitli uygulamalarda kullanılmak için polimer ve amino fonksiyonel yapılar ile kombine edilerek nanokompozitler oluşturulmuştur. Nanokompozitlerin sentezi için üç aşamalı yöntem kullanılmıştır. Öncelikle Fe_3O_4 nanopartikülleri üretildi ve ardından glisidil metakrilat ile magnetit nanopartiküllerin yüzeyi kaplanmıştır. Üçüncü aşamada ise yüzeyde bulunan epoksi halkaları ile 1,2,4-Triazol, 3-Amino-1,2,4-triazol ve 5-amino tetrazol gibi aromatik heterosiklik yapılar bağlanmıştır. Ürünlerin yapısı, morfolojisi ve elektriksel özellikleri X-ışını toz kırınımı (XRD), Zeta potansiyeli, Fourier dönüşümü kızılötesi spektroskopisi (FT-IR), Termal gravimetrik analiz (TGA), taramalı elektron mikroskobu (SEM) ile karakterize edilmiştir. FT-IR analizi modifikasyonun gerçekleştiğini kanıtlamaktadır. SEM mikrografları, Fe_3O_4 nanopartiküllerinin glisidil metakrilat ile kaplaması üzerine hafifçe toplandığını, TGA sonuçları ise numunelerin termal olarak kararlı olduğunu göstermiştir. Zeta potansiyeli ile izoelektrik noktaları belirlenerek ileriki çalışmalar için uygun ortamların belirlenmesi sağlanmıştır.

Anahtar Kelimeler: Fe_3O_4, Glisidil metakrilat, 1,2,4-Triazol, 3-Amino-1,2,4-triazol, 5-amino tetrazol

➢ *POSTER PRESENTATION*

Cucurbita pepo extracts against Pseudomonas aeruginosa Quorum Sensing

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Abstract

Bacterial drug resistance is a major health problem all over the world in treatment of several diseases. *Pseudomonas aeruginosa* is a major human pathogen that causes a wide range of clinical infections such as skin and respiratory infections. *P. aeruginosa* is responsible for 10–20% of nosocomial infections. Therefore, alternative treatment approaches are needed. Most research have alternatively focused on inhibition of bacterial communication systems called quorum sensing (QS). Many bacteria including *P. aeruginosa*, control the production of virulence factors by this communication system. Bacteria protect itself from many environmental stresses as well as antibacterials via QS. Therefore, it is important to discover QS inhibitor compounds from natural sources. *Cucurbita pepo* is known to demonstrate several biological activities such as antidiabetic, antitumor, antiinflammatory and anticancer. In this study, it is aimed to investigate QS inhibition effects of *Cucurbita pepo* against *P. aeruginosa*.
C. pepo leaves were extracted with methanol. Crude extracts were tested against *P. aeruginosa* at concentrations of 120, 60 and 30 µg/ml. QS inhibition tests were performed in 96-well microplates with *P. aeruginosa* biomonitor strains: *las-gfp, rhl-gpf, pqs-qfp*. Absorbance and GFP fluorescence were measured every 30 minutes for 14 hours.
QSI screenings of *C. pepo* methanol extracts showed inhibition rates of approximately 29,24% for *las*, 30,70% for *rhl*, 39,95% for *pqs* QS system of *P. aeruginosa.* As a result, it was observed that *C. pepo* extracts have potential compounds that inhibit and of *P. aeruginosa* and can be employed in future antivirulence research.

Keywords: *Pseudomonas aeruginosa, Cucurbita pepo, quorum sensing.*

➢ *POSTER PRESENTATION*

Can vitamin E and carotenoid reduce *β*-Lactoglobulin allergenicity? An answer from *ex vivo* study

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Abstract

Vitamin E and β-carotene powerful antioxidants capable of direct free radical scavenging were selected for their role in improving the allergic state both as antioxidants and immune modulators. Therefore, we investigated here whether pretreatment with a combination of vitamin E and β-carotene can ameliorate *β*-Lactoglobulin-induced intestinal changes in a murine model of intestinal anaphylaxis.
Three-to four-week-old female Balb/c mice randomized into three equal groups were assigned to daily treatment with: 0 mg as a positive control group or 20 mg and 30 mg/kg of *β*-carotene and vitamin E respectively. Two weeks after beginning the supplementation protocol, mice were sensitized intraperitoneally with *β*-Lactoglobulin (*β*-Lg). Negative control group did not undergo intraperitoneal sensitization. Throughout the supplementation protocol, weight was monitored weekly in all mice. Intestinal anaphylactic responses measured as an increase in the short circuit current (Isc) and epithelial conductance (G) a measure of passive ion permeability were studied *ex vivo* in Ussing chambers. Specimens were also processed for histological analysis.
Feeding vitamin E associated with *β*-carotene was shown to significantly decrease *β*-Lg-induced intestinal anaphylactic responses indicating a reduction of the secretory response. Epithelial permeability was also decreased in the vitamin E and *β*-carotene-supplemented mice as compared with the positive control group. Our analysis of histological sections revealed that antioxidants treatment clearly reduced the microscopic lesions caused by *β*-Lg sensitization.
Our findings provide evidence that the combination of different antioxidants might be the successful approach to guarantee the total abolishment of both anaphylactic response and the epithelial permeability caused by *β*-Lg sensitization.

Key words *β*-carotene . *β*-Lactoglobulin . Anaphylactic response . Epithelial permeability. Vitamin E . Ussing chambers.

➢ *POSTER PRESENTATION*

Clay Reinforced Poly(AAm-*co*-Stb) Nanoarchitectures: Structural and Thermal Properties

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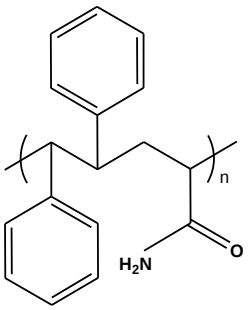
Abstract

Polymer matrix-based nanocomposites have recently attracted considerable attention in the field of nanotechnology such as barrier and membrane separation, flammability resistance, thermal reinforcements and biomedical applications. The combination of polymers and organic nanofillers is an important way of achieving the enhanced properties of a particular material. Various functional organic copolymers have been used to prepare functional polymer/copolymer-clay nanocomposites [1,2]. It was expected that presence of the clay affects the structure and the properties of the obtained nanocomposites and these organic-inorganic hybrid materials would show better mechanical and thermal properties. In the present work effect of the organically surface modified montmorillonite (O-MMT) nanoclay (3, 6 and 10 wt.%) on to the thermal properties and nanostructure-composition-property relationships of the poly(acrylamide-*co*-*trans*-stilbene) [poly(AAm-*co*-Stb)] (Scheme 1) and poly(acrylamide-*co*-*trans*-stilbene)-organo-MMT [poly(AAm-*co*-Stb)-O-MMT] nanocomposites were investigated. Thermal properties of the functional copolymer and its nanocomposites were analyzed by thermal methods (TGA and DSC) including certain thermal transitions. As regarding to the thermoanalytical analysis, it has been observed that nanocomposites have higher thermal stability then copolymer at higher temperatures.



Scheme 1. Structure of poly(AAm-*co*-Stb)

Keywords: Nanocomposite, nanoclay, thermal stability.

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➢ *POSTER PRESENTATION*

Siyez Buğdayında (*Triticum monococcum*) *In vitro* Çalışmalar İçin Etkin Tohum Sterilizasyon Yönteminin Belirlenmesi

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Özet

In vitro çalışmalarda etkin bir sterilizasyon yöntemi, bitki materyalinin sadece yüzeyini değil bitki materyali içerisinde yer alan olası enfeksiyon kaynaklarına karşı da etkin bir sterilizasyon sağlaması gerekmektedir. Bu nedenle tarla kaynaklı tohum içi patojen bulaşmalarına karşı yapılacak tohum sterilizasyon yöntemleri farklı tohum ön uygulamalarını gerektirmektedir. Bu çalışma siyez buğdayı tohumunda karşılaşılan tohum içi fungal ve bakteriyel patojenlere karşı etkili bir tohum sterilizasyon yönteminin belirlenmesi amacıyla yürütülmüştür. Çalışmada, tohumlar farklı zamanlarda 50 °C'de ya da oda sıcaklığı şartlarında %2'lik NaOCl'de belirli sürelerde (5, 10, 20, 25, 30, 35 dakika) inkübe edilmiştir. Devamında NaOCl (%20, %30, %40, %42, %100), $HgCl_2$ (%0,1), EtOH (%70) ve Captan 50 WP (fungusit) kimyasallarının tekli ya kombinasyonları şeklinde sterilizasyon işlemine alınmıştır. Tohumlar her bir sterilizasyon uygulamasının ardından içerisinde çift kat kurutma kâğıdı bulunan steril kapaklı cam petri kaplarında (80x15mm) 20 ± 0,5 °C karanlık ortamda çimlenmeye bırakılmıştır. Sonuçlar tohum ön uygulamaları ile uygulanan sterilizasyon yöntemlerinin tohumlardaki kontaminasyon oluşumları ve tohum canlılığı üzerine çok farklı etkilerinin olabileceğini, en az kontaminasyon oluşumunun ise 10 dakika %42 NaOCI ile muamele edilen tohumlardan elde edildiğini göstermiştir. Çalışma sonuçları aynı zamanda tarla şartlarında kontamine olan siyez buğday tohumlarının *in vitro* şartlarda kullanılmasını sağlayacak yeni sterilizasyon tekniklerinin geliştirilmesine ihtiyaç olduğunu göstermiştir.

Anahtar Kelimeler: Siyez buğdayı, kontaminasyon, sterilizasyon

➢ *POSTER PRESENTATION*

Potentiation of the neuritogenic effects of PC-12 Adh Cells with Trichostatin A and RG108 combination

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Abstract

Neuropathological characteristics of neurodegenerative disorders, such as Alzheimer's or Parkinson disease, are atrophy or loss of specific neurons in the specific brain areas. The lack of regeneration is due to a combination of factors: death of injured neurons, reduced capacity of regeneration, lack of necessary trophic molecules to support growth, and the presence of an environment hostile for any growth line. Neuritogenic substances hold the promise of therapeutic efficacy in the treatment of neuronal injuries by the virtue of their ability to stimulate outgrowth of neurites from neuronal cells. Using small-molecule products like epigenetic drugs in combination, which can activate silenced genes, regulate chromatin remodelling and transcription, for eliciting neuritogenic activity or in combination with neurotrophic substances is currently been focused as an alternative approach. In this study, the effects of a novel epigenetic drug, DNA methyltransferase I inhibitor RG108 and histone deacetylase inhibitor Trichostatin A (TSA) combination, were investigated on neuronal differentiation and neurite outgrowth of the PC-12 Adh cell line. For this purpose, differentiated PC-12 Adh cells were treated with 50 nM nerve growth factor (NGF), 50 nM TSA and 100 nM RG108 in various combinations and neurite outgrowth was observed with immunofluorescence staining with anti-beta III tubulin antibody and determined by quantitative neurite outgrowth analysis on this cell line. As a result, the combination of 50 nM NGF + 100 nM RG108 + 50 nM TSA has significant effects on neuronal differentiation and neurite elongation. With further investigations, our results will contribute to the literature in terms of epigenetic mechanisms in neurodegenerative diseases.

Keywords: neurite outgrowth, epigenetics, RG108, Trichostatin A, neurodegeneration.

➢ *POSTER PRESENTATION*

Poliklorlu Bifeniller (PCB) ve Su Ürünleri

Semra Küçük

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Özet

Poliklorlu bifeniller (PCB), ilk 1881 yılında sentezlenmiştir ve doğada ilk kez 1914 yılında bazı kuşların tüylerinde tespit edilmiştir. Ticari olarak üretimi 1927 yılında ABD'nin Alabama Eyaletinde Anniston Ordu Donatım şirketi tarafından olmuştur. 1935 yılından itibaren İtalya, Fransa, Almanya ve Japonya'da çeşitli firmalarda PCB üretimi yapılmıştır. Bu zamanlarda, insanlarda PCB kaynaklı sağlık problemleri ortaya çıkmaya başlamıştır. Örneğin, 1933 yılında üretim fabrikalarındaki işçilerde yüz ve vücutlarında akne benzeri oluşumlar görülmüştür. Bu tür problemler ortaya çıkmasına rağmen günümüzde halen PCB'lerin sanayide kullanımı devam etmektedir. Sanayide transformatörlerde, ısı iletimi ve hidrolik sistemlerde, vakum pompalarında, floresan lambaların balastlarında, boyalarda, yapıştırıcılarda ve karbonsuz kopya kağıtlarında, hareketli akşamlarda kullanılan yağlarda yapımında kullanılmaktadır. PCB'lerin doğal kaynağı bulunmamaktadır ve tamamı sentetik olarak elde edilmektedir. Katı, sıvı, renksiz veya hafif sarı renkte olanları vardır. Evsel ve endüstriyel aktiviteler sonucu çevreye bırakılan PCB atıklarına akut veya kronik maruziyet sonucu direkt veya indirekt olarak canlı organizmalarda toksik etkilere sahip olmaktadır. Bu maddeler maruziyet yoluna, süresine ve doza bağlı olarak birey üzerinde yüksek veya düşük derecede toksik etki göstermektedir. PCB'ler hücre ölümüne neden olabilecek kadar büyük bir hücre hasarı oluşturabilirler. Ya da PCB'lerin metabolizması sonucu oluşan metabolitler DNA, RNA ve proteinler gibi bir takım makro moleküllere kovalent bağlanarak mutajenik veya kanserojenik etki gösterebilmektedir. Bu konuda yapılmış birçok araştırma bulunmaktadır. Zira, bu maddeler dünya üzerinde yaşayan tüm canlıları olumsuz etkilemekte ve onların sağlığını ciddi şekilde bozabilmektedirler. Bu nedenle, PCB'lerin tanımına, özelliklerine, mekanizmasına,tespit yöntemlerine ve insan sağlığına etkilerine hakkında bilgi verilerek su ürünlerine üzerine etkileri hakkında yapılmış araştırmalara bu çalışmada yer verilmiştir.

Anahtar Kelimeler:Oleogel, emulgel, multi-component, stability, rheology, property.

➢ *POSTER PRESENTATION*

Theoretical investigation of the antioxidant activity of resveratrol analogues

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Abstract

Free radicals are produced daily by the body during cell metabolism and functional activities and play an important role in cell signaling, apoptosis, gene expression and ion transport. Oxidative stress is an imbalance between the antioxidant defense system and free radicals, following the excessive production of the latter, causing many diseases: cancer, cardiovascular disease and several infections. There is a great deal of work on chemical compounds exhibiting antioxidant activity because of their important role in preventing oxidative stress diseases. They are also in demand in the pharmaceutical, cosmetic and food industries. Thus, we conducted a theoretical study of antioxidant activity to derive information relating to the chemical reactivity of resveratrol and its analogues. The calculated molecular descriptors allowed us to establish the different mechanisms of the antioxidant action of the selected molecules, in both gas and in ethanol phases. The calculations were carried out via the Gaussian 09 program using the DFT theory.

Keywords: antioxidant activity, resveratrol, molecular descriptors, DFT, Gaussian 09.

➢ *POSTER PRESENTATION*

Theoretical study of the inhibition of the alpha-glucosidase activity by betulinic acid derivatives

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Abstract

According to the world health organization (WHO), diabetes kills more than 1.6 million people per year. There are several types of diabetes drugs including α-glucosidase inhibitors which are given for the treatment of type 2 diabetes (or non-insulin-dependent diabetes). The type 2 diabetes is a disease characterized by high blood sugar. It generally affects the elderly as well as obese people. The inhibition of biological targets involved in certain pathologies is one of the most widespread therapeutic strategies. Thus, a large class of drugs consists of enzyme inhibitors. In this study, we were interested in α-glucosidase inhibitors which are used in the treatment of type 2 diabetes. A recent study has shown an inhibitory effect of certain derivatives of betulinic acid, vis-à-vis this enzyme. We used the techniques of computational chemistry, in particular molecular docking, to carry out an in-silico study of the action of the selected betulinic acid derivatives on α-glucosidase.

Keywords: α-glucosidase, betulinic acid, molecular docking, DFT, protein-ligand interactions.

➢ *POSTER PRESENTATION*

The effects on neuronal differentiation of Trichostatin-A and RG108 combinations using Real Time Cell Analysis

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Abstract

Neurodegeneration, the slow and progressive dysfunction and loss of neurons and axons in the central nervous system, is the primary pathological feature of acute and chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, stroke and multiple sclerosis. The regulation of neuronal differentiation is crucial in developing therapies after nerve injury or in neurodegenerative diseases. Recently, there have been several report suggesting correlations between histone acetylation and other epigenetic regulation including DNA and histone methylation. Histone deacetylase inhibitors is an important therapeutic target in epigenetic mechanisms. DNA methylation anomalies in neurodegenerative diseases have also attracted considerable attention in recent year because this methylation is a crucial epigenetic modification of the genome that is involved in regulating many cellular processes including neurodegeneration. In the present study, we have investigated the roles of RG108, a DNA methyltransferase inhibitor, and Trichostatin-A (TSA), a histone deacetylase inhibitor, in differentiation of NGF-induced PC-12 cells. In this study, it was aimed to determine the effects of TSA and RG108 combinations on PC-12 cell viability by WST-1 method and to evaluate the effect of the selected combination on cell differentiation using Real Time Cell Analysis System (RTCA DP). According to RTCA DP results, we demonstrated that the combination of 50 nM NGF + 100 nM RG108 + 50 nM TSA has significant effects on neuronal differentiation in PC-12 cells. According to neuronal differentiation results, TSA and RG108 combination may serve as a potential candidate for future treatment of neurodegeneration. Therefore, additional studies are needed to support this result.

Keywords: neuronal differentiation, PC-12, Trichostatin-A, RG108

➢ *POSTER PRESENTATION*

Potential application of Lactic acid bacteria in the modulation of the immune response in Balb/c mice immunized by β-lactoglobulin

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Abstract

Lactic acid bacteria strains belong to beneficial bacteria. They are normal inhabitants of the healthy gut microbiota and exert a positive role in human health following oral administration. They improve the balance of the microbial community in the intestine, confer protection against potential pathogenic bacteria, and prevent and/or cure intestinal diseases. The effect of Lactic acid bacteria on murine intestine and systemic response are presented. 48 Balb/c mice were divided in four lots of 12 mice each. During an initial period of 18 days, the animals from the first and second lot received via an oral way suspension of 0.3 mL contain 10^8 CFU/mL of *Lactococcus Lactis* and *Enterococcus facium*. The third (positive control) and fourth lot (negative control) received 0.3 mL of saline solution. Later, mice from the first, second and third lots are immunized via intra-peritoneal way using β-Lactoglobulin (β-Lg). They were sacrificed on the 35th day and histologic studies were conducted on their jejunum fragments. The immune response was explored using murine model of allergy through measuring IgG antiβ-Lg levels by a colorimetric Enzyme-Linked Immunosorbent Assay (ELISA) and by in vivo anaphylactic reactions in β-Lg sensitized mice. Our results demonstrated that *Lactococcus Lactis* and *Enterococcus facium* has not modified the aspect and the structure of the villi, compared to the negative control group. Furthermore, the intra-epithelial lymphocytes infiltrations are little marked. The immune response study shows that challenging mice with β-Lg produce a pronounced allergic reaction. However, the clinical manifestations of the *Lactococcus Lactis* and *Enterococcus facium* fed groups were significantly less important than those from the β-Lg sensitized group. In conclusion, our lactic acid bacteria protect the intestinal epithelium integrity by maintaining the structure of the *villi* and have the ability to modulate the systemic immune response to β-lactoglobulin.

Keywords: β-Lactoglobulin – Balb/c –IgG–intestinal epithelium -–Lactic acid bacteria, anaphylactic reaction.

➢ *POSTER PRESENTATION*

Tahinde *Salmonella* spp. ve *Listeria* spp. Gelişiminin Biyokontrol Potansiyelinin Araştırılması

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Özet

Üretime uygun susam tohumlarının (*Sesamum indicum* L.) tekniğine uygun olarak kabukları ayrıldıktan ve fırında kurutulup kavrulduktan sonra değirmende ezilmesi ile elde edilen ürün, tahin olarak tanımlanmaktadır. Tahin, düşük su aktivitesi, (a_w:0,16-0,25) nedeniyle dayanıklı bir ürün olarak bilinmesine rağmen, susam tohumları, büyüme, depolama veya işleme sırasında *Salmonella* spp. ve diğer bazı patojen mikroorganizmalar ile kontamine olabilmektedir. Bu çalışmada *Lactobacillus plantarum* ve *Lactobacillus alimentarius* 3 adet referans patojen mikroorganizmaya karşı oluşturduğu antagonistik etki tespit edilmiştir. Ayrıca piyasadan toplanan 10 farklı tahin örneğinde *Salmonella* spp. ve *Listeria* spp. varlığı araştırılmıştır. Daha sonra patojen mikroorganizma içermeyen örneklerden biri seçilerek içerisine farklı kombinasyonlarda antagonistik laktik asit bakterileri, *S. typhimurium* ve *Listeria monocytogenes* 6 log kob/g olacak şekilde ilave edilmiş ve 28 gün oda sıcaklığında depolanmıştır. Aynı depolama ve bakteri sayımları, *S. typhimurium* tespit edilen bir örneğe de antagonistik bakteri ilavesi yapılarak gerçekleştirilmiştir. Yapılan analizler sonucunda *L. plantarum*'un *S. typhimurium, L. monocytogenes* ve *Escherichia coli*' ye karşı oluşturduğu inhibisyon zon çapları sırasıyla 24, 27 ve 29 mm, *L. alimentarius*'un oluşturduğu ihnibisyon zon çapları ise sırasıyla 11, 17, 15 mm olarak ölçülmüştür. Piyasa örneklerinden bir tanesinde *Salmonella enterica* serovar *typhimurium* (2,35 log kob/g) tespit edilmiştir. Depolamada her iki patojen açısından en yüksek antagonistik aktiviteyi *L. plantarum* göstermiştir. Depolama sonunda *S. typhimurium* sayısının %34,2 azalmasını, *L. monocytogenes'*in tamamen yok olmasını sağlamıştır. Her iki antagonistik bakterinin ve her iki patojenin de ilave edildiği örnekte ise 3. hafta sonunda *L. monocytogenes* üremesi gözlenmemiştir. Ayrıca içerisinde *S. enterica* serovar *typhimurium* tespit edilen piyasa örneğinde ise *L. plantarum*'un antagonistik aktivitesi ile 3. hafta sonunda üreme gözlenmemiştir. Bu çalışma tahinde görülen patojenlerin antagonist ilavesi ile biyokontrolü üzerine yapılmış ilk çalışma niteliği taşımaktadır.

Anahtar Kelimeler: Tahin, *Salmonella* spp., *Listeria* spp., antagonist, *Lactobacillus plantarum*

➢ *POSTER PRESENTATION*

The investigation of the biocatalytic oxidation reaction of racemic 1-phenylethanol with Baker's yeast

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Abstract

Different enantiomers of chiral substances which are very important in the pharmaceutical industry cause different effects in drugs. While an enantiomer has pharmacological importance, undesirable enantiomer is called as impurity and it may cause side effects and toxic effects. Therefore, separation of these enantiomers is very important for the pharmacology industry. The aim of this study is the production of enantiomerically pure 1-phenylethanol, which is commonly used in the production of pharmaceutical active substances, via the biocatalytic deracemization process containing sequential oxidation-reduction reactions. For this purpose, some parameters affecting the oxidation reaction of rac-1-phenylethanol were investigated in the first step. Baker's yeast (BY) was used as an enzyme source in the reaction. First, the effect of cell concentration for low substrate concentration (1 mM rac-1-phenylethanol) was investigated. As a result, 100% enantiomeric excess and 50% conversion were obtained at the cell concentration of 200 g/L. After the cell concentration was determined, investigations were made at different pH values of the reaction environment. In these studies with 0.05M Tris-HCl buffer, pH 8 was obtained as the suitable pH. Then, in order to obtain high product concentrations, investigations were carried out at higher substrate concentrations (5 mM, 7 mM, 10 mM, 12 mM) with BY. It was observed that when the substrate concentration increased, the conversion and enantiomeric excess values decreased. Considering that the reason for this result may be the cell concentration, experiments were carried out at different cell concentrations (200 g/L, 300 g/L, 500 g/L) with a substrate concentration of 5 mM. But no increase in conversion and enantiomeric excess values were observed. As a result, in the oxidation reaction experiments with BY; the highest enantiomeric excess was obtained as 100%(S) at 1 mM substrate concentration, 200 g/L cell concentration and pH=8.

Keywords: 1-phenylethanol, deracemization, biocatalytic oxidation, Baker's yeast, enantioselectivity.

➢ *POSTER PRESENTATION*

Gene Targeting Studies of the Malaria Parasite DNA Photolyase gene using CRISPR-Cas9 Genome Editing Technology.

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Abstract

Ultraviolet (UV) light damages DNA by converting two adjacent thymine bases into pyrimidine dimers which are potentially mutagenic, carcinogenic, or lethal. In all organisms, except mammals, this damage is repaired by the photolyase enzyme. Dr. Aziz SANCAR has won the Noble Prize for the characterization of the role of a homolog of the photolyase enzyme in higher eukaryotes in the control of the circadian rhythm. Surprisingly, no research has been conducted on the photolyase gene of the malaria parasite or of any other parasitic protozoa. Recently, it was confirmed that a genetically- regulated circadian rhythm tightly controls the development and growth of malaria parasites according to external light stimuli. However, the genetic factors that control this system have not yet been discovered. In order to investigate the role of the malaria parasite DNA photolyase enzyme in the mechanism of the circadian rhythm regulation of the malaria parasite, in addition to its putative function as a DNA repair enzyme, we utilized a novel CRISPR-Cas9 system with enhanced-specificity Cas9 function, to limit the genome off-target endonuclease activity, to investigate the function of DNA Photolyase by targeted gene knockout deletion and knock-in C-terminal GFP tagging. The DNA photolyase knockout parasites failed to grow normally compared to WT parasites, with even more severe growth attenuation when perturbations of ambient light conditions were applied. Moreover, the DNA photolyase knockout parasites failed to recover after UV light exposure compared to WT parasites. Thus, we are initially confirming the role of malaria parasite DNA photolyase in the regulation of the responses to the light/ dark cycle, and an additional function in DNA repair due to UV light exposure. Therefore a more in-depth investigation is needed to detail the molecular function of the DNA photolyase in the malaria parasite and in other pathogenic microorganisms.

Keywords: Malaria, *Plasmodium berghei*, DNA Photolyase, UVB DNA damage, Circadian rhythm, CRISPR-Cas9

➢ *POSTER PRESENTATION*

Apelin Hormonunun Yapısı ve İşlevleri

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Özet

Yağ dokusu vücuda enerji sağlama, yağda eriyen vitaminleri depolama, fiziksel koruma ve ısı üretimi fonksiyonlarına sahiptir. Özellikle beyaz yağ dokusu protein sinyallerini ve adipokin adı verilen faktörleri salgılayan önemli bir endokrin organdır. Adipokin ailesinin peptit yapıdaki hormonlarına yeni eklenen apelin, bağ dokunun özel bir tipi olan adipoz dokudan salgılanmaktadır. Apelin, 1998 yılında sığır midesinin özsuyundan ayırt edilmiştir. Apelin, G-protein kenetli (APJ) reseptörünün endojen bir ligandıdır ve etkilerini APJ'ye bağlanarak ortaya koymaktadır. Apelin ve APJ ekspresyonunun merkezi sinir sisteminin yanı sıra akçiğer, kalp ve meme bezi gibi dokular da fazla olduğu belirtilmektedir. Apelin hormonu, apelin-10, apelin-11, apelin-12, apelin-13, apelin-15, apelin-17, apelin-19 ve apelin-36 gibi çeşitli izoformlara sahiptir. Apelin ve APJ, bir sinyal yolu oluşturarak birçok sistem organlarının (kalp, böbrek, anterior hipofiz gibi) fonksiyonlarında etkilidir. Ayrıca, damar endoteli gibi çeşitli dokularda da yaygın olarak eksprese edilmektedir. Örneğin, kardiyovasküler sistemde kan basıncı ve vasküler tonusun düzenlenmesi, kardiyak kontraktilite ve kalp hızı üzerine etki etmektedir. Bunların dışında, Apelin ve APJ, anjiyogenezis, apoptozis, inflamasyon, glukoz metabolizması, vücut sıvı ve enerji dengesi, açlık ve yemek alımının düzenlenmesi gibi çeşitli etkiler göstermektedir. Apelinin dolaşımda endokrin etki göstermesinin yanında, nörotransmiter olarak da parakrin bir etkisinin olduğunu gözlenmiştir

Bu derlemenin amacı başta kardiyovasküler fonksiyonlar, üregenital sistemdeki değişiklikler, enerji metabolizması, insülin duyarlılığı ve solunum sistemi üzerinde birçok etkisi bulunan ve adipokin ailesine yeni eklenen apelin hormonunun yapısı ve işlevleri hakkında bilgi vererek apelinin rolünün daha anlaşılmasını sağlamaktır.

Anahtar Kelimeler: Apelin, Adipokin, APJ

➢ *POSTER PRESENTATION*

One-step electrochemical fabrication of modified electrode for detection of L-tyrosine

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Abstract

L-Tyrosine (Tyr) is a non-essential amino acid synthesized from phenylalanine and incorporated into proteins in human and herbivore bodies. It is a basic component of proteins and is vital for establishing and maintaining a positive nitrogen balance in the human body. It is a vital precursor of neurotransmitters such as L-dopa, dopamine, norepinephrine, and epinephrine that important for in the mammalian central nervous system. A low level of Tyr could cause albinism and alkaptonuria. However, a high level of Tyr induces Parkinson's disease, depression, and mood disorders. Change of Tyr concentration is also linked to several other diseases such as atherosclerosis and lung diseases [1]. Thus, the selective and rapid determination of Tyr is very important.

The detection of tyrosine is usually accomplished by spectrophotometric, fluorimetric, liquid chromatography-mass spectrometry, gas chromatography–mass spectrometry, high-performance liquid chromatography [2]. These methods could be pretty accurate, but complex sample preperation procedures are necessary and therefore they are relatively expensive and time-consuming.

In this study, it was aimed to determine L-tyrosine electrochemically by developing a modified electrode. Electrochemical properties of L-tyrosine were investigated by cyclic voltammetry method. Optimization studies for L-tyrosine determination were carried out by differential pulse voltammetry method. For the characterization of the modified electrode prepared; cyclic voltammetry and electrochemical impedance spectroscopy methods were used. The morphological properties of the electrode were examined by scanning electron microscopy. Thus, the first measurements will be completed by developing a sensor for the electrochemical determination of Tyr and the analytical aspect of the study will be improved in our future works.

Keywords: L-Tyrosine; modified electrode; cyclic voltammetry; differential pulse voltammetry; sensor.

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➢ *POSTER PRESENTATION*

Antioxidant and cytotoxic properties of aqueous and alcoholic extracts of Spinacia turkestanica on breast cancer (MCF-7 cell line)

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Abstract

Turkestan spinach due to its effective compounds including vitamins A, B, C, D, K, folic acid, high unsaturated fatty acids including Palmitic acid, Lnoleic acid and Linolenic acid, Hexadecatrienoic acid, Chlorophyll, β-Karoten,Lycopene, Flavonoids constitutes, Vitamin E and so on. In this study, the cytotoxic effects of aqueous and hydroalcoholic extracts on MCF-7 breast cancer cell line were investigated. In addition, aqueous and hydroalcoholic extracts were evaluated for their antioxidant activity and phenolic compounds content.Turkestan spinach was obtained from Isfahan Agricultural and Natural Resources Research Center in Iran. Then, hydroalcoholic aqueous extracts of this plant were prepared. MCF-7 cell line was cultured in RPMI-1640 medium containing 10% cow serum in incubator with 5% CO2 and incubated under different concentrations of aqueous and hydroalcoholic extracts for 24, 48 and 72 hours. MTT assay was used to calculate cell viability in the presence and absence of extracts and optical absorption was measured by ELISA at 540 nm. The antioxidant activity of these extracts was measured by DPPH method. Extracts of this plant were also analyzed for total phenol content using Folin Siocalto reagent. Gallic acid was used as the standard compound and total phenol was calculated in mg / g gallic acid. According to the statistical results of this study, there is a cytotoxic effect of Turkestan spinach on MCF-7 carcinoma. The alcoholic extract of this plant showed the highest toxicity in less time than the aqueous extract. Also, total phenol content and antioxidant power of both extracts were measured. Alcoholic extract had the highest antioxidant and phenol potency, which was significant difference between the two extracts. The DPPH results also showed that there is a direct relationship between the amount of phenol antioxidant activity and the higher the amount of phenol, the more the free radicals reduces the extract strength.

Keywords: Cancer; Spinach; ELISA; Extract; Antioxidant

➢ *POSTER PRESENTATION*

Environmentally secure clay-humic composition against plant pests

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Abstract

The most important indicator of the effectiveness of acaricidal drugs is the long-term protective effect, sufficient to block the period of the development cycle of the pest. We have obtained an acaricidal preparation of prolonged acaricidal action based on montmorillonite clay (on which pyrethroid is adsorbed) and potassium humate obtained from peat. Unlike traditional acaricidal preparations, which protect plants from pests for 12-15 days, the period of acaricidal activity of the developed suspension preparation is 20-22 days.

The prolonged action of the developed preparation is ensured due to the gradual release of adsorbed synthetic pyrethroid from the interplanar space of a clay mineral. The presence of potassium humate helps stabilize the water-clay suspension (since it is a structure-forming agent), and ensures reliable fixation of the working solution of the preparation (1% water-clay suspension) on the surface of plant leaves.

The acaricidal activity of the developed formulations was studied in the field against peach aphids Import acaricide "Aktara" (manufactured by the Swiss company Singeta) was taken as a reference standard. The effectiveness of the tested preparations was evaluated by taking into account mortality of aphids 2, 5, 8 and 14 days after spraying.

According to the test results, the effectiveness (mortality of pests on the 14th day after spraying) for the developed clay-humic composition is 94.5%, and in the case of the imported acaricide "Aktara"- 96%. Maximum content of pyrethroid (cypermethrin) in peach fruits is 0.02 mg / kg, while the maximum permissible concentration of cypermethrin in fruits is 10 mg / kg.

By increasing the period of acaricidal activity of the developed composition, the frequency of the necessary treatment of plants is reduced, therefore, the economic costs of protecting against pests are reduced by about 2.5 times.

The developed composition can be successfully included in the system integrated plant protection.

Keywords: acaricidal preparation, clay-humic**,** plant pests

3.2. FULL-TEXT PAPER

➢ ***POSTER PRESENTATION***

α-Amanitin tayini için nanopartikül temelli immüno analiz testinin geliştirilmesi

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Özet

α-Amanitin, düşük moleküler ağırlığa sahip en ölümcül mantar toksinlerinden biridir. Karaciğere ve böbreklere zarar verir ve insanlar için ölümcül olduğu bilinmektedir. Bu çalışmada, α-amanitin toksininin tespiti için bir immünolojik test geliştirilmiştir. Tasarlanan immüno analiz sisteminde anti-amanitin antikoruna bağlı manyetik demir oksit nanopartikülleri ve altın nanopartikülleri prob olarak kullanılmıştır. Yöntem, antikor bağlı manyetik demir oksit nanopartiküllerine bağlanmak için, α-amanitin içeren numune ile α-amanitin bağlı altın nanopartiküllerinin arasındaki rekabeti ve eş zamanlı olarak nanopartiküllerin manyetik ayrımını içermektedir. Testte renk değişimi çıplak gözle izlenebilmektedir, bir UV-Vis spektrofotometre ile konsantrasyon belirlenebilmektedir ve test dakikalar içinde tamamlanabilmektedir. Bu çalışma ile önerilen immünolojik test, mantarlardaki toksinlerin taranması için uygun bir araçtır. Bu basit immünolojik test, toksinlerin, çevresel kirleticilerin ve ilaçların hızlı tespiti için, büyük bir potansiyele sahiptir.

Anahtar Kelimeler: Amatoksinler, α-Amanitin, Manyetik demir oksit nanopartikülleri, Altın nanopartiküller

The development of nanoparticle-based immunoassay test for detection of α-amanitin

Abstract

α-Amanitin is one of the deadliest fungal toxins with low molecular weight. It damages the liver and kidneys, and it is known to be lethal for humans. In this study, for the detection of α-amanitin toxin, an immunoassay was developed. In the designed immunoassay system, anti-amanitin antibody bound magnetic iron oxide nanoparticles and gold nanoparticles were used as probes. The method involves the competition between the α-amanitin-containing sample and the α-amanitin-bound gold nanoparticles for binding to the antibody-bound magnetic iron oxide nanoparticles and simultaneously magnetic separation of the nanoparticles. In the assay, the colour change can be observed with the naked eye, the concentration can be determined with a UV-Vis spectrophotometer and the test can be completed within minutes. The immunoassay proposed through this study is a convenient tool for the screening of toxins in mushrooms. This simple immunoassay has great potential for fast detection of toxins, environmental pollutants, and drugs.

Keywords: Amatoxins, α-Amanitin, Magnetic iron oxide nanoparticles, Gold nanoparticles

GİRİŞ

Funguslar morfolojik olarak yüksek çeşitlilik sergileyen organizmalardır. İçerdikleri çok çeşitli sekonder metabolitler, farmakolojik etken maddeler, toksinler ve enzimler nedeniyle, tıp, gıda, tarım, ilaç ve çevre alanlarında kullanılmaktadır. Makrofunguslar, taksonomik olarak Basidiomycetes'lere dahildir. Yenebilir mantarların yanında, bazı mantarlar ölüme neden olabilecek güçlü toksinler üretmektedirler. Toksik mantarların çoğu, Basidiomycotina'ya dahildir, *Amanita* (Hymenomycetes, Amanitaceae), *Inocybe* (Cortinariceae), *Panaeolus* (Coprinaceae) ve *Russula* (Russulaceae) önemli cinsleri arasındadır. Asya kıtasında 190'dan fazla zehirli mantar türü bildirilmektedir. Bunların 40'tan fazlası çok toksiktir ve ölüme neden olabilmektedir. Halüsinojenik ve zehirli mantarlarda çok kesin olarak türlerin tanımlanması, klinik çalışmalarda, teşhis ve tedavide olduğu kadar, adli amaçlar için de önemlidir. Makrofungusların karakterizasyonu için morfolojik ve mikroskobik inceleme yapılmaktadır, ancak bu yaklaşımlar güvenilir değildir, çevre koşullarına yüksek bağlılıktan dolayı sınırlıdır. Mantar toplanmasında zehirli türler ile yenebilir mantarların ayırt edilememesi temel sorundur, bununla birlikte, göz ardı edilmemesi gereken bir husus, sadece zehirli mantarların değil, aynı zamanda bazı yenebilir mantarların da tehlikeli olabildiğidir. Literatürde, *Pleurotus ostreatus, Volvariella volvacea, Flammulina velutipes, Agaricus bisporus* gibi pek çok yenebilir mantarların da bazı toksinleri içerdikleri (Woo-Sik ve ark., 2014), gastrointestinal, hepatik ve kardiyak toksisitesi gibi çeşitli belirtilerle seyreden zehirlenmelere sebep oldukları, çok sayıda çalışma ile tespit edilmiştir (Jin ve ark., 2014).

Zehirli mantarların içerdikleri toksinler yedi kategoride sınıflandırılır; bunlar siklopeptitler, giromitrin, koprin, muskarin, ibotenik asit, muskimol, psilosibindir. Dünya genelindeki tüm ölümcül mantar zehirlenmelerinin % 90'ından fazlası siklopeptidler içeren, küresel çapta geniş bir yayılma gösteren ve istilacı bir tür olarak değerlendirilen, *Amanita phalloides* türünden kaynaklanmaktadır (Tanahashi ve ark., 2010). *Amanita* mantarlarının içerdiği amatoksinler, bisiklik oktapeptitlerdir. Amatoksinler, dokuz farklı bileşikten oluşmaktadır. α-Amanitin, γ-Amanitin, amaninamid, amanullin ve proamanullin nötr amatoksinler olarak sınıflandırılırken, β-Amanitin, ε-Amanitin, amanin ve amanullinik asit, asidik amatoksinlerdir. Amatoksinler, suda çözünebilir, pişirme veya kurutma yoluyla tahrip edilemeyen, büyük bir ısı kararlılığına sahip, asit degredasyonuna karşı dirençli toksinlerdir. Amatoksinlerin etki mekanizması, hepatositlerde ökaryotik RNA polimeraz II'nin bağlanması ve inhibisyonu yoluyla, DNA transkripsiyon işleminin güçlü inhibitörleri olmasına dayanmaktadır. Özellikle karaciğer ve böbreklerde, nekrozu indükleyerek, hızla organ hasarına yol açmaktadır. (Filigenzi ve ark., 2007)

Amatoksinlerin tespit edilmesi için literatürde mevcut yöntemler arasında, kütle spektrometresi, ince tabaka kromatografisi, kapiler zon elektroforezi ve yüksek performanslı sıvı kromatografisi bulunmaktadır (Garcia ve ark., 2015). Bu tekniklerin en önemli avantajı, yüksek hassasiyette ve spesifik sonuç elde edilebilmesidir. Ancak, dezavantajlı yönleri ise, uzun ön hazırlık ve işlem süresi gerektirmeleri, karmaşık prosedürler içermeleri ve yüksek ekipman maliyeti ve personel eğitimi gerektiren yöntemler olmalarıdır. Ayrıca bu seçkin teknikler yalnızca laboratuvar ortamında çalıştırılabilmektedir.

İmmüno analizler, bir antikor ve bir antijen arasındaki etkileşime dayanan biyoanalitik yöntemlerdir, hızlı algılama süresi, çok çeşitli koşullarda uzun süreli stabilite ve eğitimli personel gerektirmemesi ile, saha testlerinde kolaylık sağlayan, kullanıcı dostu bir formata sahiptirler (Rong-Hwa ve ark., 2010).

İmmüno analizler, yarışmalı veya sandviç tipte çalıştırılabilir. Antijenik yüksek moleküler ağırlıklı moleküller için, sandviç tipi immüno analizler uygulanabilirken, amatoksinler gibi küçük moleküllerin tayini için bu yöntem uygun değildir. Bu sebeple sunulan çalışmada, fungal α-amanitin toksini ile kaplanmış olan altın nanopartiküllerinin, manyetik demir oksit nanopartiküller üzerindeki anti-amatoksin antikoruna bağlanmak üzere, numunelerde aranacak olan α-amanitin molekülleri ile yarışmasına dayanan bir test yöntemi geliştirilmiştir. Numunelerde α-amanitin bulunması durumunda, ilk aşamada manyetik nanopartiküllerin üzerindeki anti-amanitin antikoru ile bağlanma gerçekleşmiştir. Devamında uygulanan α-amanitin kaplı altın nanopartiküller, manyetik kürelere bağlanamayarak, çözeltide kalmaktadırlar ve konsantrasyonları UV-Vis spektrofotometre kullanarak belirlenebilmektedir. Numunelerde α-amanitin bulunmaması durumunda ise, manyetik nanopartiküllerin üzerindeki anti-amanitin antikoruna, altın nanopartiküllerin üzerinde konjuge halde bulunan α-amanitin bağlanmaktadır. Altın nanopartiküllerinin, manyetik nanopartiküllere bağlanarak çözeltiden uzaklaştırılması mümkün olmaktadır.

MATERYAL VE METOD

Materyaller

Manyetik demir oksit nanopartikülleri (Karboksil tanıtılmış, partikül boyutu 30 nm), 1-Etil-3-(3-dimetilaminopropil) karbodiimid, konjugasyon kiti ile birlikte Ocean NanoTech, LLC (San Diego, CA 92126, ABD) firmasından temin edilmiştir. Anti-amatoksin antikoru (Rabbit amatoxin polyclonal antibody), MyBioSource Inc (San Diego, CA 92195-3308, ABD) firmasından satın alınmıştır. α-Amanitin, Altın nanopartiküller (5 nm çapında, karboksilik asit fonksiyonelleştirilmiş, PEG 3000 kaplı, OD 50), Sığır serum albumin, sulfo-N-hidroksi süksinimid, sodyum klorür, sodyum periyodat, sodyum borohidrit ve kullanılan diğer bütün kimyasallar, Sigma-Aldrich (St. Louis, MO, ABD) firmasından satın alınmıştır.

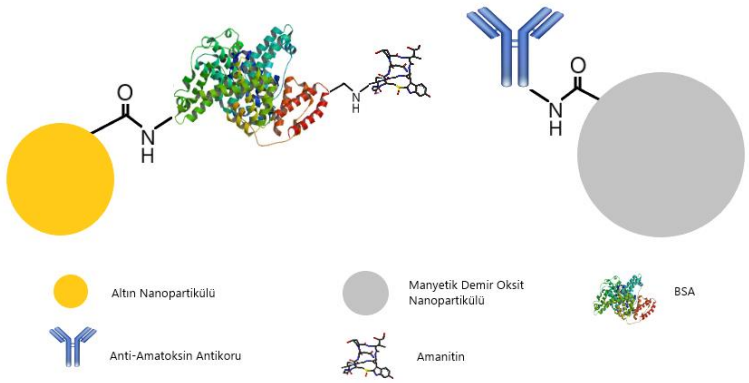
Manyetik Demir Oksit Nanopartiküllerinin Anti-Amatoksin Antikoru ile Konjugasyonu

Anti-amatoksin antikoru ile, manyetik demir oksit nanopartiküllerin konjugasyonu için karbodiimid kimyasından faydalanılmıştır. Konjugasyon protokolü için üreticinin talimatları takip edilmiştir. Prosedür şu şekildedir: 1.7 ml'lik düşük protein bağlayan santrifüj tüpleri içine, 10 µl (5 mg/ml) demir oksit nanopartikülü çözeltisi eklenmiştir ve 5 µl aktivasyon tamponu ilave edilmiştir. Tüpe, 4.25 µl anti-amatoksin antikoru (3 mg/ml, PBS tamponu içinde) ve 10 µl aktivasyon tamponu eklenmiştir. 3 µl EDC (50 mg/ml, aktivasyon tamponu) eklenmiştir ve 2.5 saat boyunca, oda sıcaklığında sürekli karıştırarak inkübe edilmiştir. Süre sonunda, 0.5 µl sönümleme tamponu eklenerek karıştırılmıştır ve 30 dakika sürekli karıştırılarak oda sıcaklığında inkübe edilmiştir. 14000 rpm'de 20 dakika santrifüj edilerek süpernatantı uzaklaştırılmıştır. 25 µl yıkama/depolama tamponunda resüpanse edilmiştir. İkinci kez, 14000 rpm'de 20 dakika santrifüjlenerek

yıkanmış ve süpernatant uzaklaştırılmıştır. 1 µl yıkama tamponu eklenmiştir. 50 µl, %5 (w/v) BSA çözeltisi eklenerek, 30 dakika inkübe edilmişir. Yıkama/depolama tamponu ile tekrar yıkanarak ve santrifüjlenerek süpernatant uzaklaştırılmıştır. Yıkanan nanopartiküller, 10 µl yıkama/depolama tamponu eklenerek, kullanılana kadar 4°C'de saklanmıştır. Sonuç çözeltinin yüklenen antikor miktarı 26 µg/mg demir oksit nanopartikülüdür.

Karboksil Tanıtılmış Altın Nanopartiküllerinin α-Amanitin ile Konjugasyonu

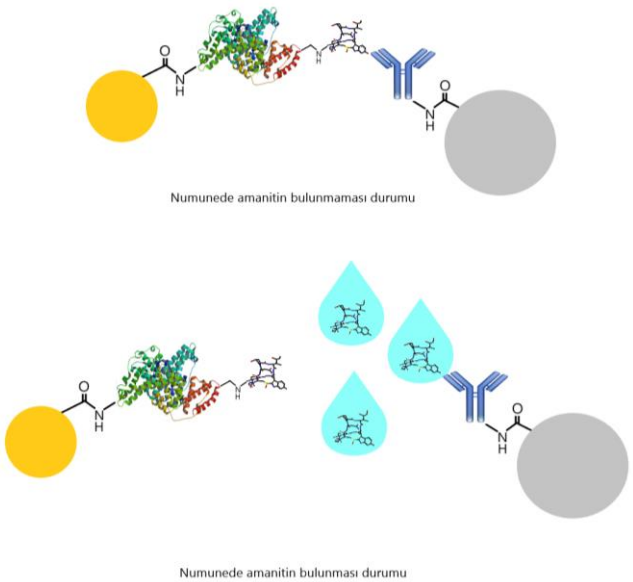
Düşük protein bağlayan özel 1.7 ml'lik santrifüj tüpleri içine, 10 µl (OD 50, ultra saf suda) karboksil tanıtılmış altın nanopartikülü çözeltisi eklenmiştir ve 10 µl EDC/NHS karışımı çözeltisinden (EDC 30 mg/ml ve NHS 36 mg/ml, MES tamponunda) ilave edilmiştir. 30 dakika oda sıcaklığında inkübe edilmiştir. 1 ml PBST tamponu eklenmiş ve vortekslenmiştir. 14000 rpm'de 20 dakika santrifüjlenerek, süpernatantı uzaklaştırılmıştır. 10 µl BSA (10 mg/ml, PBS tamponu içinde) eklenmiştir. 3 saat boyunca, oda sıcaklığında sürekli karıştırarak inkübe edilmiştir. Süre sonunda, 1 ml PBST tamponu eklenerek karıştırılmıştır ve 14000 rpm'de 20 dakika santrifüj edilerek süpernatantı uzaklaştırılmıştır. Nanopartiküller, 20 µl yıkama/depolama tamponu eklenerek, kullanılana kadar 4°C'de saklanmıştır. Nanopartiküller, santrifüj edilerek, süpernatantı uzaklaştırılmıştır ve 10 µl pH 9.0, 0.05 M karbonat tamponunda resüspanse edilmiştir. α-Amanitinin yapısındaki terminal hidroksiller üzerinden periyodat oksidasyonu ile BSA yapısındaki amin grupları arasındaki konjugasyon işlemi, Bever ve ark., 2018'e göre gerçekleştirilmiştir (Bever ve ark., 2018). Prosedür şu şekildedir: α-amanitin (0.5 mg) 250 µL ultra saf suda çözülmüştür. $NaIO_4$ (109 µL, 40 mM suda) eklenmiştir ve çözelti rengi, açık sarıdan, koyu sarı renge dönüşene kadar, 20 dakika boyunca karanlıkta oda sıcaklığında karıştırılmıştır. Aktive edilen α-amanitin çözeltisi (11.7 µL), BSA kaplı altın nanopartiküller üzerine eklenmiştir ve 2 saat oda sıcaklığında karanlık ortamda karıştırılmıştır. $NaBH_4$ (0.83 µL, 0.1 M, 10 mM NaOH içinde) eklenmiştir ve sonuç karışımı 2 saat oda sıcaklığında karanlıkta karıştırılmıştır. İnkübasyon sonunda, 25 µl yıkama/depolama tamponu ile iki defa yıkanarak, santrifüjlenmiş ve süpernatant uzaklaştırılmıştır. Yıkanan nanopartiküller, 20 µl yıkama/depolama tamponu eklenerek, kullanılana kadar 4°C'de saklanmıştır. Elde edilen antikor ve toksin konuge edilen nanopartiküller Şekil 1'de şematize edilmiştir.



Şekil 1. Altın ve demir oksit nanopartiküllerine α-amanitin ve anti-amatoksin antikoru konjugasyonu

Yarışmalı İmmuno Analiz Sisteminin Tasarımı

Anti-amatoksin antikorları ile hazırlanan sistemin ilk aşamasında, süspanse demir oksit nanopartikülleri, α-amanitin içeren sıvı numune ile inkübe edilmiştir. İkinci aşamada, sisteme altın nanopartikülleri eklenmiştir. Numunede α-amanitin bulunmuyorsa, altın nanopartikülleri, demir oksit nanopartiküllerine bağlanmaktadır ve çözelti şeffaf kalmaktadır. Dış bir manyetik alan uygulanarak, manyetik demir oksit nanopartikülleri, altın nanopartiküllerle bağlı halde, çözeltiden kolayca uzaklaştırılmaktadır. Numunede α-amanitin bulunması durumunda ise, demir oksit nanopartiküllerine bağlı olan anti-amatoksin antikorlarına, numunedeki serbest α-amanitin molekülleri bağlanmaktadır. Sisteme altın nanopartiküllerinin eklenmesi ile, numunedeki α-amanitin konsantrasyonuyla ters orantılı olarak, işgal edilmiş olan bağlanma yerleri nedeniyle, altın nanopartikülleri bağlanmaksızın, çözeltide kalmaktadır. Oluşan renk değişimi, çıplak gözle izlenebilmektedir ve toksin konsantrasyonuyla ters korelasyon içindedir. Süpernatantın UV-Vis absorpsiyon spektrası alınarak, sıvı analiz çözeltisindeki altın nanopartikül miktarı belirlenmiştir. Tasarlanan analiz sistemi, numunede α-amanitin bulunmaması ve bulunması durumu için Şekil 2'de şematize edilerek açıklanmıştır.



Şekil 2. Numunede α-amanitin bulunmaması ve bulunması durumunda sistemin çalışması

Prosedür Optimizasyonu

Test için optimum altın ve demir oksit nanopartiküllerinin dozlarının araştırılması için, demir oksit nanopartiküllerinin miktarı 10 µl'de sabit tutulurken, eklenen altın nanopartiküllerinin miktarı, 1, 5, 10, 15, 20 µl olarak değiştirilmiştir, 20 dakika boyunca inkübe edilmiştir. Manyetik ayırımdan sonra süpernatant UV-Vis spektrofotometre ile analiz edilmiştir. Deneyler çift tekrar olarak çalışılmıştır. Altın nanopartiküllerinin, manyetik nanopartiküllere tutunarak, tamamen süpernatanttan ayrıldığı en düşük konsantrasyonu belirlenmiştir.

Optimum Test Zamanının Belirlenmesi

Sistem, iki aşamada çalıştırıldığından, her iki aşama için optimum test zamanı araştırılmıştır. İlk aşamada ortamda serbest α-amanitin olmaksızın, altın nanopartikülleri ve demir oksit nanopartiküllerinin bağlanma süresi, inkübasyon süresi 0, 1, 5, 10, 20 ve 30 dakika olarak değiştirilerek incelenmiştir. Belirlenen tespit süresi, ikinci aşamada uygulanmıştır. Sistemin ikinci aşaması için, numune içindeki serbest α-amanitin moleküllerinin manyetik demir oksit nanopartikülleri tarafından yakalanma zamanının belirlenmesi için, α-amanitin içeren numune olarak ultra saf su kullanılmıştır ve test farklı inkübasyon süreleri (1, 5, 10, 20 ve 30 dakika) boyunca çalıştırılmıştır. Farklı test süreleri sonunda renk değişimleri ve spektrofotometre taramaları değerlendirilmiştir ve optimum yakalama ve tespit süresi tanımlanmıştır.

Tuz Konsantrasyonunun Etkisinin Belirlenmesi

Sistemin yanlış sonuç vermesine sebep olabilecek agregasyonların oluşmasına engel olmak amacıyla, testin optimum çalışacağı tuz konsantrasyonunun belirlenmesi için, α-amanitin çözeltisine 0, 0.01, 0.05, 0.1 ve 0.5 M son derişimlerinde olmak üzere, sodyum klorür eklenerek test çalıştırılmıştır. Farklı tuz konsantrasyonlarında elde edilen sonuçlar, spektrofotometre taramaları ile değerlendirilmiştir ve sistemin optimum tuz konsantrasyonu belirlenmiştir.

α-Amanitin Tespit Prosedürü

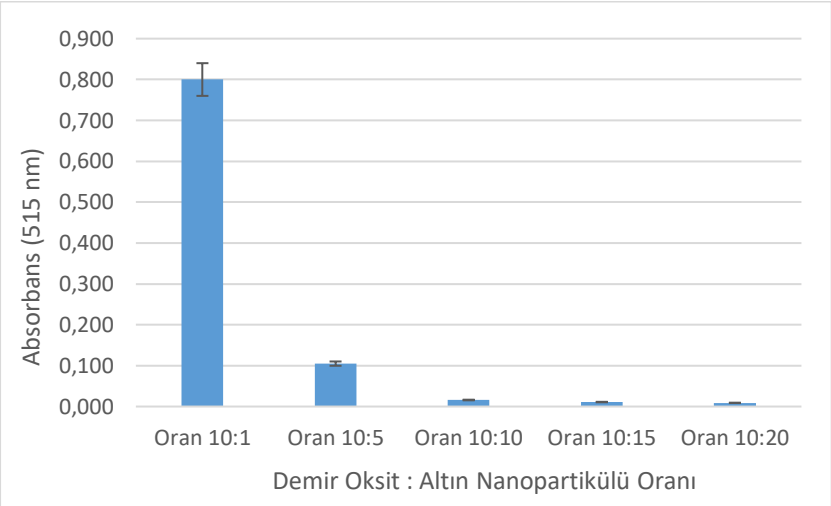
Farklı konsantrasyonlarda (0.05, 1.0, 2.0, 5.0, 10.0, 20.0, 30.0, 50.0, 100.0 µg/ml) α-amanitin içeren, 20 µl ultra saf su içindeki çözeltiler, antikor konjuge edilmiş 10 µl demir oksit nanopartikülleri ile 10 dakika inkübe edilmiştir. Daha sonra, 10 µl α-amanitin konjuge edilmiş altın nanopartikülleri eklenmiş ve 10 dakika boyunca karıştırılarak inkübe edilmiştir. İnkübasyondan sonra, oluşan kompleks yapılar, manyetik olarak uzaklaştırılmıştır ve süpernatant, UV-Vis spektrofotometre ile incelenmiştir.

Mantar Özütünde Testin Çalıştırılması

Kültür mantarlarının özütünün hazırlanması için, 50 gram mantar, 250 mL ultra saf su içinde bir mutfak robotu kullanılarak parçalanmış ve süzülmüştür. Mantar özütü içine, optimum α-amanitin miktarı dozlanmıştır ve test çalıştırılmıştır. Test tamamlanarak, manyetik ayırmadan sonra, süpernatant spektrofotometre ile ölçülmüştür.

BULGULAR ve TARTIŞMA

Altın ve demir oksit nanopartiküllerinin optimum dozları, demir oksit nanopartiküllerinin miktarı 10 µl'de sabit tutulurken, sisteme 1, 5, 10, 15, 20 µl altın nanopartikülü eklenmiş ve 20 dakika boyunca inkübe edilerek araştırılmıştır. Altın nanopartiküllerinin, manyetik nanopartiküllere tutunarak, tamamen süpernatanttan ayrıldığı en düşük konsantrasyon belirlenmiştir. Şekil 3'de görüldüğü üzere, 10 µl altın nanopartikülü eklendiğinde, neredeyse tamamı çözeltiden uzaklaştırılabilmiştir, altın nanopartikülünün miktarının arttırılması sonucu anlamlı derecede etkilememiştir. Bu nedenle bu analiz için 10 µl demir oksit ve 10 µl altın nanopartikülü optimum dozlar olarak belirlenmiştir.

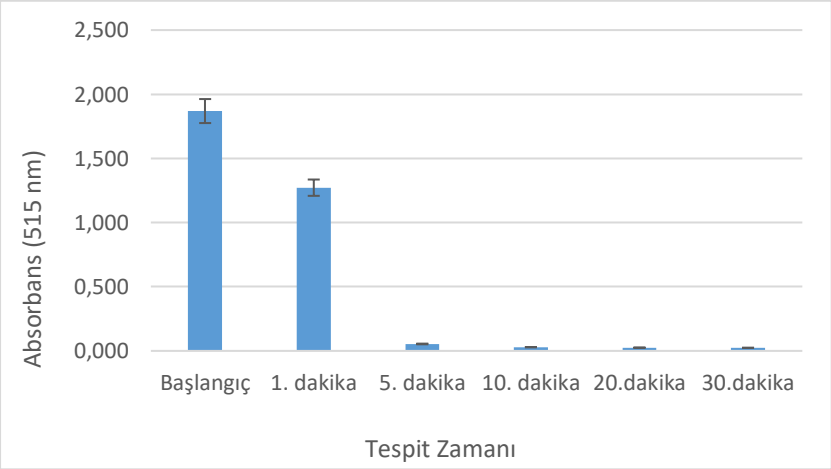


Şekil 3. Optimum demir oksit: altın nanopartikülü dozunun belirlenmesi

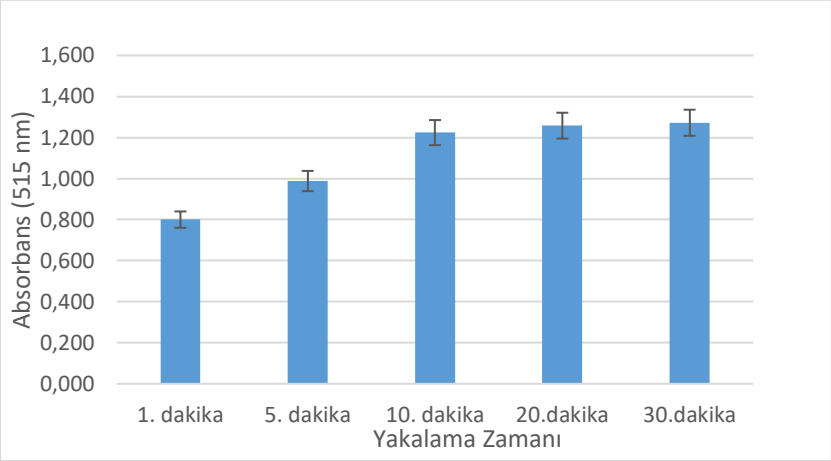
Optimum Test Zamanının Belirlenmesi

Tespit süresi, ortamda serbest α-amanitin olmaksızın, altın nanopartikülleri ve demir oksit nanopartiküllerinin bağlanması için inkübasyon süresi 0, 1, 5, 10, 20 ve 30 dakika olarak değiştirilerek incelenmiştir. Farklı tespit sürelerinin etkisi Şekil 4'de sunulmuştur. Çözeltinin renginin tamamen kaybolması 10 dakika içinde gerçekleşmiştir.

Numune içindeki serbest α-amanitin moleküllerinin manyetik demir oksit nanopartikülleri tarafından yakalanma zamanının belirlenmesi için, α-amanitin içeren numune olarak, ultra saf suda 5.0 µg/ml α-amanitin çözeltisi kullanılmıştır ve test farklı inkübasyon süreleri (1, 5, 10, 20 ve 30 dakika) boyunca çalıştırılmıştır. İlk aşamada belirlenen tespit süresi, bu aşamada uygulanmıştır. Optimum yakalama süresi 10 dakika olarak belirlenmiştir. Farklı yakalama sürelerinin etkisi Şekil 5'de sunulmuştur.



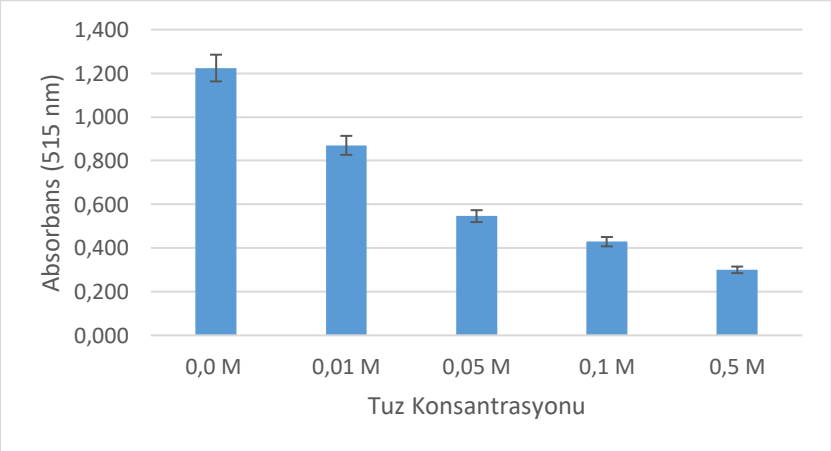
Şekil 4. Optimum tespit süresinin belirlenmesi



Şekil 5. Optimum yakalama süresinin belirlenmesi

Tuz Konsantrasyonunun Etkisinin Belirlenmesi

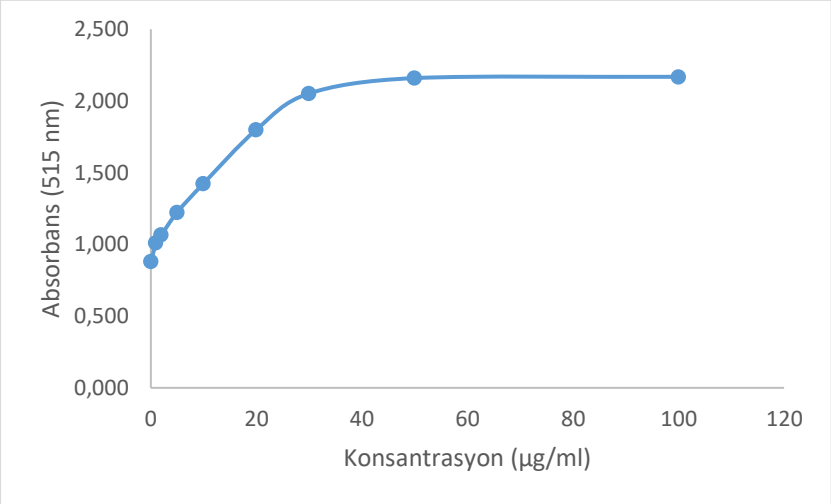
Testin optimum tuz konsantrasyonunun belirlenmesi için, α-amanitin çözeltisine 0.00, 0.01, 0.05, 0.10 ve 0.50 M son derişimlerinde olmak üzere, sodyum klorür eklenerek test çalıştırılmıştır. Farklı tuz konsantrasyonlarında elde edilen sonuçlar, Şekil 6'da sunulmuştur. 0.01 M ve daha yüksek tuz içeriği durumunda, absorbans azalma göstermiştir, bu nedenle tuz içermeyen şartlarda deneyler sürdürülmüştür.



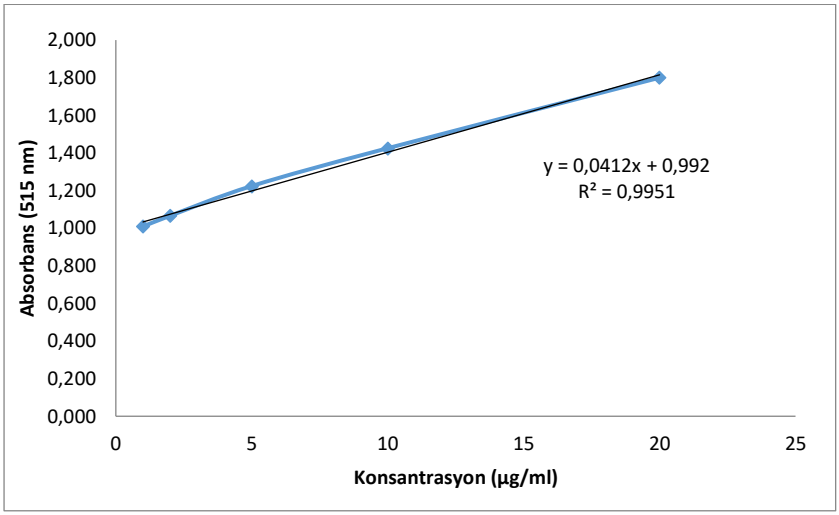
Şekil 6. Tuz konsantrasyonunun etkisi

α-Amanitin Tespit Prosedürü

Geliştirilen test prosedürü ile, α-amanitin tayininin açıklanabilmesi için, 20 µl ultra saf suda, farklı α-amanitin konsantrasyonları (0.05, 1.0, 2.0, 5.0, 10.0, 20.0, 30.0, 50.0, 100.0 µg/ml) dozlanan çözeltiler, 26 µg/mg anti-amanitin antikoru konjuge edilmiş 10 µl demir oksit nanopartikülleri ile 10 dakika inkübe edilmiştir. Daha sonra, 10 µl α-amanitin konjuge edilmiş altın nanopartikülleri eklenmiş ve 10 dakika boyunca karıştırılarak inkübe edilmiştir. İnkübasyondan sonra, oluşan kompleks yapılar, manyetik olarak uzaklaştırılmıştır ve süpernatant, UV-Vis spektrofotometre ile incelenmiştir. Sonuçlar Şekil 7'de sunulmuştur. 0.05-5.0 µg/ml aralığında süpernatant transparanttır, 10.0 µg/ml ve üzerindeki konsantrasyonlarda renk daha koyudur. 515 nm'deki absorbanslar ölçülerek grafiğe aktarılmıştır. 1.0-20.0 µg/ml aralığında doğrusal bir değişim sergilemiştir. 30.0 µg/ml ve üzerindeki konsantrasyon değerlerinde, önemli bir absorbans artışı elde edilmemiştir. Kalibrasyon eğrisi 1.0-20.0 µg/ml aralığı için, y=0.0412 x + 0.992 denklemi ve 0.9951 korelasyon katsayısı ile elde edilmiştir ve Şekil 8'de sunulmuştur. Tespit sınırı 1.0 µg/ml olarak belirlenmiştir.



Şekil 7. Absorbans değişimi



Şekil 8. Kalibrasyon eğrisi

Mantar Özütünde Testin Çalıştırılması

Mantar özütü içine, 5.0 µg/ml α-amanitin dozlanmıştır ve test çalıştırılmıştır. Test tamamlanarak, manyetik ayırmadan sonra, süpernatant spektrofotometre ile ölçülmüştür. Örneklerin renkli ve opak olması, özellikle gözle yapılan gözlemler için sonuçları etkileyebilen bir faktördür. Numune renginin etkisini değerlendirmek için, kültür mantarı içinde çalıştırılan sistemde, manyetik ayrımdan sonra süpernatant görüntüsü alınmıştır ve spektrofotometrik ölçümü yapılmıştır. Renk farkı, kontrolden net olarak ayırt edilebilmektedir, renkli numunelerde de başarıyla çalışabileceği gösterilmiştir.

SONUÇ

Bu çalışmada, ölümcül fungal toksinlerden biri olan α-amanitin tayinine yönelik, hızlı, kolay uygulanabilir ve çıplak gözle gözlenebilen bir analiz yöntemi geliştirilmiştir. α-Amanitinin sekiz aminoasitten oluşan, düşük moleküler ağırlıklı, prolin içeren bisiklik yapısı nedeniyle immüno analiz yöntemi geliştirmek zordur. Konjugasyon işlemleri için sıklıkla tercih edilen fonksiyonel gruplardan yoksundur. Yapısal olarak bu dezavantajlı durumdan kaynaklı olarak, çoğu amatoksin tayini çalışmasının, α-amanitin dışındaki amatoksinler üzerine yoğunlaştığı ve α-amanitin çalışmalarının geri planda kaldığı görülmektedir. α-Amanitin, kanser hastalığıyla mücadelede kullanım potansiyeli olan bir bileşik olduğundan, son yıllarda yoğun olarak, farklı bağlayıcı moleküller ve ligandlar kullanılarak, antikorlara bağlanmaya çalışılmaktadır. Geliştirdiğimiz analiz yönteminde ise, α-amanitin molekülünün nanopartiküllere konjugasyonu için, toksine özgün bir poliklonal antikor kullanılmıştır, böylece antikorun, sistemde hem yakalayıcı bir prob, hem de aynı zamanda bir toksin bağlayıcı ligand işlevi görmesi sağlanmıştır. Farklı miktarlarda α-amanitin içeren su ve mantar özütü örneklerinde, α-amanitin konsantrasyonuyla korelasyon içinde olan bir renk değişimi ve renk yoğunluğu elde

edildiğinden, çıplak gözle toksin varlığı tespit edilebilmektedir. Arazi çalışmalarında, herhangi bir analitik cihaza ihtiyaç olmaksızın, toksin varlığının belirlenebilmesi, geliştirilen testin en önemli faydalarından biridir. Analiz, antikor-antijen etkileşimine dayanan bir yönteme dayanmaktadır. Kolorimetrik tayin yaklaşımlarında, genellikle sadece altın nanopartiküllerinin optik özelliklerden faydalanıldığı görülmektedir. Bu çalışmada ise, altın nanopartiküllerinin, tanecik boyutuna bağlı renk değiştirme özelliği ile birlikte, sisteme demir oksit nanopartikülleri de dahil edilerek, bu taneciklerin manyetik özelliklerinden de faydalanılmıştır. Bu şekilde, çözeltiden toksinin yakalanması ve uzaklaştırılması, eş zamanlı olarak sağlanabilmektedir ve literatürde benzer bir toksin tayini yaklaşımı mevcut değildir. Testin kullandığı yenilikçi yaklaşım, başka toksinlerin, uyuşturucu maddelerin ve ilaç etken maddelerinin tayinlerinin gerçekleştirilmesinde yüksek bir potansiyele sahiptir.

TEŞEKKÜR

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➢ ***POSTER PRESENTATION***

Optimization of THP-1 Monocyte / Macrophage Polarization Using PMA

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Abstract

The tumor microenvironment continues to be studied as an important topic in tumor biology for a long time. The microenvironment defines non-cancer cells and structures within tumor tissue. Cells in this tumor microenvironment are known as cells of a wide variety and of many origins, including endothelial cells, cancer associated fibroblasts (CAFs), tumor associated macrophages (TAMs) or dendritic cells. Especially, TAMs are cells that are at the center of the important relationship between chronic inflammation and cancer. TAMs can differentiate into M1 and M2 macrophages depending on the stimuli in their environment, and this differentiation is very important for co-culture studies. Studies have shown that PMA is the most effective differentiation agent to obtain THP-1 monocyte-derived macrophages, but in these studies, many PMA concentrations were applied during different incubation times. Therefore, our study was conducted to determine the optimal PMA concentration to be used to differentiate THP-1 human monocyte cells into M0 macrophage cells. Firstly, the effect of PMA concentrations (1, 10, 100 and 1000 ng/mL) on cell viability at 24-hour incubations with the WST-1 method was investigated. Later, the appropriate PMA concentration was investigated by determining the levels of CD-14 and CD-68 antibodies by flow cytometry in THP-1 and THP-1 origin macrophage differentiation. According to the flow cytometry results, the lowest CD-14 (5.5%) and the highest CD-68 (58.2%) levels were determined as the appropriate concentration to be used to differentiate 100 ng/mL PMA THP-1 cells. In addition, it was determined that 100 ng/mL PMA had no toxic effect on the differentiation of THP-1 cells to M0 macrophage. As a result of these activities will be held to the light macrophage differentiation studies to be conducted in the future.

Keywords: THP-1, macrophages, PMA

INTRODUCTION

Cancer is defined as the uncontrolled proliferation of cells due to various genetic and epigenetic disorders and is the second leading cause of death in the world after cardiovascular diseases. The reason cancer is a complex mechanism; proliferation, induced angiogenesis, invasion and activation of metastasis, resistance to cell death, and immunological mechanisms by the tumor microenvironment (Siegel et al., 2019, Denisenko et al., 2018). The microenvironment defines non-cancer cells and structures within the tumor tissue, and 5-40% of the tumor mass in solid tumors consists of tumor-associated macrophages (Tsai et al., 2014; Sousa et al., 2015). Tumor-associated macrophages are cells at the center of the important relationship between chronic inflammation (Mosser and Edwards, 2008; Martinez and Helming, 2009), cancer and play a key role in the development of cancer progression, angiogenesis, cancer metastasis, and drug resistance (Tsai et al., 2014). Macrophages can express pro-tumoral and anti-tumoral functions in the tumor microenvironment due to their ability to differentiate into different phenotypes in response to different microenvironmental signals. This is a distinctive feature of monocyte-macrophage-derived cells from other microenvironmental cells, which complicates the role they play in tumor development (Mantovani et al., 2014). Macrophages are studied as two functionally separate forms; pro-inflammatory subgroup (classically activated, type I or M1) and anti-inflammatory / regulatory subgroup (alternative activation, type II or M2) (Mills et al., 2000; Anderson and Mosser, 2002; Foey, 2014). In our study, we aimed to optimize M0 macrophage differentiation, which is the basis of all these M1 and M2 macrophage transformations. In this context, THP-1 monocyte cells were used, a human leukemia monocytic cell line that is widely used to study monocyte / macrophage functions, mechanisms, signaling pathways, and nutrient and drug action. This cell line is a common model for investigating the modulation of monocyte and macrophage activities. Therefore, THP-1-derived macrophages are one of the most viable

options to study cell-cell interaction in vitro and are proposed as an important model in studies to better mimic in vivo conditions (Chanput et al., 2014). THP-1 human monocyte cells; It has been confirmed that PMA is differentiated into macrophage (M0) cells by various stimulating agents, such as phorbol 12-myristate 13-acetate (PMA), 1α, 25-dihydroxy vitamin D3, macrophage colony stimulating factor (M-CSF), and PMA It has been explained to be the most effective differentiation agent to achieve (Chanput et al., 2013; Bastiaan-Net et al., 2013; Chanput et al., 2014). In our study, it was aimed to determine the optimized PMA concentration to be used for the differentiation of THP-1 monocyte cells into THP-1 origin macrophage cells (M0 macrophage).

MATERIALS AND METHODS

Cell lines and cell culture

THP-1 Human Monocyte Cells (ATCC; TIB-202 ™) were obtained from the American Type Culture Collection. THP-1 cells were grown in an RPMI 1640 medium supplemented with 2 mm L-glutamine and 10% fetal bovine serum, 1% penicillin/streptomycin at a temperature of 37ºC in a humidified incubator with a 5% CO2 atmosphere. Powdered PMA (Catalog No: P8139, Sigma) was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution and it was diluted to the required concentrations. A total of 70–80% confluent cells were used in the cell culture experiments.

WST-1 cytotoxicity assay

In order to obtain non-cytotoxic concentrations of the PMA, the viability of THP-1 cells was measured by using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt (WST-1) assay (Roche, Mannheim, Germany). The test is based on the cleavage of the tetrazolium salt WST-1 in formazan by mitochondrial dehydrogenases in viable cells. The formazan dye was quantified in a scanning multi-well spectrophotometer by measuring the absorbance of the dye at 420 nm. THP-1 cells were plated onto 96-well culture plates at 1×10^4 density per well. After 24 h, the cells were treated with (1, 10, 100 and 1000 ng/mL) concentrations of PMA for 24 h. After the incubation period, the cell proliferation reagent WST-1 (10 µL per well) was added to the wells; and absorbances were measured after 3 h using a Cytation 3 cell imaging multi-mode reader at 420 nm (Biotech, Winooski, VT, USA). The measured absorbances directly correlated to the number of viable cells. The cell viability rates were expressed as a percentage of the controls (Engür and Dikmen, 2017).

Determination of THP-1 human monocyte cell differentiation studies into THP-1 derived macrophage (M0) by flow cytometry

To determine the appropriate concentration of PMA to be used in M0 polarization / the specific surface antibody specific to the monocyte macrophage type was selected, analyzes were made by flow cytometry method. In this context, CD-14 (Catalog No: 367116, Biolegend) was used as the monocyte marker and CD-68 (Catalog No: 333806, Biolegend) was used as the M0 marker. THP-1 was incubated with concentrations of PMA for 24 hours to optimize the differentiation of human monocyte cells into M0 macrophage cells. Afterwards, they were left to a 48-hour rest incubation with fresh medium to eliminate the effect of PMA. "Biolegend Cell Surface Immunofluorescence Staining Protocol" was applied for CD-14 and CD-68 surface antibody. Finally, the samples analyzed on a BD Accuri™ C6 Flow Cytometry and at least 10,000 cells were analyzed per sample.

Statistical analysis

Graphics were drawn using GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego California USA) and statistically analyzed with one-way analysis of variance (ANOVA) and Tukey's post hoc test. The results are expressed as mean ± standard deviation and the means of three independent experiments (n=8 for cytotoxic assays), *$p<0.5$, **$p<0.01$, ***$p<0.001$ ****$p<0.0001$ were considered to be significant compared to the control group. $p>0.5$ values were accepted as non-significant.

RESULTS and DISCUSSION

WST-1 results

Cells were cultivated in 96-well plates in medium with 10,000 cells per well, and the effect of PMA concentrations (1, 10, 100 and 1000 ng / mL) on cell viability at 24-hour incubations was investigated by the WST-1 method.

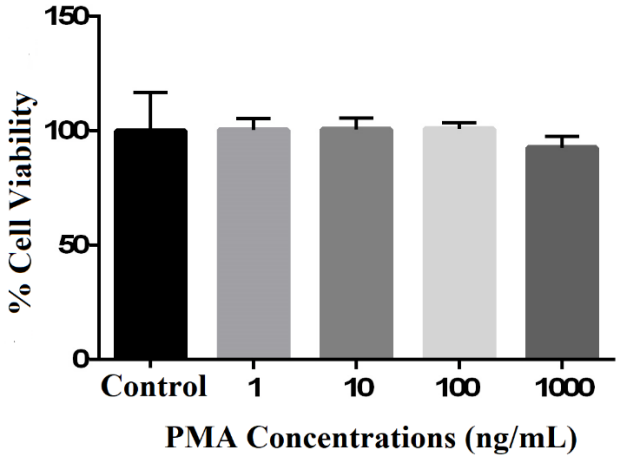

Figure 15. The cells were treated with different concentration of PMA for 24 h and cell cytotoxicity was determined by WST-1 assay. Results are expressed as mean ± standard deviation and the means of three independent experiments (n=8), Control: 0.1% DMSO

The viability values (%) at 24 hours in THP-1 cells were calculated as 100.44, 100.65, 100.98 and 92.66 at PMA concentrations of 1, 10, 100, 1000 ng / mL, respectively, compared to the control. Statistically, significance was not determined in any group compared to the control group. According to this result, it was determined that 100 ng / mL PMA to be applied during 24 hours of incubation in the differentiation of THP-1 cells to M0 macrophage has no toxic effect (**Figure 1**).

In some studies, for PMA concentration in THP-1 cells; It was observed that 30 ng/mL (Bongiovanni et al., 2015), 10 ng / mL (Smith et al., 2015), 500 ng / mL were used (Sueki et al.,2014). For this reason, the 100 ng / mL PMA concentration that we used and did not show cytotoxic effects in cells is among the PMA concentrations used in these studies.

Evaluation of CD-14 and CD-68 antibody levels in THP-1 and THP-1 origin macrophage transformation by flow cytometry method

In order to determine the most appropriate PMA concentration to be used in differentiating THP-1 human monocyte cells into M0 macrophage cells, 5, 10, 20 and 100 ng / mL concentrations of PMA were applied during the 24-hour incubation period. Later, the cells were left to a 48-hour rest incubation with fresh medium to eliminate the effect of PMA. As the control group, CD-14 and CD-68 levels were determined in THP-1 cells by flow cytometry without PMA concentrations and the results are shown in **Figure 2** and **Table 1**. For the control of differentiation, CD-14 (THP-1 marker) and CD-68 (M0 marker) antibodies were determined in flow cytometry method in M0 cells and the results are shown in **Figures 3,4** and **Table 1**.

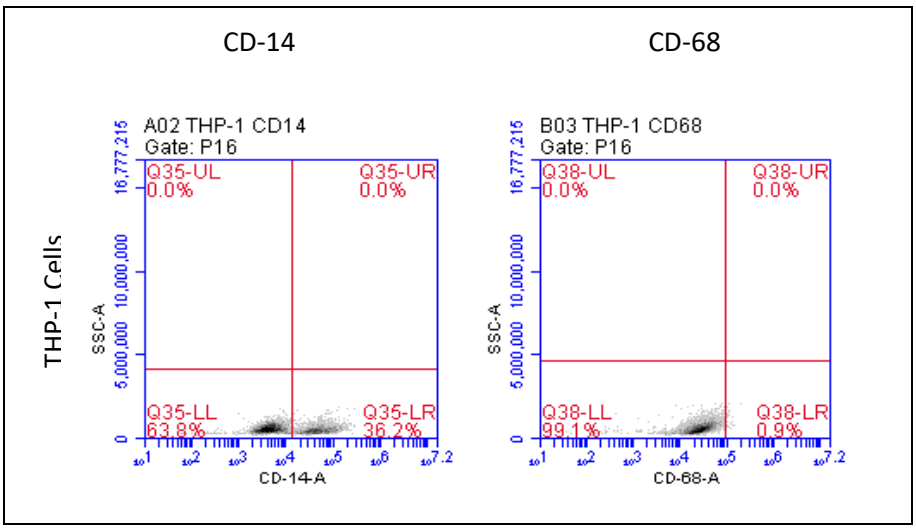

Figure 2. CD-14 and CD-68 levels determined by flow cytometry in THP-1 cells (control).

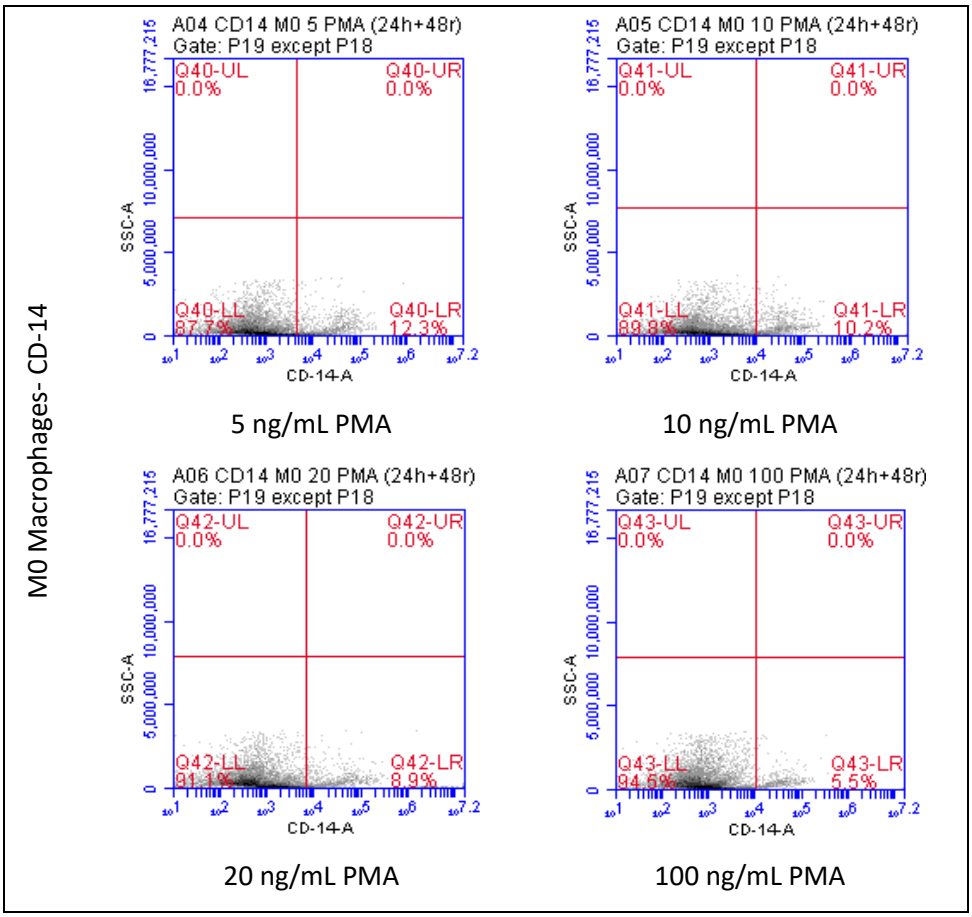

Figure 3. Determination of CD-14 levels by flow cytometry in THP-1 origin macrophages (M0) differentiated by application of PMA at different concentrations.

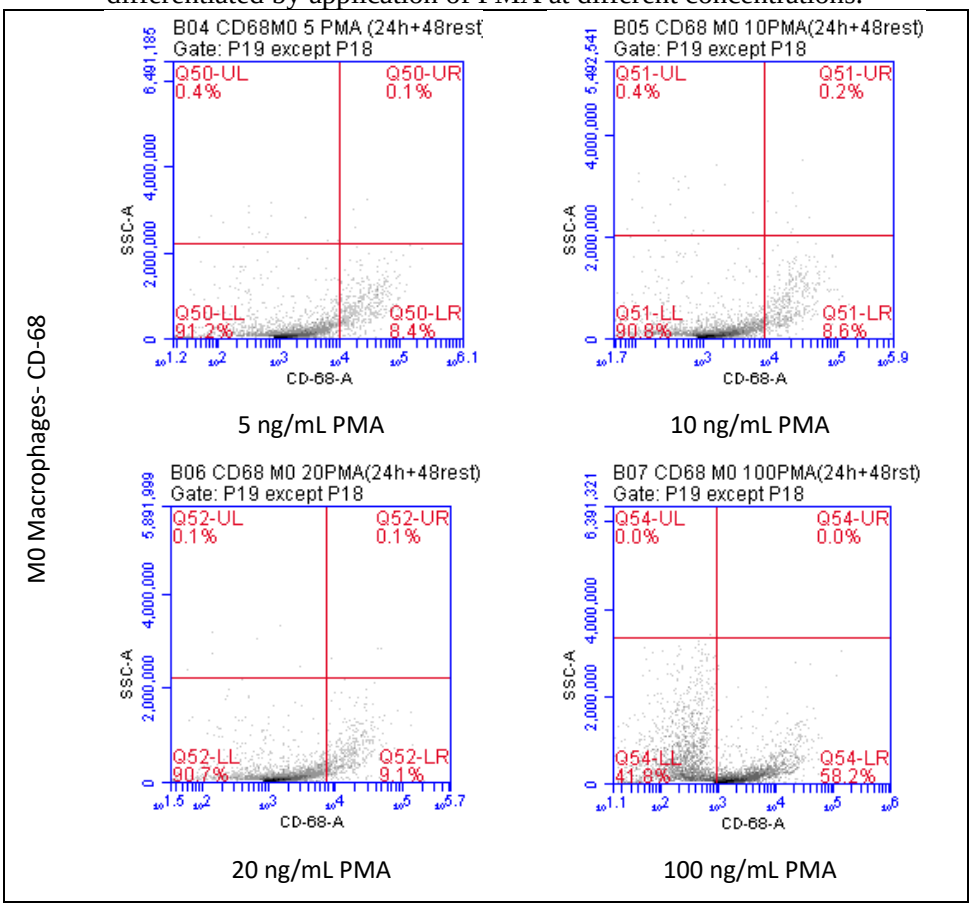

Figure 4. Determination of CD-68 levels by flow cytometry in THP-1 origin macrophages (M0) differentiated by application of PMA at different concentrations.

Table 1. *CD-68 and CD-14 levels in THP-1 origin Macrophages (M0) differentiated by administration of different concentrations of PMA.*

	THP-1	M0			
PMA (ng/mL)	THP-1	5	10	20	100
CD-14 (THP-1 Marker)	%36.2	% 12.3	%10.2	%8.9	%5.5
CD-68 (M0 Marker)	%0.9	%8.4	%8.6	%9.1	%58.2

As seen in Figure 2 and Table 1, levels of CD-14, a marker specific to THP-1 cells, were found to be 36.2% positive in the control group. CD-68, a macrophage marker, was not found in our THP-1 control cells (0.9%). This result shows us that our THP-1 cells that we used in our experiments are healthy and usable. CD-14 and CD-68 levels were analyzed to determine the PMA concentration to be used in the macrophage differentiation process. According to the flow cytometry results, the lowest CD-14 (5.5%) and the highest CD-68 (58.2%) levels and 100 ng / mL PMA were determined as the concentration we would use to differentiate THP-1 cells (**Figures 3,4** and **Table 1**).

When a comprehensive literature review is made, in in vitro studies of macrophage cells and cancer cells in co-culture medium, THP-1 human monocyte cells; It has been reported to differentiate into macrophage cells by various stimulating agents such as phorbol 12-myristate 13-acetate (PMA), 1α, 25-dihydroxy vitamin D3, macrophage colony stimulating factor (M-CSF). According to the literature, PMA has been declared to be the most effective differentiation agent for obtaining THP-1 monocyte-derived macrophage (Chanput et al., 2013; Bastiaan-Net et al., 2013; Chanput et al., 2014). The PMA concentration and incubation time we determined in our study are in parallel with many studies. In a study conducted, 100 ng / mL PMA concentration was applied for the differentiation of THP-1 cells into M0 macrophage at an incubation period of 48 hours (Zhang et al., 2015). In a study in which differentiation of THP-1 cells into macrophage was carried out, especially in parallel with our results, 100 ng / mL concentration of PMA was applied for 24 hours of incubation and then the cells were left to rest incubation for 24 hours (Chanput et al., 2013). Apart from these studies, PMA has been used in many different concentrations for THP-1 cells to differentiate into M0 macrophage in many studies, respectively. These include 24-hour incubation with 30 ng / mL PMA (Bongiovanni et al., 2015), 96-hour incubation with 500 ng / mL PMA (Sueki et al., 2014), 24-hour incubation with 150 nM PMA (Genin et al., 2015) and reported as 48 hours incubation with 100 nM PMA (Zhou et al., 2015).

CONCLUSION

As a result, the study presented shows that PMA concentrations used in differentiation of THP-1 cells into macrophage have no toxic effects in the range of 1-1000 ng/mL. In addition, according to our flow cytometry results, 100 ng/mL PMA was determined as the concentration that can be used in future studies for polarization. In the light of the data we obtained from this study, M0 macrophage polarization was realized from THP-1 monocyte cells, and we obtained an optimized differentiation model for our next M1 and M2 macrophage polarization studies and co-culture models.

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➢ ***POSTER PRESENTATION***

Evaluation of cell viability in a *Chlamydia pneumoniae*-induced neuroinflammation co-culture model

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Abstract
Chlamydia pneumoniae is a mandatory intracellular gram-negative pathogen that causes a wide variety of pulmonary diseases, usually mild and atypical. *Chlamydia pneumoniae* can cause permanent infections, and there is growing evidence that this type of infection is associated with neurodegenerative central nervous system diseases, especially Alzheimer's disease (AD). Increased anti-*Chlamydia pneumoniae* antibodies in the blood of Alzheimer's patients was detected and it was shown that systemic infection caused by this pathogen increased the risk of AD 5 times by inducing neuroinflammatory pathways. Neuroinflammation can be defined as a complex set of local immune responses that mediate dealing with a threat to the neuronal microenvironment. The first indicator of neuroinflammation is microglia activation. Unlike other members of the chlamydia family, inoculation of *Chlamydia pneumoniae* inclusions and isolation of the bacteria from samples are difficult. Therefore, designing an *in vitro Chlamydia pneumoniae*-induced neuroinflammation co-culture model was aimed. As the first step to this approach, cytotoxic effects of drugs effective on epigenetic mechanisms (Trichostatin A (TSA), RG108 and Givinostat) and the antibiotic Rifampin at a concentration of 200, 100, 50, and 12,5 µM, whose effects were desired to be examined in this model, and lyophilized *Chlamydia pneumoniae* supernatant and lipopolysaccharide (LPS) at a concentration of 200, 100, 50, and 12,5 µg/ml were investigated on HMC3 human microglia and SH-SY5Y human neuroblastoma cells for 24 and 48 hours in this study by using WST-1 method. In addition to this method, the effects of the compounds were also investigated by real-time cell analysis system in low concentrations (100 nM). As a result, no significant cytotoxic effect was found at low concentrations in all concentration groups. This study is a preliminary study for the use of real-time cell analysis system in neuroinflammation models.
Keywords: *Chlamydia pneumoniae*, neuroinflammation, cytotoxicity.

INTRODUCTION

Alzheimer's disease (AD) is the main cause of dementia worldwide, with 75-80% of total dementia cases; and 5% of the population over 65 years of age and 30% of the population over the age of 85 (Reitz et al., 2011). The incidence of the disease has been increasing in recent years due to the prolongation of life expectancy, among other reasons. It is also estimated that this incidence will increase approximately every 20 years (Joshi & Morley, 2006). AD is a progressive neurodegenerative disease that usually presents first mild cognitive impairment and then dementia. It is characterized by loss of memory and other cognitive functions and changes in behavior, mood, and personality. These disorders interfere with the daily life of the patient and place a serious burden on families and the health system (Terracciano & Sutin, 2019).

Chlamydia pneumoniae is an obligate intracellular gram-negative pathogen that causes common human respiratory tract infection. Interestingly, previous studies have shown that *Chlamydia* infections tend to become chronic, and these data are clinically important not only in terms of persistent respiratory diseases but also in relation to diseases such as atherosclerosis caused by blood vessel inflammation (Uruma et al., 2005). In many studies conducted to date, the relation of *Chlamydia pneumoniae* infection with inflammatory diseases such as asthma and atherosclerosis has been proven definitively, and the evidence that this type of infection is associated with neurodegenerative central nervous system diseases, especially AD, is gradually increasing (Shima et al., 2010).

Neuroinflammation is a response involving all existing cells in the central nervous system, including neurons, macroglia and microglia. There are factors such as genetic background, trauma, environmental factors and age that activate microglia and complex neuroinflammatory pathways. An example of these factors is LPS, an endotoxin found in the outer membrane of Gram-negative bacteria, which induces a systemic inflammatory response via TLR signaling (Lyman et al., 2014).

In the literature, there are many neuroprotective effect studies in which microglia activated by an inducing agent such as LPS or amyloid β are co-cultured with the SH-SY5Y human neuroblastoma cell line (DaSilva et al., 2019). However, similar *in vitro* studies with *Chlamydia pneumoniae* have commonly been studied on infected cells (Boelen et al., 2007). In studies conducted with infected cells, it is not possible to distinguish between living and dead bacteria, and it is not possible to limit the analysis to living cells (Rogers et al., 2010). Therefore, this study was a preliminary study to establish a neuroinflammation model suitable for molecular pharmacological experiments.

MATERIALS AND METHODS

Cell Culture Studies

SH-SY5Y (*American Type Culture Collection* (ATCC), CRL-2266) is a human bone marrow derived neuroblastoma cell line which is often used as a human neuronal cell model. The SH-SY5Y cell line was incubated in Eagle's Minimum Essential Medium (EMEM) growth medium containing 10% fetal bovine serum (FBS) and 1% penicillin / streptomycin until reaching a density of 70-80% in a 37 ^{0}C incubator containing 5% CO_2. To induce the differentiation of SH-SY5Y cells into neuronal fenotype, current medium was changed with 1% FBS, 1% penicillin / streptomycin and 10 µM retinoic acid (RA) containing EMEM differentiation medium and cells were incubated for 5 days (Shipley et al., 2016).

HMC3 (ATCC, CRL-3304) is a human microglia cell line transformed from human fetal brain-derived primary microglia culture. HMC3 cells were grown in EMEM growth medium containing 10% FBS, 1% penicillin / streptomycin in a 37 ^{0}C incubator containing 5% CO_2 until the density reached 70-80%.

Determination of Non-Cytotoxic Concentrations with the WST-1 Method

WST-1 (4- [3- (4-Iodophenyl) -2- (4-nitrophenyl) -2H-5-tetrazolio] -1,3-benzene disulfonate), is a tetrazolium salt which is used to measure cell proliferation, cell viability and cytotoxicity in mammalian cells. The experimental protocol is based on the conversion of WST-1 tetrazolium salt to formazan salt by cellular mitochondrial dehydrogenases. As the number of living cells increases, the activity of mitochondrial dehydrogenases and the resulting amount of formazan salt increases. Apart from incubation, it is faster and more sensitive than other cytotoxicity measurement methods, as it does not involve washing and solubilization steps (Engür & Dikmen, 2017).

Before doing planned optimization experiments with *Chlamydia pneumoniae*-induced neuroinflammation co-culture model, pre-screening of cytotoxicity was performed in wide concentration ranges with the WST-1 method. For this study, SH-SY5Y and HMC3 cells were incubated in EMEM growth medium containing 10% FBS and 1% penicillin / streptomycin at 37 ^{o}C in an incubator with 5% CO_2. After the cells reached 80% density, they were counted in the Cedex XS (Innovatis, USA) device with Trypan blue dye and seeded in 96-well plates with $5x10^3$ cells per well and incubated for 24 hours for the adherence of the cells to the plates. After incubation, the current medium was replaced with 100 µl of medium containing; 200, 100, 50, 25 and 12.5 µM TSA, Givinostat, RG108 and Rifampin and 200, 100, 50, 25 and 12.5 µg / ml LPS and lyophilized *Chlamydia pneumoniae* supernatant. Medium containing 0.1% DMSO was used as the control group and the cells were incubated for 24 and 48 hours. At the end of the incubation periods, 10 µl of WST-1 solution was added to the existing medium on the plates and the cells were incubated for 4 hours. Subsequently, absorbance values of the plates were read in Cytation 3 Cell Imaging Multi-Mode Reader at 540 nm wavelength. 8 replicates for each concentration were measured. The average absorbance values of control wells were accepted as 100% and % cell viability values of other concentrations were calculated (Kaya-Tilki et al., 2016).

Monitoring cell viability in *Chlamydia pneumoniae*-induced neuroinflammation co-culture model with real time cell analysis system

The xCELLigence Real Time Cell Analysis System is a device that displays electrical impedance as unitless cell index data (CI) using plates containing interlocking gold microelectrodes to non-invasively monitor cell viability real-time (Ke et al., 2011). It is possible to monitor cell-cell interactions in real time through the E-plate Inserts placed in special plates containing gold electrodes used in this system (Thibeault vd., 2014).

In order to monitor cell viability in real-time during the neuroinflammation co-culture model created with *Chlamydia pneumoniae*-activated microglia and differentiated SH-SY5Y cells, firstly, SH-SY5Y cells were collected in EMEM differentiation medium containing 1% FBS, 1% penicillin / streptomycin and 10 µM RA. $5x10^3$ cells per well were seeded into E-plates and incubated 5 days for differentiation. 48 hours before the co-culture model was applied by inserting E-plate Inserts inside the E-plate, HMC3 cells were inoculated in

EMEM medium containing 10% FBS and 1% penicillin / streptomycin onto E-plate Inserts at a density of $5x10^3$ cells per well. At the end of the 24 hours incubation period, the existing medium was replaced with Cpn (1 µg / ml) + IFN (10 ng / ml) containing medium for microglia activation and incubated for another 24 hours. After the incubation for activation, medium was discarded and the cells were washed with PBS. E-plate Inserts were placed on the E-plates containing differentiated SH-SY5Y cells, which were discarded and washed in the same way, and concentration groups were applied in 1% FBS and 1% penicillin / streptomycin containing EMEM differentiation medium as explained in **Table 1**.

Table 13. Concentration groups in the experiment.

Groups	**HMC3 Activation**	**Concentrations Applied After Co-Culture**
Control	Growth medium	Differentiation medium
Chlamydia pneumoniae supernatant	Cpn (1 µg/ml) + IFN-ɣ (10 ng/ml)	Differentiation medium
TSA + *Chlamydia pneumoniae* supernatant	Cpn (1 µg/ml) + IFN-ɣ (10 ng/ml)	TSA (100 nM)
Rifampin + *Chlamydia pneumoniae* supernatant	Cpn (1 µg/ml) + IFN-ɣ (10 ng/ml)	Rifampin (100 nM)
Givinostat + *Chlamydia pneumoniae* supernatant	Cpn (1 µg/ml) + IFN-ɣ (10 ng/ml)	Givinostat (100 nM)
RG108 + *Chlamydia pneumoniae* supernatant	Cpn (1 µg/ml) + IFN-ɣ (10 ng/ml)	RG108 (100 nM)
LPS	LPS (100 ng/ml) + IFN-ɣ (10 ng/ml)	Differentiation medium
TSA + LPS	LPS (100 ng/ml) + IFN-ɣ (10 ng/ml)	TSA (100 nM)
Rifampin + LPS	LPS (100 ng/ml) + IFN-ɣ (10 ng/ml)	Rifampin (100 nM)
Givinostat + LPS	LPS (100 ng/ml) + IFN-ɣ (10 ng/ml)	Givinostat (100 nM)
RG108 + LPS	LPS (100 ng/ml) + IFN-ɣ (10 ng/ml)	RG108 (100 nM)

In addition to co-culture groups, differentiated SH-SY5Y cells were also treated with only *Chlamydia pneumoniae* supernatant (1 µg / ml) or LPS (100 ng) in combination with IFN-ɣ (10 ng / ml), RG108 (100 nM), TSA (100 nM), Givinostat (100 nM) and Rifampin (100 nM) in E-plates without the E-plate Insert. Cell viability were recorded in real time during the experiment.

Statistical analysis

Graphics were drawn using GraphPad Prism 7.0 software and statistically analyzed with one-way analysis of variance (ANOVA) and Tukey's post hoc test. The results are expressed as mean ± standard deviation and the means of three independent experiments (n=8 for cytotoxic assays), $p < 0.1$ * $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ****were considered to be significant compared to the control group. $p > 0.05$ values were accepted as non-significant.

RESULTS and DISCUSSION

Evaluation of non-cytotoxic concentrations using WST-1 method

According to the WST-1 results, 12.5, 25, 50, 100 and 200 µg/ml *Chlamydia pneumoniae* concentrations decreased cell viability of HMC3 cells at a rate of 0.82, 3.95, 4.09, 6.4 and 8.04% in 24 hours; 3.32, 7.99, 19.4, 19.5 and 19.55% in 48 hours respectively, compared to the control group (**Figure 1**). The decrease in cell viability was significant at 50, 100 and 200 µg / ml *Chlamydia pneumoniae* concentrations in only 48 hours ($p < 0.01$ **). 12.5, 25, 50, 100 and 200 µg/ml *Chlamydia pneumoniae* concentrations decreased cell viability of SH-SY5Y cells at a rate of 6.54, 11.41, 12.33, 15.72 and 30.51% in 24 hours; 15.82, 24.45, 36.1, 46.75 and 53.73% in 48 hours respectively, compared to the control group (**Figure 1**). Decrease in cell viability 100 (p

<0.01 **) and 200 (p <0.0001 ****) µM at 24 hours, 12.5 (p <0.01 **), 50, 100 and 200 µM at 48 hours (p <0.0001 *** *) was significant in concentration groups.

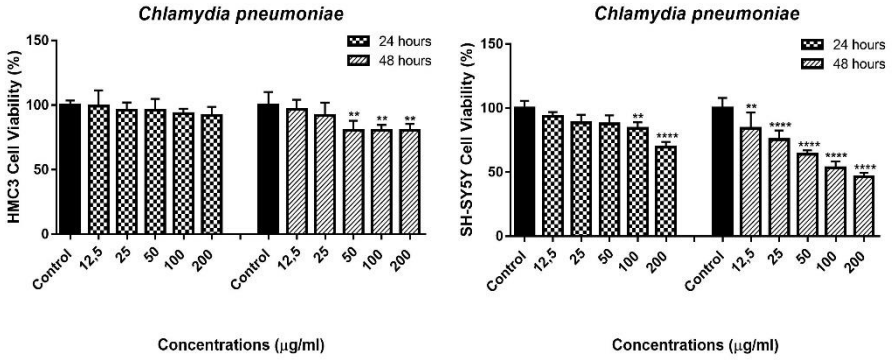

Figure 16. Cell viability values determined by WST-1 method and statistical evaluation of *Chlamydia pneumoniae* concentrations in HMC3 (left) and SH-SY5Y (right) cell lines.

12.5, 25, 50, 100 and 200 µg/ml LPS concentrations decreased cell viability of HMC3 cells at a rate of 0.88, 2.04, 3.21, 3.72 and 4.24% in 24 hours; 9.7, 12.68, 13.03, 14.02 and 14.52 % in 48 hours respectively, compared to the control group (**Figure 2**). The decrease in cell viability was not significant compared to the control group. 12.5, 25, 50, 100 and 200 µg/ml LPS concentrations decreased cell viability of SH-SY5Y cells at a rate of 6.34, 12.86, 15.29, 18.87 and 29.77 % in 24 hours; 27.11, 25, 34.9, 35.59 and 47.71% in 48 hours respectively, compared to the control group (**Figure 2**). Decrease in cell viability 25 (p <0.05 *), 50 (p <0.01 **), 100 (p <0.001 ***) and 200 (p <0.0001 ****) µM at 24 hours, 12.5, 25 at 48 hours, It was found significant in 50, 100 and 200 µM (p <0.0001 ****) concentration groups.

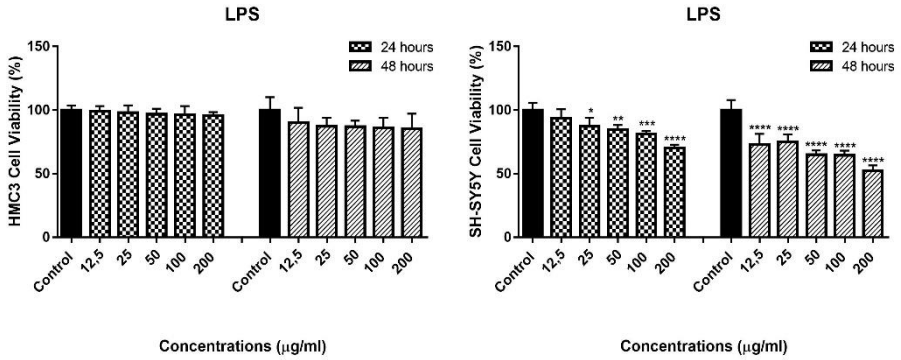

Figure 2. Cell viability values determined by WST-1 method and statistical evaluation of LPS concentrations in HMC3 (left) and SH-SY5Y (right) cell lines.

12.5, 25, 50, 100 and 200 µM TSA concentrations decreased cell viability of HMC3 cells at a rate of 34.82, 43.33, 75.06, 93.46 and 95.95% in 24 hours; 47.33, 63.16, 85.84, 93.52 and 98.54% in 48 hours respectively, compared to the control group (**Figure 3**). The decrease in cell viability was found to be significant in all concentration groups (p <0.0001 ****). It has been determined that TSA has significant cytotoxic effects on HMC3 cells depending on the increase in concentration. 12.5, 25, 50, 100 and 200 µM TSA concentrations decreased cell viability of SH-SY5Y cells at a rate of 95.9, 97.41, 98.63, 99.31 and 99.42% in 24 hours; 89.5, 94.5, 97.71, 99.41 and 99.5% in 48 hours respectively, compared to the control group (**Figure 3**). The decrease in cell viability was found to be significant in all concentration groups (p <0.0001 ****).

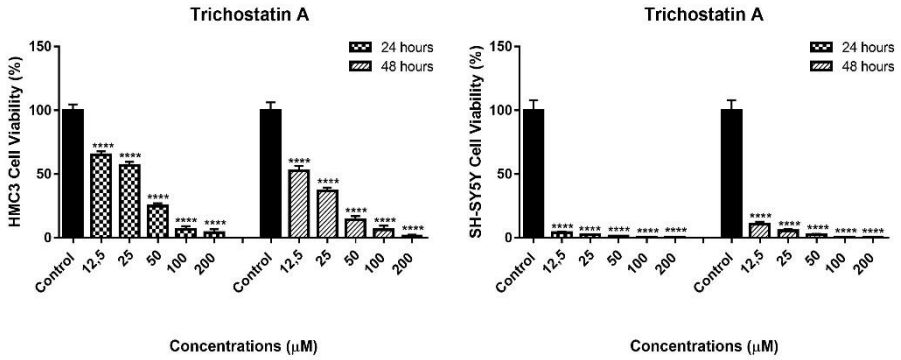

Figure 3. Cell viability values determined by WST-1 method and statistical evaluation of TSA concentrations in HMC3 (left) and SH-SY5Y (right) cell lines.

12.5, 25, 50, 100 and 200 µM Rifampin concentrations decreased cell viability of HMC3 cells at a rate of 9.36, 10.02, 10.94, 24.81 and 53.5% in 24 hours; 14.58, 17.31, 24.55, 54.56 and 65.52% in 48 hours respectively, compared to the control group (**Figure 4**). Decrease in cell viability at 100 and 200 µM ($p < 0.0001$ ****) in 24 hours, and at 12.5 ($p < 0.01$ **), 50 ($p < 0.001$ ***), 100 and 200 µM ($p < 0.0001$ **) in 48 hours were found significant. According to the cell inhibition rates, Rifampin showed significant cytotoxic effects on HMC3 cells at high concentrations, especially at 200 µM in 24 hours, and at 100 and 200 µM in 48 hours. 12.5, 25, 50, 100 and 200 µM Rifampin concentrations decreased cell viability of SH-SY5Y cells at a rate of 95.9, 97.41, 98.63, 99.31 and 99.42% in 24 hours; 89.5, 94.5, 97.71, 99.41 and 99.5% in 48 hours respectively, compared to the control group (**Figure 4**). Decrease in cell viability at 24 hours in 50 ($p < 0.001$ ***), 100 and 200 ($p < 0.0001$ ****) µM, 48 hours in 25, 50, 100 and 200 µM ($p < 0.0001$ ****) concentration groups found significant.

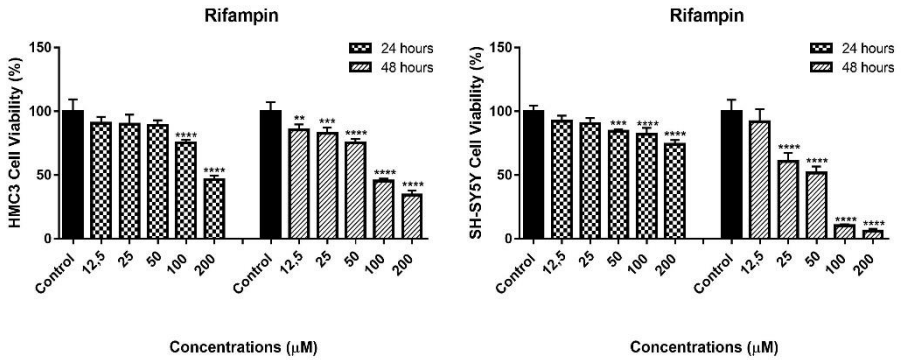

Figure 4. Cell viability values determined by WST-1 method and statistical evaluation of Rifampin concentrations in HMC3 (left) and SH-SY5Y (right) cell lines.

12.5, 25, 50, 100 and 200 µM Givinostat concentrations decreased cell viability of HMC3 cells at a rate of 14.64, 55.18, 65.06, 89.94 and 96% in 24 hours; 43.66, 97.88, 98.95, 99.45 and 99.61% in 48 hours respectively, compared to the control group (**Figure 5**). The decrease in cell viability was found to be significant in all concentration groups at both 24 and 48 hours, and it was seen that the cytotoxic effect was very high ($p < 0.0001$ ****). 12.5, 25, 50, 100 and 200 µM Givinostat concentrations decreased cell viability of SH-SY5Y cells at a rate of 52.01, 67.01, 85.61, 97.07 and 99.58% in 24 hours; 42.57, 78.56, 82.52, 97.47 and 99.67% in 48 hours respectively, compared to the control group (**Figure 5**). The decrease in cell viability was found to be significant in all concentration groups ($p < 0.0001$ ****).

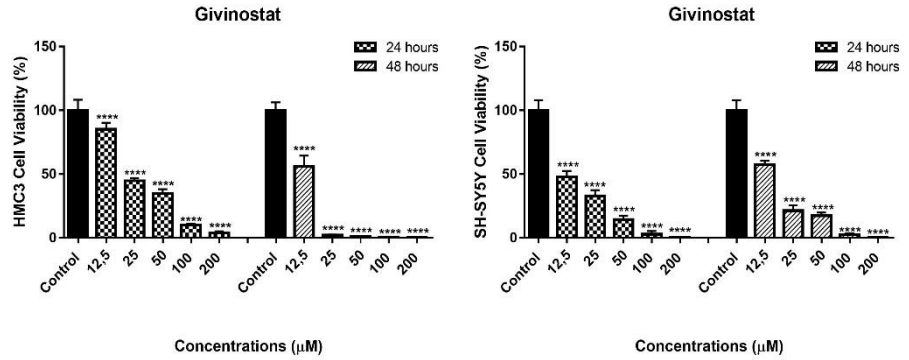

Figure 5. Cell viability values determined by WST-1 method and statistical evaluation of Givinostat concentrations in HMC3 (left) and SH-SY5Y (right) cell lines.

12.5, 25, 50, 100 and 200 μM RG108 concentrations decreased cell viability of HMC3 cells at a rate of 6.62, 7.18, 8.79, 10.15 and 13.19% in 24 hours; 15.1, 15.82, 17.95, 25.77 and 27.19% in 48 hours respectively, compared to the control group (**Figure 6**). The decrease in cell viability was significant at 200 μM ($p < 0.01$ **) in 24 hours, 12.5 and 25 μM ($p < 0.001$ ***) in 48 hours; It was found significant in 50, 100 and 200 μM ($p < 0.0001$ ****) concentration groups, but these cytotoxic effects did not inhibit cell viability more than 50%. 12.5, 25, 50, 100 and 200 μM RG108 concentrations decreased cell viability of SH-SY5Y cells at a rate of 82.47, 86.07, 91.15, 94.75 and 97.91% in 24 hours; 82.5, 93.54, 97.84, 98.86 and 99.2% in 48 hours respectively, compared to the control group (**Figure 6**). The decrease in cell viability was found to be significant in all concentration groups ($p < 0.0001$ ****).

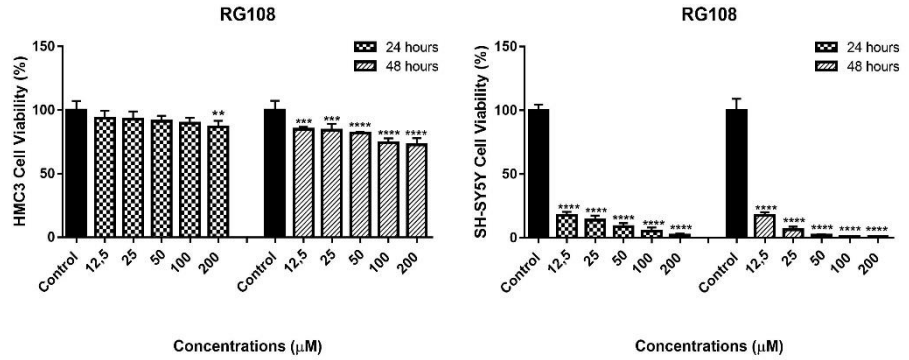

Figure 6. Cell viability values determined by WST-1 method and statistical evaluation of RG108 concentrations in HMC3 (left) and SH-SY5Y (right) cell lines.

In the literature review, a cytotoxicity study was not found on SH-SY5Y cells with *Chlamydia pneumoniae* supernatant, RG108 and Givinostat. In a study by Shakya and Chongthammakun, in which 17β-estradiol attenuates the SH-SY5Y cell proliferation-reducing effect through the Wnt signaling pathway of chronically active microglia, findings were similar to the WST-1 study with LPS (Shakya and Chongthammakun, 2019). LPS was studied in a dose range between 0.4 ng-0.4 μg / ml, and no significant decrease in viability was observed in SH-SY5Y cells treated with LPS only at this dose range. The lack of TLR4 receptors in SH-SY5Y cells was explained by the fact that only LPS administration had no effect on cell viability, although co-culture with active microglia reduced cell viability (Molteni et al., 2004). In a similar study examining the effect of luteolin on apoptosis in a co-culture model created with LPS-induced BV2 microglia and SH-SY5Y cells, it was reported that 0.1, 1.0 and 10.0 μg / ml LPS administration did not decrease SH-SY5Y cell viability (Zhu et al., 2014).

Parallel findings to TSA results were found in a study investigating TSA's cytotoxic effects on various neuroblastoma cell lines (SH-SY5Y, IMR32, SH-EP1, SK-N-AS, GOTO, SMS-KCN-69n, LA1-55n LA1-5s, SH-310 and WSN). In this study, Subramanian et al. used 0.25, 0.5, 0.75 and 1 μM TSA, and the decrease in cell viability at the end of 24 hours was approximately 40% compared to the control group. The decrease with the highest concentration (1 μM) was around 80% after 48 hours (Subramanian et al., 2007).In another study investigating the effects of TSA on dopaminergic neuronal cell (rat N27, mouse MN9D and human SH-SY5Y cell line) survival, the effects of low concentrations of TSA (10, 25, and 50 nM) were determined using trypan

blue method after 12-96 hours. Cell viability decreased approximately 90% with 50 nM TSA at the end of 48 hours compared to the control group (Wang et al., 2009). In a study investigating the effects of Vorinostat and TSA, which are HDACi on the SH-SY5Y cell line, a significant decrease in cell viability was reported with 200 and 500 nmol/L TSA with MTT analysis performed at the end of 48 hours (De los Santos et al., 2007). In a study investigating the neuroprotective effects of TSA on an Alzheimer's disease model established by using Aβ25--35 on SH-SY5Y cells, no significant decrease in cell viability was reported with MTT study conducted with 100, 80, 60, 40, 20 and 10 nM TSA concentrations (Li et al., 2020).

Findings similar to the cytotoxicity study performed with rifampin were found in a study by Wu et al. in which the protective effects of rifampin on rotenone-induced SH-SY5Y apoptosis were examined. After $5x10^3$ SH-SY5Y cells were treated with 100 µM Rifampin for 24 hours, a decrease in cell viability was detected with the Cell Counting Kit-8 cytotoxicity test, but it was not found significant (Wu et al., 2018).

Evaluation of cell viability in *Chlamydia pneumoniae*-induced neuroinflammation co-culture model with real time cell analysis system

In order to evaluate the cytotoxic effects of the concentrations in neuroinflammation co-culture cell model, the cell index values were recorded using xCELLigence RTCA-DP analysis system to monitor the viability of SH-SY5Y cells, which were differentiated for 5 days and then incubated with *Chlamydia pneumoniae* or LPS activated microglia cells for 48 hours.

The slope graph drawn according to the 5th and 7th day cell index data recorded during the neuroinflammation co-culture model created with concentration groups in **Figure 7**.

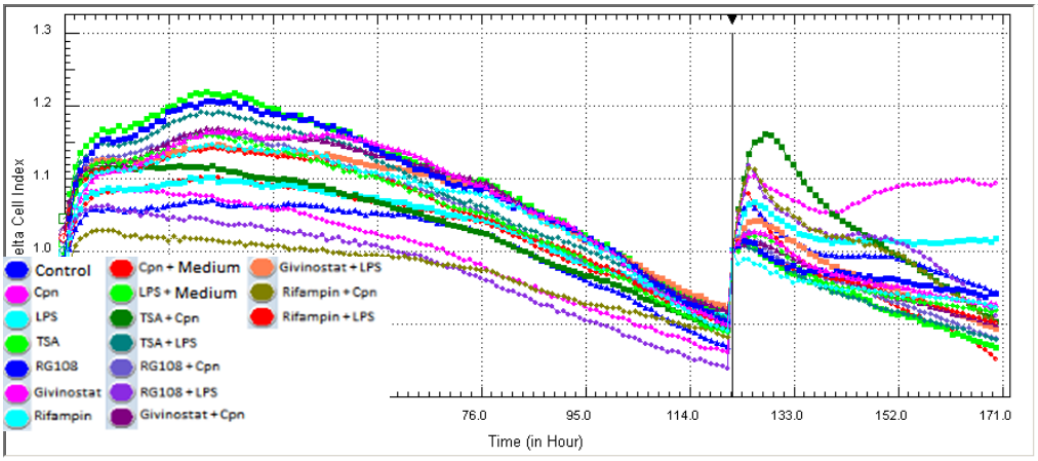

Figure 7. Graph of cell index values measured over 7 days during monitoring of differentiated SH-SY5Y cell viability in the RTCA-DP system in the neuroinflammation co-culture model (Cpn (1 µg / ml) / LPS (100 ng / ml) + IFN-ɣ (10 ng / ml) TSA, RG108, Givinostat, Rifampin (100 nM)).

As shown in the graph, there was no significant decrease in SH-SY5Y cell viability compared to the control group during differentiation and co-culture (**Figure 7, Figure 8**).

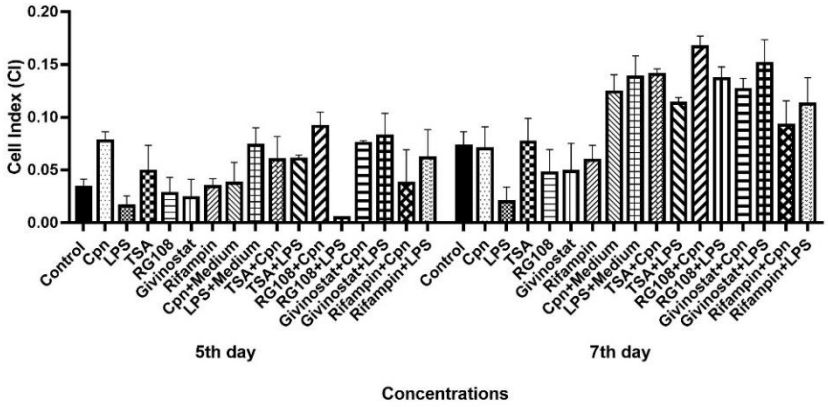

Figure 8. Graph of cell index data on 5th and 7th days after the concentrations applied in the neuroinflammation co-culture model in the RTCA DP system (n = 4, mean ± st deviation).

The rapid increase in CI values is accepted as the first stages of differentiation in SH-SY5Y cells (Dwane et al., 2013). In this first stage, the cell body enlarges and swells to cause an increase in impedance, and then as neurites elongate from the cell, the cell body shrinks and causes a decrease in impedance (Dwane et al., 2013; Kaya- Tilki et al., 2016). Co-culture studies performed in the xCELLigence real-time cell analysis system are frequently used in cancer research (Berger et al., 2017). Similar to our study, there are studies conducted with differentiated SH-SY5Y cell line and BV2 microglia cells activated by LPS or another agent (Ma et al., 2015). However, no co-culture study with activated HMC3 cell line and differentiated SH-SY5Y cells was found in the literature review, and our study is important in terms of using xCELLigence technology in modeling neuroinflammation.

CONCLUSION

In this study, cytotoxicity of epigenetic mechanism related drugs TSA, RG108, Givinostat, the antibiotic Rifampin, lyophilized *Chlamydia pneumoniae* supernatant and LPS were evaluated on HMC3 microglia and differentiated SH-SY5Y cells to determine non-cytotoxic concentrations that will be used to optimize a co-culture model. This study is a preliminary study for the use of xCELLigence technology in neuroinflammation models.

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➢ *POSTER PRESENTATION*

Damızlık Japon bıldırcınları (*Coturnix coturnix japonica*) rasyonlarına mannan oligosakkarit-beta glukan ile vitamin C ilavesinin bazı biyokimyasal parametreler üzerine etkileri

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Özet

Bu çalışma, damızlık bıldırcın rasyonuna prebiyotik (Mannan oligosakkarit-*β*-Glucan, ImmunoWall™) ve vitamin C ilavesinin serum biyokimyasal parametreler üzerine etkilerini belirlemek amacıyla yapıldı. Çalışmada 10 haftalık toplam 120 adet Japon bıldırcını (*Coturnix coturnix japonica*) kullanıldı. Hayvanlar, her birinde 6 (4 dişi+2 erkek) bıldırcın bulunan 5 tekrarlı 4 deneme grubuna ayrıldı. Deneme grupları 1. grup bazal rasyon (kontrol), 2. grup 4 g/kg prebiyotik, 3.grup 300 mg/kg vitamin C, 4. grup 4 g/kg prebiyotik+300 mg/kg vitamin C olacak şekilde düzenlendi. Deneme 8 hafta sürdü. Çalışmanın sonunda her deneme grubundan 10'ar adet hayvandan alınan kan örnekleri 3000 rpm'de 10 dakika santrüfüj edilerek serumları ayrıldı. Serumda glukoz, total kolesterol, HDL-kolesterol, LDL-kolesterol, trigliserid, total protein, albümin düzeyleri ticari test kitleri (Rel Assay Diagnostics, Türkiye) ile otoanalizör cihazında ölçülerek (Mindray BS 300, Çin) belirlendi. Ölçülen biyokimyasal parametrelerin düzeylerinde istatistiksel olarak fark bulunmadı ($p>0,05$). Sonuç olarak, damızlık bıldırcınların yemlerine 4 g/kg prebiyotik ve 300 mg/kg vitamin C'nin hem ayrı ayrı hem de kombine olarak ilavesi serum biyokimyasal parametreleri üzerinde değişikliğe neden olmamıştır.

Anahtar Kelimeler: Biyokimyasal parametreler, Damızlık bıldırcın, Kan, Prebiyotik, Vitamin C

Abstract

In this study, the effects of prebiotic (mannan-oligosaccharides and β-glucans**,** ImmunoWall™) and vitamin C supplementation alone and in combination to ration on some biochemical parameters were investigated in breeding Japon quail (*Coturnix coturnix japonica*). A total of 120, 10 weeks old, Japanese quails were used in this study. Quails were assigned to four groups with 5 replicates of 6 (4 females, 2 males) quails each group. Treatment for each group consisted of: first group (control group) received basal diet without supplementation; second group received 4 g/kg prebiotic (mannan-oligosaccharides -*β*-Glucan, ImmunoWall™); third group received 300 mg/kg vitamin C; and fourth group received 4 g/kg prebiotic (mannan-oligosaccharides, ImmunoWall™) + 300 mg/kg vitamin C. The experiment lasted 8 weeks. At the end of the study, the blood samples were taken from 10 animals of each group. The blood samples were centrifuged at 3000 rpm for 10 seconds. Serum glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, total protein and albumin were determined by a autoanalyser (Mindray BS 300, China) with diagnostic kits (Rel Assay Diagnostics, Turkey). There were no observed significant statistical differences in biochemical parameters ($P>0.05$). Thus in conclusion, the addition of 4 g/kg prebiotic and 300 mg/kg vitamin C to the feed of breeding quails both separately and in combination did not cause any changes on serum biochemical parameters.

Keywords: Biochemical parameters, Breeding quail, Blood, Prebiotic, Vitamin C

GİRİŞ

Hayvanlarda büyüme performansını artırmak ve ekonomik verimlilik için yem katkı maddeleri uygulanmaktadır (Namur et al., 1998). Önceden hayvan yemlerine büyüme performansı için antibiyotikler katılırken; Avrupa Birliği'nin almış olduğu kararla 2006 yılından bu yana antibiyotiklerin yemlere katılmasına izin verilmemiştir. Bu nedenle prebiyotikler ve probiyotikler gibi katkı maddeleri kullanılmaya başlanmıştır (Patterson and Burkholder, 2003; Shashidhara and Davegowda, 2003). Prebiyotikler kolondaki bir veya daha fazla bakterinin üremesini ve aktivitesini sağlayarak, konakçının yararına olan, sindirilemeyen gıda içerikleri olarak ifade edilmektedir. Prebiyotiklerin sağlık üzerinde pek çok faydaları vardır. Bağırsak hareketlerini düzenlediği, kan kolesterol ve trigliserit düzeylerini olumlu yönde etkilediği, minerallerin emilimini ve biyoyaralanımını artırdığı, patojen mikroorganizmaların çoğalmasını önleyerek intestinal ve ekstraintestinal enfeksiyonun gelişme riskini azalttığı ve immune sistemini güçlendirdiği bilinmektedir (Taşdemir, 2017). Prebiyotikler mannanoligosakkarid (MOS), transgalaktooligosakkarit, galaktooligosakkarit, fruktooligosakkaritleri içine alan sindirilemeyen olugosakkaritlerden oluşmaktadır. *Saccharomyces cerevisiae* hücre duvarından türetilen MOS, ne endojen sindirim enzimleri tarafından hidrolize edilir ne de konakçı tarafından emilir ve prebiyotik bir ajan olarak kabul edilir. *Saccharomyces cerevisiae* hücre duvarı %30 glukan, %30 mannan ve %12,5 proteinleri içermektedir (Yalçınkaya ve Leblebiciler, 2012). ImmunoWall™, *Saccharomyces cerevisiae* hücre duvarından türetilmiş mannan-oligosakkarit (MOS) ve β-glukanlardan oluşan ticari bir prebiyotiktir. Mannanoligosakkarit ve β-glukan prebiyotik son zamanlarda sığır (Boyd ve ark., 2016) ve civcivlerde (Awaad ve ark, 2011; Koksal ve ark., 2013; Yanık ve ark., 2018; Fadl ve ark., 2020) dahil olmak üzere çeşitli hayvanlarda sağlığı geliştirmek için bir diyet takviyesi olarak çok dikkat çekmiştir. Antioksidanlar gıda sanayinde ürünlerin besinsel değerinin korunması, dolayısıyla kaliteli gıda elde etmek için kullanılmaktadır (Atalay ve Erge, 2018). İmmun sisteminin normal faaliyetleri sonucu fazla miktarda oluşan reaktif oksijen türleri, hücre membran yapısını olumsuz yönde etkileyerek bağışıklık sistemini baskılayabilir. Bu nedenle rasyon ile yeterli miktarlarda antioksidan alımı gerekebilir. Antioksidan vitaminlerden birisi olan C vitamini sitokin üretimi ve immünoglobulin sentezini artıran enfeksiyonlara cevap olarak T-lenfosit proliferasyonunu iyileştirerek immün sistemi uyarmaktadır (Ergün, 2016). Vitamin C, vücudu oksidatif zorluklara karşı koruyan güçlü bir antioksidandır. Vitamin C'nin antioksidan etkisini ortamdan singlet oksijen, superoksit, hidroksil, hidroperoksil, lipid peroksil ve lipid alkoksil radikallerini temizleyerek gösterdiği de bildirilmiştir (Aslan, 2018). Vitamin C (l-askorbik asit), biyolojik fonksiyonlar için gereken, suda çözünür bir mikro besindir (İriş ve Çınar, 2019). Bu bilgiler doğrultusunda sunulan çalışma ile damızlık Japon bıldırcınları rasyonlarında mannanoligosakkarit-B-glukan ile vitamin C'nin ayrı ayrı ve kombine kullanımının bazı biyokimyasal parametreler üzerine etkisini ortaya çıkarmak hedeflenmiştir.

MATERYAL VE METOT

Çalışma Aksaray'ın Eskil İlçesinde bulunan TR680000273125 numaralı işletmedeki bıldırcın deneme ünitesinde gerçekleştirilmiştir. Kırıkkale Üniversitesi Hayvan Deneyleri Yerel Etik Kurul tarafından onay alınmıştır (18.06.2020/19).

Deneme Hayvanlarının Bakımı ve Beslenmesi

Çalışmada 10 haftalık toplam 120 adet damızlık Japon bıldırcını (*Coturnix Coturnix japonica*) kullanılmıştır. Aksaray'ın Eskil İlçesinde bulunan TR680000273125 numaralı işletmedeki deneme kümesine getirilen bıldırcınlar tartılarak, canlı ağırlıkları birbirine yakın olacak şekilde, 90 cm uzunluğunda, 80 cm genişliğinde ve 80 cm yüksekliğindeki katlı bataryalı kafeslere yerleştirilmiştir. Hayvanlar her birinde 6 (4 dişi+2 erkek) bıldırcın bulunan 5 tekrarlı 4 deneme grubuna ayılmıştır. Deneme grupları 1.grup bazal rasyon (kontrol), 2.grup 4 g/kg prebiyotik (Mannan oligosakkarit-*β*-Glukan, ImmunoWall™), 3.grup 300 mg/kg vitamin C ve 4.grup 4 g/kg prebiyotik (Mannan oligosakkarit-*β*-Glucan, ImmunoWall™) + 300 mg/kg vitamin C olacak şekilde düzenlenmiştir. Tüm gruplara yem ve su *ad libitum* olarak verilmiştir. Deneme boyunca kümes ısısının otalama 22-23°C düzeylerinde olmasına özen gösterilmiştir. Havalandırma fan aracılığıyla uygulanmıştır. Sular her bölmede bulunan bir adet damla tipi (nipelli) sulukla hayvanlara verilmiştir. Bıldırcınlara su, kafeslere bağlanan su deposundan temin edilerek taze ve temiz su içmeleri sağlanmıştır. Yemler her bölmede birer adet bulunan özel plastik yemliklerle verilmiştir. Deneme 8 hafta sürmüştür.

Kan Numunelerinin Alınması ve Bazı Biyokimyasal Parametrelerinin Belirlenmesi

Çalışmanın sonunda kesim öncesi hayvanlar 12 saat aç bırakılmıştır. Her deneme grubundan 10'ar adet hayvanın (5 dişi, 5 erkek) V.*jugularis* damarından antikoagulantsız tüplere kan alınmıştır. Kan örnekleri oda sıcaklığında 1 saat bekletildikten sonra 3000 rpm'de 10 dakika santrifüj edilerek serumları ayrılmıştır. Serumlar analiz edilinceye kadar -20°C de derin dondurucuda saklanmıştır. Elde edilen serum örneklerinde

glukoz, total kolesterol, HDL-kolesterol, LDL-kolesterol, trigliserid, total protein, albümin düzeyleri ticari test kitleri (Rel Assay Diagnostics, Türkiye) ile otoanalizör cihazında ölçülerek (Mindray BS 300, Çin) belirlenmiştir.

İSTATİSTİKSEL ANALİZLER

İlk olarak uygun analiz türünün belirlenmesi amacıyla, verilerin normal dağılım gösterip göstermediklerinin tespitinde Shapiro-Wilk testi uygulanmıştır. Nonparametrik dağılım gösteren serum total kolesterol, trigliserid ve HDL-kolesterol düzeyleri Kruskal-Wallis testi kullanılarak test edilmiştir. Diğer parametreler (parametric) tek yönlü ANOVA testi ile analiz edilmiştir. F değerleri anlamlı olduğunda Duncan's testi yapılmıştır. İstatistiksel değerlendirmede $p \leq 0.05$ düzeyi anlamlı farklılığın göstergesi olarak kabul edilmiştir. Verilerin istatistikî analizleri SPSS 25.0 paket programı ile yapıldı (SPSS Inc., Chicago, Illinois,). Veriler parametrik dağılımlarda ortalama değerler ve standart hata (x+Sx) olarak, non-parametrik dağılımlarda ise x+Sx, medyan ve mean rank olarak verilmiştir.

BULGULAR

Deneme de kullanılan rasyonun bileşimi tablo 1 de verilmiştir.

Tablo 1. Denemede kullanılan rasyonun bileşimi

Hammadde	%
Mısır	54,20
Soya fasülyesi küspesi	27,00
Ayçiçeği tohumu küspesi	7,00
Bitkisel yağ	4,30
Mermer tozu	5,60
Dikalsiyum fosfat	1,15
Tuz	0,35
Premiks[1]	0,25
DL metiyonin	0,15
Toplam	100,00
Besin Maddeleri Kompozisyonu	**%**
Metabolik enerji, kkal ME/kg	2902
Ham protein	20,09
Kalsiyum	2,51
Kullanılabilir fosfor	0,35
Lisin	1,00
Metiyonin	0,45
Sistin	0,37
Metiyonin + sistin	0,82

[1]Her 1 kg vitamin premiksi; 80 mg Mn, 60 mg Fe, 5 mg Cu;
1 mg I_5; 0,15 mg Se, 8.800 IU Vit A, 2.200 IU Vit D3, 11 mg Vit E,
44 mg Nikotin asit, 8,8 mg Cal-D-Pan, 4,4 mg Riboflavin, 2,5mg Tiamin,
6,6 mg Vit B_{12}, 1 mg Folik asit; 0,11 mg Biyotin, 220 mg Kolin içermektedir.

Kontrol ve deneme gruplarının kan serumunda glukoz, LDL-kolesterol, total protein ve albümin düzeyleri tablo 2'de, total kolesterol, HDL-kolesterol ve trigliserid düzeyleri tablo 3'de gösterilmiştir. Analizler sonunda elde edilen bıldırcın kan serumunda glukoz, total kolesterol, HDL-kolesterol, LDL-kolesterol, trigliserid, total protein ve albümin düzeylerinde istatiksel olarak bir farklılık görülmemiştir ($p>0.05$)

Tablo 2. Kontrol ve deneme gruplarında serum glukoz, LDL-kolesterol, total protein ve albümin düzeyleri (x ± Sx) (n=10)

Parametreler	Kontrol	4g/kg MOS-β-glukan	300mg/kg Vitamin C	4g/kg MOS-β-glukan +300 mg/kg Vitamin C	P
Glukoz (mg/dl)	219,00±16,67	199,60±12,70	196,00±14,19	187,90±10,49	0,438
LDL-kolesterol (mg/dl)	42,20±3,91	33,40±4,59	39,80±4,14	40,90±2,87	0,409
Total protein (g/dl)	3,58 ±0,15	3,76±0,18	3,68±0,16	3,91±0,10	0,472
Albumin (g/dl)	1,34±0,05	1,35±0,02	1,34±0,05	1,42±0,05	0,513

Gruplar arasında istatistiksel olarak bir farklılık bulunmamıştır (P>0,05)

Tablo 3. Kontrol ve deneme gruplarında total kolesterol, trigliserid ve HDL-kolesterol düzeyleri (n=10)

	Gruplar											
Parameters	**Kontrol**			**4g/kg MOS-β-glukan**			**300mg/kgVitamin C**			**4g/kg MOS-β-glukan +300 mg/kg Vitamin C**			
	x ± Sx	Medyan	Mean Rank	x ± Sx	Medyan	Mean Rank	x ± Sx	Medyan	Mean Rank	x ± Sx	Medyan	Mean Rank	P
Kolesterol (mg/dl)	188,50 ±19,73	174	23,00	177,90±22,18	150,50	20,90	177,40±20,88	172,50±	20,90	174,00±22,61	150,00	19,40	0,851
Trigliserid (mg/dl)	380,00±77,05	368,50	23,65	338,20±52,31	378,50	21,00	322,80±62,56	332,50	18,40	331,60±68,08	302,50	18,95	0,743
HDL-kolesterol (mg/dl)	172,15±11,22	168,85	18,70	173,36±16,09	157,15	20,20	182,54±15,00	182,80	21,80	175,57±14,32	149,55	21,30	0,937

Gruplar arasında istatistiksel olarak bir farklılık bulunmamıştır (P>0,05)

TARTIŞMA VE SONUÇ

Bu çalışma; damızlık Japon bıldırcın rasyonlarında prebiyotiklerden, MOS+β glukan ile vitamin C'nin ayrı ayrı veya kombine kullanımının bazı serum biyokimyasal parametreler üzerine etkilerini incelemek amacıyla yapılmıştır.

Kanatlıda kan parametreleri kuşların refah koşulunun önemli göstergeleridir. Çünkü hayvan diyetleri gibi eksojen faktörlere fizyolojik cevapları yansıtmaktadırlar (Sigolo ve ark.2019). Sunulan çalışmada serum glukoz düzeyleri MOS+Beta glukan ilavesiyle istatistiksel olarak önemli düzeyde değişmemiştir. Elde edilen verilerin, Konca ve ark. (2009)'nın hindilerde (1g/kg MOS), Igbal ve ark.(2018)'nın bıldırcınlar üzerinde (% 0.25, 0.50, ve 1.0% MOS) yaptıkları çalışma bulgularına uyumlu olarak; kontrol grubu ile karşılaştırıldığında MOS ilavesinin serum glukoz düzeylerinde istatistiksel olarak önemli değişikliklere sebep olmadığı gözlenmiştir. Buna karşın Speranda ve ark (2008) Bio-MOS takviyeli diyetin kontrol grubuna kıyasla sülün tavuklarında glikoz düzeylerini artırdığını ifade etmişlerdir. Igbal ve ark. (2018) Japon bıldırcın rasyonlarına 15 hafta boyunca % 0.25, 0.50, ve 1.0% MOS ilavelerinin serum trigliserid düzeylerini azalttığını belirtmişlerdir. Trigliserid düzeyindeki azalmanın, MOS ilavesi ile bağırsakta laktik asit üreten *Bifidobacteria* ve *Lactobacillus* spp. popülasyonun artışından kaynaklanabileceğini vurgulamışlardır. Bu bakterilerin bağırsaktaki laktik asit fermantasyonunu desteklediğini ve sonuçta serum trigliseridlerini azalttığını ifade etmişlerdir (Konca, 2009). Sunulan çalışmanın bulgularına uyumlu olarak Yanık ve ark. (2018) Japon bıldırcınlarının (Coturnix coturnix japonica) rasyonlarına maya hücre duvarı ekstraktı ilavesinin (200 000 mg/kg β-glukan, 180 000 mg/kg MOS) serum total kolesterol ve trigliserid düzeylerini düşürme eğiliminde olduğunu belirtmişlerdir. Rasyondaki prebiyotik türü yem katkı maddelerinin serum kolesterol düzeylerini düşürücü etkisinin genellikle artan yararlı mikrobiyal faaliyet ile ilişkilendirildiğini ifade etmişlerdir. Ayrıca, Li ve ark. (2007) ile Yılmaz-Leblebiciler ve Aydoğan (2018) broyler tavuklarda sırasıyla % 0.05 MOS ve 100 ppm MOS ilavelerinin serum trigliserid ve total kolesterol düzeyini etkilemediğini, Ibrahim (2011)'in ise Japon bıldırcın rasyonuna eklenen MOS+β-glukanın serum kolesterol düzeyini etkilemediğini ifade etmişlerdir.

Igbal ve ark. (2018) Japon bıldırcın rasyonlarına %0.25, 0.50, ve %1.0 MOS ilavelerinin serum LDL-kolesterol düzeylerinde önemli değişiklikler olmazken HDL-kolesterol düzeyinde ise önemli artışlar olduğunu belirtmişlerdir. HDL seviyelerindeki önemli değişikliklerin, MOS'un kısa zincirli yağ asitlerinin sentezi ve serbest bırakılması üzerindeki ayrıntılı etkisine bağlı olabileceği ve bunun da sonuçta serum HDL-kolesterol üzerinde önemli bir etkiye sahip olduğunu ifade etmişlerdir. Sunulan çalışmaya uyumlu olarak Stanley ve ark. (2000), Kannan ve ark. (2005), Khalji ve ark. (2011) broylerlerde, Konca ve ark. (2009) hindilerde, Igbal ve ark. (2018) bıldırcınlarda MOS ilavesinin, serum HDL kolesterol ve LDL kolesterol düzeylerini etkilemediğini belirtmişlerdir.

Albümin, karaciğer tarafından sentezlenen bir plazma proteinidir ve serum konsantrasyonu vücut savunma mekanizmasını gösterir (Kabir, 2013). Kanatlılarda, serum total proteini esas olarak albümin ve globulinden oluşur (Scholtz ve ark. 2009). Igbal ve ark. (2018) dişi bıldırcınlarda serum total protein konsantrasyonları üzerinde yüksek konsantrasyonundaki MOS (1.0%MOS)'un düşük konsantrasyonlardaki (%25MOS) kadar etkili olmadığını belirtmişlerdir. Konca ve ark. (2009) hindilerde MOS ilavesinin serum total protein ve albumin düzeylerini etkilemediğini ileri sürmüşlerdir. Fadl ve ark. (2020)'da broiler civcivlerde MOS ve β-Glukan (Agrimos®)'ın serum total protein düzeylerini artırırken, albumin düzeylerini etkilemediğini bildirmişlerdir. Japon bıldırcınlarının (Coturnix coturnix japonica) rasyonlarına maya hücre duvarı ekstraktı ilavesinin (200 000 mg/kg β-glukan, 180 000 mg/kg MOS) total protein ve albumin düzeylerini etkilemediği belirtilmişdir (Yanık ve ark.2018). Shahir ve ark. (2014)'da broiler yemlerine maya hücre duvarı ekstraktı ilavesinin serum toplam protein ve albumin düzeylerini etkilemediğini gözlemlemişlerdir. Sunulan çalışmada da yukarıdaki çalışmalara uyumlu olarak MOS+ β-Glukan ilave edilen gruplarda albumin düzeylerinde değişiklikler gözlenmezken, total protein düzeyinde sayısal artışlar gözlenmiş, fakat istatistiksel olarak farklılık bulunmamıştır.

Vitaminler ve mineraller, metabolik ve fizyolojik süreçler ve dolayısıyla hayvansal üretkenlik ve üreme performansı için çok önemli besinlerdir. Vitaminler hayvanlarda bağışıklık durumunu güçlendirir, hastalıklara ve strese direnmeye yardımcı olur (Sahin ve Kücük 2001, Sigola ve ark. 2019). Sıcaklık stresine maruz bırakılan Japon bıldırcınları yemine 250 mg/kg vitamin C ilavesinin serum glukoz ve total kolesterol düzeylerini azaltırken, total protein düzeylerini artırdığı belirtilmiştir (Şahin ve ark. 2003). Seyrek ve ark. (2004) sıcaklık stresine karşı Japon bıldırcınlarının yemlerine 150 mg/kg, 250 mg/kg ve 500 mg/kg vitamin C ilavelerinin serum albumin düzeylerini artırırken, glukoz, total kolesterol, VLDL-kolesterol ve trigliserit düzeylerini azalttığını bildirmişlerdir. Lipid mobilizasyonu ve katabolizmasının azaltılmasında birkaç metabolik yol bulunmaktadır. Birincisi kuşlara askorbat ilave edildiği zaman kortikoid sekresyonu azalır, lipoprotein ve doku lipazı uyarılamaz. Sonuç olarak, dokulardan lipidler ve kolesterol mobilize olamaz. İkinci

olarak askorbat, mikrozomal 7α-hidroksilasyonunu kontrol ederek kolesterolün safra asitlerine dönüşümü için gereklidir. Bu reaksiyon, karaciğerde kolesterol katabolizmasının hız sınırlayıcı aşaması olduğundan, askorbik asit eksikliği, bu reaksiyonun belirgin şekilde yavaşlamasına neden olarak karaciğerde ve kanda kolesterol birikmesine yol açar (Naidu, 2003).

Askorbat takviyesinin kolesterolün safra asitlerine dönüşümünü hızlandırarak, karaciğer ve serumdaki kolesterol konsantrasyonlarını azaltacağı vurgulanmıştır. Kolesterol kanda lipoprotein kompleksleri (VLDL, LDL ve HDL) tarafından taşındığı için, kolesterol ve lipoprotein konsantrasyonları pozitif korelasyon göstermektedir (Linne ve Ringsrud, 1999). Sunulan çalışmada bıldırcınların yemlerine 300 mg/kg vitamin C ilavesi serum total kolesterol ve trigliserit seviyelerini sayısal olarak azaltırken, istatistiksel olarak önemli bulunmamıştır. Prebiyotik ile kombine olarak Vitamin C verilen gruplarda ise glukoz, total kolesterol, trigliserid düzeylerinin kontrol grubuna kıyasla sayısal olarak azaldığı, total protein ve albumin değerlerinin arttığı gözlenmiştir.

Sonuç olarak, damızlık bıldırcınların yemlerine 4 g/kg prebiyotik ve 300 mg/kg vitamin C'nin ayrı ayrı ve kombine olarak ilave edilmesi serum biyokimyasal parametreleri üzerinde istatistiksel olarak değişikliğe neden olmamıştır. Bu durumun, kullanılan katkı maddesinin ve vitaminin dozlarına bağlı olabileceği düşünülmektedir. Kanatlı hayvan beslemede prebiyotik ile birlikte vitamin kullanım düzeyinin saptanması ile ilgili çalışmalara ihtiyaç duyulmaktadır.

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