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Editors

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Dear Scientist,

The sixth International Congress on Advances in Veterinary Sciences & Technics (icavst) was organized in Aksaray, Türkiye. We are very happy for organizing this congress in such a beautiful city and country that we have strong historical ties.

We wanted to make this conference little bit special by bringing scientist together from different disciplines of veterinary area and also to open new research and cooperation fields for them. In this sense, we desired to bring the distinguished scientist together to get know each other and to develop and implement new joint projects.

The scientist joined the congress was from different country and mostly from Turkey. Total over the one hundred scientists were registered in the congress. The total number of submissions were 43 and after a careful evaluation 34 submissions were accepted by our scientific committee and 2 of them were accepted as poster presentation and 32 of them were accepted as oral presentation and all those presentations was taken place in the conference booklet.

We would like to send our special thanks to Mr. Musa Köse and Mr. İsmet Uzun, ZENITH Group workers for their special efforts. and finally, the most importantly I would like to thank to all the participants individually who came from far away to join this conference.

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Abbreviation
FVM: Faculty of Veterinary Medicine

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FULL TEXTS 2022

INVITED SPEAKERS

THE FUTURE OF ANTI-INFECTIVES: BEYOND CONVENTIONAL ANTIBIOTICS

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Nowadays, antibiotics are widely used worldwide with many therapeutic applications. Since the initial commercial production of antibiotics in 1930s starting with successful commercialization of penicillin's (as model β-lactam antibiotic) followed by the discovery of tetracyclines group (tetracycline, oxytetracycline, and chlorotetracycline), many antibiotics have been discovered by different groups of researches worldwide. For more almost 100 years for now antibiotics saved life of many people worldwide. It was estimated that if antibiotics were not existed in treatment protocol, the number of deaths could be tripled. However, with the extensive use of antibiotics with miss-use and miss-dose problems in addition of the extensive uses of antibiotics in non-medical fields such agriculture, aquaculture, and animal feeding many problems have been created. Continuous exposure of human body to subclinical doses of antibiotics, lead to the development of new generation of microbes which are resist to many known antibiotics. In addition, extensive uses of antibiotics in human body can lead to the significant reduction of natural human microbiomes (probiotics) which play significant role in general human health. Therefore, the need of applying other natural anti-infectives which cannot lead to microbial resistance over time without inhibition of neutral microbiota is needed. In this lecture, the new trends of anti-infective development will be presented, providing a futuristic view of novel antimicrobial bioactives of the future.

Keywords: Antibiotics, Anti-infective, Future

BIOPROCESSING AND BIOCATALYSIS FOR A POST COVID WORLD

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Current climate change concerns and our post COVID world continue to change the way we used to live, sometimes opening opportunities for sustainable societies by deploying better human creations. For instance, biotechnology approaches are mentioned as key option in several worldwide initiatives, including the UN Sustainable Development Goals, COP26 and more. Bioprocessing and biocatalysis are major parts of biotechnology for materials transformation. Biomass instead of petroleum, coal or controversial food feedstocks can produce cheaper, safer, faster and environmentally friendlier products and services. For instance, advanced biofuels, bioplastics, biooil, sugars, biofertilizers, proteins and foods to cite a few. In the current bioeconomy, the global biotechnology market grew 2.9% in 2022 and could be US$2.44 Trillion in 2028. The biotechnology market contributes 2.7% to the GDP in OECD countries. By 2030, the biotechnology market could be greater in non-OECD countries; more than 50% of total world agricultural output and 35% of chemicals and related output would depend on biotechnology. Over 40 countries have a national strategy related to bioeconomy and 13 have a dedicated bioeconomy strategy. Bioprocessing and biocatalysis are key players to build up a more sustainable future offering a better quality of life to people worldwide.

Keywords: Bioprocessing, biocatalysis, COVID

ORAL PRESENTATIONS

LOW COST BIOETHANOL PRODUCTION FROM VARIOUS PLANT BIOMASS BY NANO-COUPLED RECOMBINANT B-GLUCOSIDASE FROM THERMOANAEROBACTERIUM THERMOSACCHAROLYTICUM

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With an increasing demand of an alternating source of energy for industries and transportations, thermostable cellulases are considered to be an important enzymes for the saccharification of cellulosic material for biofuel production. β-glucosidase is an essential cellulase enzyme and plays a key role in the degradation of cellulosic biomass and produce simple sugars that can be converted into biofuel. In the current research work, cloning, expression, purification and characterization of β-glucosidase was carried out from Thermoanaerobacterium thermosaccharolyticum into E. coli. β-glucosidase gene from thermophile T. thermosaccharolyticum was amplified through PCR and later cloned in pET-21a(+) vector by utilizing standard procedures. β-glucanase was expressed in E. coli and enzyme thus obtained was purified by ammonium sulphate precipitation and immobilized metal ion affinity chromatography with 2.74 fold purification having 31.87 U mg-1 specific activity and recovery of 29.42%. Molecular weight of the purified β-glucanase was 75 kDa as determined by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Effect of temperature, pH, metal ions, organic solvents on the purified enzyme was analyzed. The enzyme was stable upto 80°C with a broad pH range of 4-9, with optimum temperature 70°C and pH 6.5. The enzyme activity was increased in the presence of metal ions especially Mg+2 and Ca+2, reduced activity in the presence of Cu and Mn and was highly affected by EDTA. However, an addition of 30% Isopropanol and absolute ethanol resulted in decrease of enzyme activity to 66% and 62% respectively. Saccharification ability of β-glucosidase was tested on various pre-treated biomass that were analyzed by scanning electron microscopy for the removal of lignins and disruption of cellulose contents. The highest saccharification percentage was observed with sugarcane baggase (18.4%), Hazelnut cob (15.8%), Hazelnut shell (13.1%) and Rhododendron (10.6%) after 72 h of incubation at 55°C with 25 units of enzyme. Immobilization of purified β-glucosidase enzyme with magnetic nanoparticles showed better saccharification results and reusability. With atleast 50% enzymatic activity, the immobilized β-glucosidase was reused 13 times for the process of saccharification. Maximum bioethanol production (3.18±0.05 g/L) was obtained utilizing yeast strain of Wickerhamomyces anomalus. The results suggest that recombinant β-glucosidase can be used in the bioconversion of natural biomasses into simple sugars which could be efficiently used in the biofuel industry for the bioconversion of raw biomass into bioethanol. The immobilized β-glucosidase can further facilitate in the saccharification by repeated usage and help in the reduction of the cost of the biofuel production process significantly.

Keywords: B-Glucosidase, Plant Biomass, Nano-Particles, Thermoanaerobacterium Thermosaccharolyticum

Support / sponsor note: This study is supported by Higher Education Commission (HEC), Pakistan

SCREENING OF FUNCTIONALIZED RICE HUSK BIOCHAR WITH CYCLODEXTRIN FOR CARBAZOLE ABSORPTION IN WASTEWATER

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Aromatic chemicals accumulate more and are maintained longer in marine organisms than alkanes. The octanol and water partition coefficients significantly determined the build-up of aromatic hydrocarbons. Hydrocarbons with a greater molecular weight are released more slowly after absorption. Water body degradation is not just an indicator of environmental degradation, but also poses a threat to the ecosystem. In this study, a novel material, rice husk biochar functionalized with cyclodextrin (RHB-CD), was examined to improve the adsorption capability for heterocyclic aromatic hydrocarbons (HAH) removal from wastewater. Biochar from rice husk was functionalized with cyclodextrin by adjusting the parameters of biochar mass (5-10g), cyclodextrin concentration (1-5% w/v), and sonication time (1-5 min). The adsorption capability of functionalized RHB-CD was compared to that of raw biochar. Using Fourier Transform Infrared Spectroscopy (FTIR), the qualitative characterization of functional groups was determined. One would anticipate RHB-CD to lead the path for new ecologically friendly methods of wastewater treatment based on the results. The study suggests the types of bonding that modify the photochemical and photophysical properties of the guest molecules in the inclusion environment, while concurrently visualizing the new window of knowledge for the host-guest inclusion complex.

Keywords: Functionalized Biochar, Aromatic Hydrocarbon, Wastewater, Carbazole, Cyclodextrin

Support / sponsor note: This study is supported by MRUN Young Researcher Grant Scheme (MYRGS) 2019

MECHANISM OF YEAST FROM CACAO BEAN FERMENTATION TO INHIBIT THE GROWTH OF CACAO PATHOGENIC MOLD

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Decreasing of cacao production in Indonesia caused by several things, including disease attacks that caused by the molds. Chemical fungicides caused serious health problem and affect the sustainability of agro-ecosystem. The alternative fungicides are the use of yeast as a biological agent. This study aimed to determine the yeast isolates from cacao bean fermentation from the State University of Jakarta Culture Collection (UNJCC) which have the potential to inhibit the growth of pathogenic molds on cacao pods and the mechanism of their inhibition. All test isolates were subjected to molecular identification and analysis based on the ITS area for mold isolates and the D1/D2 region for yeast in the PCR process as well as morphological characterization. The tests carried out were antagonistic test using multiple culture technique, activity test for volatile compounds using uniform petri dish technique, and detection of killer toxin compounds. The data were analyzed using one-way ANOVA and Chi-square test. Molecular identification and morphological characterization, antagonist test, inhibitory activity test of volatile compounds, and detection of killer toxin were carried out on 4 yeast isolates from the UNJCC collection. The identification results showed that the yeast isolates UNJCC Y-158, UNJCC Y-159, UNJCC Y-160, UNJCC Y-161 were Rhodotorula alborubescens, Meyerozyma guilliermondii, and Pichia kudriavzevii, respectively. A total of 4 yeast isolates (UNJCC Y-158, UNJCC Y-159, UNJCC Y-160, UNJCC Y-161) had the potential to inhibit the growth of molds F. decemcellulare UNJCC F9, F. solani UNJCC F18, and C. siamense UNJCC F14. Based on antagonism test, Pichia kudriavzevii UNJCC Y-160 had the best inhibiton against F. decemcellulare UNJCC F9 dan F. solani UNJCC F18, while the best inhibition against C. siamense UNJCC F14 produced by P. kudriavzevii UNJCC Y-160 and P. kudriavzevii UNJCC Y-161. Based on volatile test, the best inhibition against F. decemcellulare UNJCC F9 produced by M. guilliermondii Y-159, P. kudriavzevii Y-160 and P. kudriavzevii Y-161, while the best inhibition against F. solani UNJCC F18 produced by P. kudriavzevii UNJCC Y-160. The best inhibition against C. siamense UNJCC F14 produced by P. kudriavzevii UNJCC Y-160 and P. kudriavzevii UNJCC Y-161. Two isolates of yeast UNJCC Y-160, UNJCC Y-161 were able to produce killer toxins against fungi F. decemcellulare UNJCC F9 and F. solani UNJCC F18 through detection of killer toxin compounds.

Keywords: Yeast, Mold, Antagonist, Volatile, Killer Toxin
Support / sponsor note: Hibah Penelitian Kolaborasi UNJ

SATEERA®-BASED SANITARY PAD AS POTENTIAL NATURAL DEODORIZER

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The amount of menstrual flow differs depending on individual female. In average, women excrete around 100 to 300 cc and the amount of pure blood thereof is 30 to 50 cc. Cervical mucus or vulval sebaceous glands and fused endosporium are naturally mixed with several vaginal bacterial which causes peculiar smell due to the exudates and causes some restriction for women outdoor activities and comfortabilities. The currently and widely used female sanitary pad have simple function of absorbing menstrual flow by adopting different thickness and materials depending on the amount of menstrual flow, but could not eliminate the peculiar smell of the blood. Most of female sanitary pads do not allow leakage of moisture by the exterior vinylcover, and this is the same as window-blocked bathroom, thus likely to result in fungal or bacterial multiplication. The new invention aims to develop the best design of cotton-based sanitary pad incorporated with medicinal/herbal Sateera® extract due to the claim of its medicinal and deodorized properties. Microbial contamination tests has been conducted and the results obtained show that the sanitary pad sample incorporated with Sateera® successfully prevents any growth of microbial colony. The findings give an indication that the Sateera®-based sanitary pads were good in inhibiting microbial growth and puts a light as a natural deodorizer.

Keywords: Sateera®-based Sanitary Pad, deodorizer, antimicrobial

PLATFORM TECHNOLOGY FOR PLASTIC WASTE BIOREMEDIATION USING MICROBIAL APPROACH

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Due to its widespread usage in agriculture, architecture and construction, health, and consumer products, plastics play an essential role in every area of the economy across the world. They are the foundation of many businesses since they are utilized in the production of a variety of commodities such as defense materials, sanitary wares, tiles, plastic bottles, fake leather, and a variety of other home items. Food packaging, pharmaceutical packaging, detergent packaging, and cosmetic packaging all employ plastics. Per year, almost one trillion single-use plastic bags are used across the world, or roughly two million every minute. Plastic pollution poses a severe danger to the planet's environment and human existence. The buildup of plastic on land and at water has sparked interest in degrading these polymers. As a result, in order to lessen the environmental impact of plastics, appropriate biodegradable technologies must be used. Microbes that can digest plastic waste, particularly non-biodegradable polymers like polyethylene, polypropylene, polystyrene, polyvinyl chloride, and polyurethane, are one way to solve this problem. Many bacteria have the ability to break down plastics. However, while this biodegradation process is not fully efficient, it is a way forward from traditional plastic waste treatment. More research on microorganisms that digest polyvinyl chloride is needed since this type of polymer is the most difficult to decompose. This work focuses on the origins of plastics, strategies for managing plastic trash, and the biodegradation of plastic waste utilizing various microbes.

Keywords: Plastic Types, Waste, Bioremediation, Microbes, Degradation

SPRAY DRYING EVALUATION FOR DEVELOPMENT OF PINEAPPLE PREBIOTIC POWDER AND PREBIOTIC PROPERTIES

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The pineapple fiber is known to have a prebiotic effect that may improve intestinal health and improve the immune system. The pineapple fiber in powder form is effortless to consume, has a better shelf life, and is easy to transport. However, the spray-dried pineapple powder is often affected by its final quality such as loss of the nutritional value associated with the harsh processing conditions. Therefore, the aim of this study is to optimize the processing parameters of the spray drying with intention to retain most of the pineapple nutrition. Iinlet temperature was varied at 150 and 180 oC with maltodextrin concentrations of 25, 30 and 35% (w/w) as encapsulator. The results show that spray drying at the optimum condition of 150 oC and 25% (w/w) of maltodextrin resulted in the pineapple powder recovery of 22.3±0.10%, moisture content (MC) of 6.46±0.25%, solubility of 94.72±0.05%, hygroscopicity of 12.94±0.30%, the antioxidant activity of DPPH scavenging activity (EC50) of 30.9±0.30 mg/ml, and total phenolic acid (TPC) of 18.88±0.31mg/g of gallic acid. Scaling up of the process using a pilot-scale spray dryer with a capacity of 30 L/hr yielded 83.52% of powder recovery and produced a better quality of pineapple powder with retention of more antioxidant activity (31.33±0.22, mg/ml) and TPC (9.33±0.33 mg/g Gallic acid). The pineapple prebiotic powder exhibited significant prebiotic index of 3.435, promoting the growth of Lactobacillus lactis. The pineapple prebiotic powder developed from this study is highly potential dietary fiber as prebiotic and contains high antioxidant to improve the intestinal health and general health, respectively.

Keywords: Prebiotic, Pineapple, Spray Drying, Antioxidant
Support / sponsor note: This study is supported by Sateera Biotech Sdn Bhd

THE EFFECT OF DIFFERENT AGRICULTURAL WASTES AND PH ENVIRONMENTS ON YIELD AND NUTRITIONAL QUALITY OF MEDICINAL MUSHROOM (LENTINUS EDODES)

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In this study, Lentinus edodes (Shiitake) mushroom which has medical characteristics has been cultivated on the agricultural wastes. In the study, hazelnut shell (Corylus sp.) (HS), oak wood (Quercus sp.) (OW) and beech wood (Fagus orientalis) (BW) sawdust were used from agricultural wastes. Each waste was ground to 3-4 mm and made to compost material. The compost materials were soaked for several days until its moisture reaching 70%. Formulations were formed with combination of 100% and 50% of each agriculture waste. For pH balance, lime (CaCO3) were added at 1% ratio to dry compost weight. pH values of the compost combinations were measured by 6.64, 6.55, 6.35, 6.58, 6.63, and 6.43 for 100 % of HS, 100% OW, 100% BW, 50% HS: %50 BW, 50% HS :50% OW and 50% OW : 50%BW, respectively. The formed compost formulations were sterilized by autoclaving at 121 ° C at 1 atm pressure. At least 3 composts replicates were prepared from each waste type. After sterilization, 2% Lentinus edodes mycelium compared to dry compost weight was inoculated in microbiological safety cabinet with UV. The Composts were kept in the incubation chamber at a relative humidity of 80% and a temperature of 26 ° C. The composts which completed the development of micelles were stored in ice water for 24 hours. After ice water application, each compost was expected to fruit body. After fructification, the yields/microbiological efficiences were calcilatuled and nutrition quality was determeined. Accordin to the results obtained from the study, the highest yield and biological activity were determined in the environment where the pH of oak sawdust was 6.55. Hazelnut shells and combinnations have the highest values in terms of total nitrogen and protein. There was no significant difference between compost combinations in terms of total energy, carbohydrate and fat ratios.

Keywords: Lentinus Edodes (Shiitake), Medicinal Mushroom, Hazelnut Shell, Beech Sawdust, Oak Sawdust, Ph

HISTOPATHOLOGICAL EVALUATION OF THE PROTEIN TYROSINE PHOSPHATASE AND TENSIN HOMOLOGUE (PTEN) GENE CAUSING ENDOMETRIAL CARCINOMA

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Objectives: Protein tyrosine phosphatase and tensin homolog (PTEN) (BZS, MHAM, MMAC1, PTEN1, TEP1), a lipid and protein phosphatase, is one of the most frequently mutated genes in cancer. This gene is identified as a high-frequency mutated tumor suppressor in numerous cancers. Among the mutated and cancer-causing genes in endometrial cancers, the PTEN gene ranks first with 78.9%. The aim of this study is to determine the histological changes of this gene on the Endometrium.

Research Methods: It has been determined that the PTEN gene is effective in the endometrium from the cBioPortal site (78.9%). After the gene was identified, histopathological analysis was performed on the tissue images of 75 patients obtained from cBioPortal and Human Protein Atlas. Histopathological analyzes were performed on immunohistochemistry (Antibody HPA031335) and Hematoxylin-eosin stained images.

Results: As a result of the analysis, the rate of white-skinned women developing endometrial cancers caused by the PTEN gene was 56%. According to the localization areas of endometrial cancers, tumor localizations could not be fully defined as 73.3%, in anterior endometrium (20%), and posterior endometrium (6.7%). Endometrial hyperplasia was common in histopathological evaluations. When endometrial cells were evaluated, pathological changes were observed in cells in the stroma (10-25%), smooth muscle cells 65-80% and secretory cells (5-10%). As a result of the analysis, it was determined that cancer cases were 69 years old and above and the prognosis was not good.

Conclusion: According to the results of the study, the PTEN gene causes the most endometrial cancers. Pathological changes, especially in muscle cells, are a remarkable feature. Considering this feature, it shows us that endometrial thickening is very important in doctors' controls.

Keywords: Endometrial Hyperplasia; Endometrial Carcinoma; PTEN; Histopathology

THE EFFECT OF SUPPLEMENTATION OF BETA-ALANINE AND HIGH INTENSITY INTERVAL SWIMMING TRAINING ON LACTIC ACID AND ACTH HORMONE IN RATS: A PRELIMINARY REPORT

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Purpose: The purpose of this study was to determine the effect of beta-alanine supplementation and high intensity interval swimming training on lactic acid and adrenocorticotropic hormone (ACTH) levels in rats.

Method: Twenty-nine albino male rats (age: 12 weeks) were included as subjects in this study. Rats were divided into four groups as beta-alanine (n=7), training (n=8), beta-alanine+training (n=8), and control group (n=6). Rats performed high-intensity interval swimming training for 5-week (adaptation for one week, swimming training for four weeks, 5 days/week). During the training period, rats received Beta-alanine supplementation according to body weight measured every week for 5 days a week. Lactic acid and adrenocorticotropic hormone (ACTH) levels in rats were determined from blood samples taken after high-intensity swimming test performed at the end of the swimming exercise period. One-way ANOVA was used to determine difference between groups.

Results: A significant difference was found between ACTH levels of the groups ($F(3,28)= 3,881$, $p=0,021$). ACTH levels were significantly higher in beta-alanine, training and beta-alanine+ training groups than in the control group ($p<0,05$). A significant difference was found between lactic acid levels of the groups ($F(3,28)= 5,943$, $p=0,003$). It was found that lactic acid level was higher in beta-alanine, training and beta-alanine+ training groups than the control group ($p<0,05$). In addition, lactic acid levels were significantly lower in beta-alanine and beta-alanine+training groups than in the training group ($p<0,05$).

Conclusion: It was revealed that beta-alanine supplementation, swimming training and swimming training together with beta-alanine supplementation increased the ACTH level, while beta-alanine supplementation decreased the lactic acid level in the rats.

Keywords: Rat, Swimming, Beta-Alanine, Lactic Acid, ACTH Hormone
Support / sponsor note: SELÇUK ÜNİVERSİTESİ ARAŞTIRMA FONU

COMPUTATIONAL PREDICTION OF THE METABOLIC ALTERATIONS SHARED BY ALZHEIMER'S DISEASE AND TYPE 2 DIABETES

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Alzheimer's disease (AD) is a type of dementia that causes impairment in memory, reasoning, and thinking. Type 2 diabetes (T2D) is common in the general elderly population and is significantly associated with a higher risk of dementia. However, metabolic alterations responsible for this association are largely unknown. In this study, we aim to predict metabolic alterations in hippocampus region of the brain by predicting the activation of the metabolic reactions based on the gene expression levels of control, AD, and T2D samples from healthy individuals and in vivo disease models (Mus musculus and Rattus norvegicus). The associated transcriptomic datasets were retrieved from Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). The data was mapped on genome-scale metabolic network models using the Integrative Metabolic Analysis Tool (iMAT) to create sample-specific metabolic networks. For each reaction in the metabolic network, the active/inactive reaction ratio across disease and control samples was calculated to predict affected reactions and their pathway associations. We predicted a set of relevant reactions/pathways to be affected between control-AD or control-T2D and commonly affected pathways in both diseases. Bile acid, fatty acids, cholesterol, glycosphingolipid, steroid, inositol phosphate metabolism, chondroitin sulfate, and keratan sulfate metabolisms are commonly affected pathways in both AD and T2D patients. Metabolic alterations indicate T2D to be a risk factor for AD. In conclusion, mapping transcriptome data on genome-scale metabolic networks to predict the condition-specific activity of reactions enabled the identification of metabolic relations between AD and T2D.

Keywords: Alzheimer's Disease, Type 2 Diabetes, Sample-Specific Metabolic Networks, Transcriptome.

MICROWAVE ASSISTED EXTRACTION AND PHYTOCHEMICAL ANALYSIS OF PEPEROMIA PELLUCIDA FOR TREATMENT OF DENGUE

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Dengue is among the most widespread mosquito-borne diseases and it is endemic in many tropical and sub-tropical parts of the world. To date, no effective antiviral or vaccine is available for this chronic disease. Peperomia pellucida (P. pellucida) has demonstrated positive results against chronic diseases due to the presence of phytochemicals, mainly phenolic compounds. The extraction process of bioactive compounds increases the efficient collection of extracts with high bioactivity. Microwave-Assisted Extraction (MAE) is a "green technology" widely employed for plant matrix. In this work, the impact of temperature (60–150 ◦C) and extraction time (5–25 min) on the extraction yield and individual compounds concentrations were evaluated. Furthermore, the phytochemical analysis in 10 extracts was performed by spectrophotometer in order to know its total phenolic content. The results show that 145 ◦C, 15 min, was the best extraction condition. Temperature and extraction time have been shown to be potential factors in affecting MAE for obtaining bioactive compounds from P. pellucida.

Keywords: Dengue virus, Peperomia Pellucida leaves,Microwave-assisted extraction, Phenolic compound

Support / sponsor note: The study was supported by research grants as follows: 08G23, 08G19, 00L26 from Universiti Teknologi Malaysia.

TREATED COCOA BEAN (THEOBROMA CACAO L.) EXTRACTS AND ITS ANTIBACTERIAL PROPERTIES

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Theobroma cacao L. has been shown to possess antibacterial activity against foodborne pathogens. Fermentation of cacao beans is very important to develop flavour precursor and develop antibacterial compounds resulted from the biochemical changes inside the beans. This study was conducted to evaluate the antibacterial activity of treated cocoa extract against several foodborne pathogens namely Bacillus cereus, B. subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella Typhimurium, Staphylococcus aureus and Listeria monocytogenes. Cocoa beans were treated with Candida sp.-, and Blastobotryst sp.- as starters. Temperature and pH changes were monitored during fermentation. The cocoa beans were extracted using methanol, ethanol and hexane as solvents and screened for antibacterial activities using disc diffusion test. Gas chromatography-mass spectrometry (GCMS) analyses have exhibited several active compounds including caffeine, theobromine, gamma-tocopherol, hexadecanoic acid and gamma-tocopherol in all three fermentations. Selected cocoa extract was elected to examine the microfloral reduction in strawberries after treated with different extract concentration (0.05%, 0.50% and 5.00%) and at different exposure time (5 min and 10min). It is revealed that the natural microflora in strawberries resulted in reduction of at least 2 log10 CFU/mL after treatment with different concentration of extract. Overall, treated cocoa extract possessed substantial antibacterial characteristics and can potentially develop as natural food sanitizer and preservatives

Keywords: Antibacterial Agents, Foodborne Pathogens, Fermented Cocoa Beans, Theobroma Cacao L.

ULTRASONIC TREATMENT, HIGH SPEED MIXING AND THERMAL PASTEURIZATION FOR SELECTED TROPICAL FRUITS

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Fruits undergo various foods processing in order to enhance the organoleptic properties, nutrient content and extend its shelf life. The aim of this study is to determine the effect of food processing parameters such as thermal pasteurization, ultrasonic treatment and high speed mixing on total phenolic contents and antioxidant properties of tropical fruits. Five tropical fruits: banana, watermelon, mango, papaya and pineapple were selected. The fruits were prepared into puree and treated with three different processing conditions i: thermal pasteurization, ii: ultrasonic treatment, iii: high speed mixing. The fruits samples were analysed for the total phenolic contents and DPPH radical scavenging activity assay. The results clearly demonstrated that ultrasonic was the best treatment for total phenolic contents and antioxidant activity. Papaya was found to be the richest sources of antioxidant compounds. The results suggest that all selected tropical fruits have shown potential as sources of natural antioxidants.

Keywords: Pasteurization, Ultrasonic, Mixing, Total Phenolic Content, Antioxidant Properties

Support / sponsor note: This study is supported by Institute of Bioproduct Development

RECOVERY OF PHENOLIC-RICH CONDENSATE FROM PINEAPPLE WASTE BIOMASS AS POTENTIAL RUBBERWOOD PRESERVATIVE AGENT

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Improper management of lignocellulosic biomass generated from agricultural activities would lead to serious environmental problems. Pyrolysis offers a simple yet efficient alternative technique where pyroligneous acid (PA) is a major by-product obtained during slow pyrolysis of lignocellulosic biomass. PA has a potential to be used as rubberwood preservative in replacing conventional wood preservative such as boron and copper-chromium-arsenate which is toxic to human and posed a threat to the environment. In this study, the potential antitermites and anti-fungal properties for PA obtained from the pyrolysis of pineapple waste biomass were investigated. PA from pineapple waste biomass showed insignificant inhibition properties against both Pycnoporus sanguineus and Coriolus versicolor, but were successful in inhibiting the growth of both Aspergillus niger and Botryodiploidia theobromae for 7 days when applied at 70% and 100% concentrations. PA also exhibited good antitermites properties based on its ability to achieve 100% mortality of Coptotermes curvignanthus after one-week incubation, using non-diluted PA. GC-MS results on dichloromethane extract of PA revealed the presence of phenolic compounds and phenol with ortho-substituents such as 2,6-dimethoxyphenol and 2-methoxy-4-methylphenol. Both compounds have been reported to play an important role in termiticidal activity by previous study. The result showed that PA from pineapple waste can act as antifungal and antitermite agent but not as anti-wood decaying fungi agent. This result can be used as a good preliminary indication for future application of PA from pineapple waste as wood preservative.

Keywords: Pyroligneous Acid; Rubberwood; Antifungal; Anti-Termites; Phenols

THE DEVELOPMENT OF MANGROVE WOOD VINEGAR-BASED SANITARY PAD

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Mangrove wood vinegar or pyroligneous acid also known as Sateera® is a by-product of charcoal made from the billets of Rhizophora. Mangrove wood vinegar exhibits a high degree of antimicrobial activity against various microorganisms along with the significant antioxidant activity. It has been traditionally used as a deodorizer, fertilizer, sterilizer and antimicrobial agent. In this study, the mangrove wood vinegar was spray-dried with the addition of a different percentage of maltodextrin to produce the best Sateera® extract powder. The physical properties of the resulting powder were analysed based on its loss of drying and hygroscopicity index. The different percentages of Sateera® extract powder (batch 1: 100 g Sateera® extract powder, batch 2: 200 g Sateera® extract powder) were incorporated into the third layer of the sanitary pad. The physical properties of the Sateera®-based sanitary pad were evaluated after 1 week exposed to room temperature. It is shown that the pad was stable in terms of its physical properties. The absorption index of the Sateera®-based sanitary pad was investigated by using gelatine solution to imitate the viscosity of a menstrual fluid. It is revealed that the Sateera®-based sanitary pad exhibits a comparable absorption index (batch 1: 2.98 ± 0.09; batch 2: 2.98 ± 0.04) with the commercial sanitary pad (2.89 ± 0.10). The Sateera® incorporated sanitary pad shows a promising result to be commercialized in the market with increased absorbency index for prolonged refreshness.

Keywords: Sateera®, Sanitary Pad, Mangrove Wood Vinegar

Support / sponsor note: This research is supported by Sateera Biotech Sdn Bhd

EFFECT OF HOMOGENIZATION SPEED AND DURATION IN VIRGIN COCONUT OIL EMULSION

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The homogenization process is an essential step during the preparation of virgin coconut oil emulsion for smaller particle sizes, thus increasing the emulsion's physical stability. This study is purposely to evaluate the effect of the process parameters of homogenization speed and duration by using a rotor-stator homogenizer. The virgin coconut oil was used to be encapsulated using Tween 80 as the surfactant at the range of homogenization speed (5,000 to 25,000 rpm) and in the range of duration (5 to 25 minutes). The interfacial tension (IFT), rheology properties, and particle size were studied to evaluate the emulsion formation. The results showed that the speed of 15,000 rpm in 10 minutes was the optimum condition for good rheology properties with the smallest particle sizes, d50 (1.31± 0.34 µm). The interfacial tension indicated no significant effect at the selected range of homogenization speed ad duration. In conclusion, the speed, and duration of homogenization affected the rheology properties and particle size of the VCO emulsion

Keywords: Virgin Coconut Oil, Emulsion, Homogenization, Rheology Properties

Support / sponsor note: This study is suported by UTMPRQJ1300000.2851.00L39 IIIG and 4C437 GRANTS

BIOPROCESS OPTIMIZATION SCALING UP PLATFORM DEVELOPMENT FOR BIOMASS AND SPORES PRODUCTION BY BACILLUS AMYLOLIQUEFACIENS

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Production of high cell mass and spore of Bacillus amyloliquefaciens requires extensive study for the development of a suitable medium composition and cultivation strategy. Therefore, the aim of this study was to optimize the B. amyloliquefaciens cultivation medium for supporting high cell mass and spore production in fulfilling the demand for industrial production. At first, different production media were screened to select the best medium which supports the highest cells growth and spore production. The best medium was found to have the following composition: glucose, 25.0 g L-1; yeast extract 15.0 g L-1; and CaCl2, 2 g L-1. This medium yielded the highest cell mass and spore number of 3.21 g L-1 and 1.7x10^9 in shake flask cultivation. MgSO4 and MnSO4.H2O were added to the medium with 0.5 and 0.1 g L-1 respectively, due to their high effect on spores' production. The medium composition was further optimized using two approaches: One-factor-at-a-time (OFAT) and statistical method using response surface methodology (RSM). The produced biomass using optimized OFAT, and statistical optimized medium were 3.86 g L-1 and 4.74 g L-1, respectively. For the purpose of further industrialization, cultivations were carried out using batch mode in a 16-L, 150-L and 1500-L stirred tank bioreactor under uncontrolled pH conditions, with maximum biomass achieved at 5.66 g L-1, 5.85 g L-1 and 4.23 g L-1 respectively. In conclusion, the results suggest that the production medium was successfully optimized with an increment of cell mass about 53% using the RSM method. In addition to that, the process was successfully scaled up from 16-L up to 1500-L bioreactor achieving final yield of 4.23 g L-1. Whereas the final cell count and spore of Bacillus amyloliquefaciens obtained from the spray dried powder produced were 2.9x10^15 CFU/mL and 2.5x10^15 spores mL-1.

Keywords: Bacillus Amyloliquefaciens, Biorocess, Optimization, Cell Biomass, Spores Production

Oral Presentation

INVESTIGATION OF THE THERAPEUTIC EFFECT OF FULLERENE C60 AGAINST HEART TISSUE DAMAGE BY SOME PROTEIN SIGNALING PATHWAYS AND HISTOPATHOLOGICAL BIOMARKERS

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In this study, the effect of fullerene C60 nanoparticle on some protein signal pathways and histopathological parameters against 7,12-dimethylbenz [a] anthracene (DMBA) induced heart tissue damage in Wistar albino female rats was investigated. The animal experiments part of this study was conducted in the Firat University Experimental Animal Research Center (FUDAM) with the permission of the Firat University Animal Experiments Ethics Committee dated 18.03.2021 and numbered 2021/05. In this study, 60 Wistar albino female rats (n = 60, 8 weeks old) were used. These rats were divided into 4 groups and each group included 15 rats. Groups: (1) Control Group: Fed with standard diet; (2) C60 Group: C60 (1.7 mg/kg bw, oral gavage); (3) DMBA Group: DMBA (45 mg/kg bw, oral gavage); (4) C60 and DMBA Group: C60 (1.7 mg/kg bw, oral gavage) and DMBA (45 mg/kg bw, oral gavage) group. The rats were decapitated after 16 weeks and their heart tissues were taken and examined. Expression levels of p53 and HO-1 proteins in heart tissue were determined by western blotting technique. In addition, heart tissues were evaluated by histopathologically. As a result, p53 and HO-1 protein expression levels were significantly increased in the groups. C60 + DMBA compared to the group DMBA. According to the histological results, inflammatory cell formation, edema and streak loss were reduced in the C60 + DMBA treated treatment groups compared to the DMBA groups.

Acknowledgement: This work was supported by Scientific Research Projects Coordination Unit of Firat University. Project number: FF. 20.07. In addition, this study was supported by the Council of Higher Education (CoHE) 100/2000 Biotechnology priority field doctoral project and The Scientific and Technological Research Council of Turkey (TUBITAK) 2211/C program.

Keywords: Fullerene C60, Heart Tissue, HO-1, P53.

EVALUATING THE ACTIVITY AND STABILITY OF PROTEASE IMMOBILIZED ONTO CHITOSAN AND SILICA GEL BEADS AGAINST SOME METALLIC IONS, ORGANIC SOLVENTS AND DETERGENT

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As the use of proteases in industrial applications increases, the need for proteases with good operational stability and improved stability against various chemicals in the reaction medium is increasing. Objectives: In study, it was aimed to biochemical characterization and immobilization of protease onto chitosan and silica gel beads support by glutaraldehyde-mediated cross-linking and to determine its potential for use in the detergent industry. Methods: The pH/temperature, kinetic, operational stability, organic solvent and metal ion stability studies and compatibility in surfactants and commercial solid and liquid laundry detergents of free and immobilized enzyme were evaluated. The synthesized material was characterized via SEM and FTIR. Results: The optimum pH of the free and immobilized protease was determined as 7 and 10, respectively, and the optimum temperatures were determined as 40°C and 50°C. The immobilized enzyme exhibited good operational stability, retaining half of its activity after the 5th hydrolysis cycle and 94% of its relative activity at +4°C at the end of the 5th week. The immobilized enzyme had higher activity than the free enzyme and increased its activity by more than 18%, especially in the presence of Mg2+ ions for 1 hour. It was also increased approximately 4.4 and 3.4 fold in the medium containing ethanol and xylene respectively. In the presence of Tween 80, where the free enzyme was lost about 43% of its activity, the immobilized enzyme increased more than 6.5 fold and it is more stable and shows better activity in commercial solid detergents. Conclusions: The stability of the immobilized enzyme in various chemical agents, metallic ions, organic solvents, surfactants and commercial detergents, and its adaptation to harsh washing conditions that require alkali and high temperatures have been enhanced. The synthesized immobilized protease with its superior characteristics can be evaluated in various industrial applications, especially in the detergent industry.

Keywords: Chitosan, Silica Gel, Protease, Immobilization, Detergent Industry
Support / sponsor note: This study is supported by Scientific Research Projects Coordination Unit of Igdir University (Project no TMY1121Y40)

Oral Presentation

STABILITY OF THE HOUSEKEEPING GENE B-ACTIN TO TEMPERATURE INCREASE IN RAINBOW TROUT

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Housekeping genes are used as internal standarts in gen expression studies. These genes guidies the interpretation of the expression of target genes since these genes show stable and specific level of expression. However, deviations in the stability of housekeeping genes were observed depending on the differences of genes or organisms that leads to misinterpretation of the results of the study. In the present study, the response of β-actin, that is the most commonly used control gene in expression studies, to heat stress in Rainbow trout species were studied. According to the findings, heat stress did not cause a significant change on the expressions of β-actin gene in this species ($P>0.05$). β-actin can be used as a housekeeping gene in thermal stress in rainbow trout.

Keywords: Gene Expression, Rainbow Trout, Beta (B)-Actin, Thermal Stress

DETERMINATION OF THE EFFECT OF PLANT GROWTH REGULATORS ON SECONDARY METABOLITE CONTENTS IN S. SCLAREA

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Plants have been used for various purposes from past to present. Among these purposes, treatment with traditional methods is also included. With the development of alternative medicine, the active ingredients in the plants were determined and it was determined in which area they could have therapeutic properties, and the interest and need for medicinal plants increased. With the development of biotechnology, various methods are used to increase plant production in in vivo and in vitro environments. Plant growth regulators have an important place in this sense. Plant growth regulator applications affect plant yield depending on application methods, amounts and application times, and valuable metabolites in plants can be produced in a short time and in greater amounts. Salvia sclarea has antibacterial, antiseptic, antifungal, anticarcinogenic, anti-inflammatory, etc. It is a medicinal plant with high economic value. Although secondary metabolites are not produced as much as primary metabolites in plants, they are compounds that have at least as important a place as them. Many studies have been carried out using plant growth regulators to produce valuable metabolites of S. sclarea by tissue culture methods, and a great deal of yield has been achieved. In addition, studies on the effect of plant growth regulators on the production of metabolites of S. sclarea in vivo are limited. Plant growth regulators are known to be widely used in agriculture. In this study, 4 different cytokines (BAP, KIN, TDZ, m-T) were applied at 3 different concentrations (25, 50, 100 mg/L) to S. sclarea plants grown in pots containing 3:7 perlite/peat in the plant growth room. As a result of the application, the changes in the secondary metabolite contents of the plants were examined by GS/MS method. As a result, it was determined that the secondary metabolite contents of the leaf extracts of S. sclarea plant differed quantitatively and qualitatively depending on the type and concentration of cytokinins applied.

Keywords: Plant Growth Regulators, Secondary Metabolite, Cytokinin

EVALUATION OF THE APOPTOTIC EFFECT OF TANNIC ACID ON CANINE MAMMARY CARCINOSARCOMA CELLS

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Mammary tumors are pathological cases that originate from the mammary gland and canine mammary tumors have an important role in in the treatment of human mammary tumors due to the similarities with human mammary tumors. Tannic acid (TA) has drawn attention due to its anticancer properties specifically inducing apoptosis in some types of cancers. In the veterinary field, there is no study yet on the effectiveness of TA in canine mammary tumors. For this purpose, we aimed to evaluate the apoptotic effect of TA on canine mammary carcinosarcoma (CMCS) cells which were obtained from an 11-year-old female with the complaint of a mass (3-5 cm). We determined the potential therapeutic effect of TA on CMCS cells through the viability WST-1 assay, Annexin-V analysis and Acridine Orange (AO) staining. Our findings demonstrated that TA significantly inhibited cell viability and caused apoptotic cell death with characteristic apoptotic morphology. Therefore, our results suggest that TA-based therapy could be a promising strategy for the treatment of CMCS cells. However, more studies are needed to elucidate the therapeutic effects of TA on different subtypes of canine mammary tumors with advanced molecular analysis.

Keywords: Tannic Acid, Apoptosis, Canine Mammary Tumor

DETERMINATION OF THE EFFECTS OF TRAF2 AND NCK-INTERACTING PROTEIN KINASE (TNIK) INHIBITOR ON PI3K/AKT/MTOR SIGNALING IN CANINE LIPID-RICH CARCINOMA CELLS

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Mammary gland tumors have the highest incidence among dogs. Lipid-rich carcinoma is a very rare histological type of mammary tumor. However, it is generally observed in young female dogs and the success of current treatment options is limited. Therefore, innovative approaches are required for the treatment of canine mammary tumors. Traf2 and Nck-Interacting (TNIK) is a member of the mitogen-activated serine/threonine-protein kinase family that regulates Wnt signaling pathway. Additionally, the phosphatidylinositol-3-kinase (PI3K)/Akt and mammalian targeting of rapamycin (mTOR) signaling pathways is crucial for many aspects of cancer cell growth. Therefore, we aimed to determine the effect of NCB-0684 as a TNIK inhibitor on PI3K/Akt/mTOR signaling in canine lipid-rich carcinoma cells via determination of Akt and mTOR mRNA levels with RT-PCR analysis. Our findings demonstrated that NCB-0684 inhibited Akt and mTOR gene expression levels at higher concentrations. However, the effectiveness of TNIK inhibitor is changed dependently its concentration. Thus, our results claim that NCB-0684 could suppress PI3K/Akt/mTOR signaling. However, further investigations need to clarify the inhibitory activity of TNIK inhibitor on PI3K/Akt/mTOR signaling in canine mammary tumors

Keywords: Canine Mammary Tumor Cells, TNIK Inhibitor, PI3K/Akt/Mtor Signaling

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ANTIBACTERIAL EFFICACY OF ALTERNATIVE AND CONVENTIONAL ENDODONTIC IRRIGANTS ON S. MUTANS AND E. FAECALIS

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Objectives: Effective removal of microorganisms from the root canal system plays an important role in the success of endodontic treatment. Sodium hypochlorite and chlorhexidine are commonly used irrigation solutions in endodontics, but due to their disadvantages, the search for alternative irrigation solutions continues. This study aimed to evaluate the in vitro antibacterial activities of alternative and conventional endodontic irrigation solutions against Enterococcus faecalis and Streptococcus mutants.

Methods: The following solutions were evaluated: 5.25% sodium hypochlorite (NaOCl); 2% chlorhexidine (CHX); 0.02% (200 ppm) hypochlorous acid (HOCl); 0.1% Polyhexanide (PHMB). Sterile saline was used as a negative control. Antiseptics were impregnated on disk-shaped filter papers. Then, these discs were placed first separately and then in pairs to investigate the synergistic effects of antiseptics when used alone or together, and the zone diameters were measured by the Kirby Bauer disc diffusion method.

Results: In S.mutants, 20mm inhibition zone diameter was measured for both CHX and HOCl when used alone, and the highest antimicrobial activity was observed. Approximately the same efficacy was observed in NaOCl (6mm) and PHMB (5mm). The highest efficacy was observed in CHX+HOCl (20mm) in combined use. In E.faecalis, the highest antimicrobial activity was observed in CHX as a 5mm inhibition zone diameter when used alone, and in CHX+HOCl (12mm) and CHX+PHMB (10mm) when used in combination.

Conclusions: Alternative endodontic irrigation solutions had different antimicrobial effects on test microorganisms. The use of hypochlorous acid, which is an alternative irrigation solution, together with chlorhexidine may provide microbiological advantages in clinical use.

Keywords: Antimicrobials, Chlorhexidine, Endodontics, Irrigation, Microbiology, Sodium Hypochlorite

Oral Presentation

PREPARATION AND CHARACTERIZATION OF ANTIBACTERIAL COMPOSITES CONTAINING HEXAGONAL BORON NITRIDE NANOPARTICLES

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Hexagonal boron nitride nanoparticles are accessible to process and are used in many antibacterial studies with low toxicity materials. The use of hexagonal boron nitride nanoparticles, which are used as an antibacterial material in the research, is shallow in the composite content. In addition, many studies show it is effective in antibacterial studies. In the study, hexagonal boron nitride and hexagonal boron nitride nanoparticles coated with silica will be modified with PGMA polymer to form an antibacterial, chemically resistant, high thermal stability, and non-toxic composite.

As a result of this study, it was aimed to find the most effective concentration of hexagonal boron nitride and hexagonal boron nitride nanoparticles coated with silica to be used in the composite. With the data obtained from this study, it is predicted that this material with high stability and non-toxicity can be easily used in future antibacterial studies and dental composite applications. In this way, it is thought that it can shorten the duration of long-lasting antibacterial studies and contribute positively to the preparation process of the studies. In addition, it aims to use this material, which is proven effective in dental applications, non-toxic, effective, and highly stable in dental examinations.

Keywords: Hexagonal Boron Nitride, Glycidyl Methacrylate, Silica, Antibacterial, Nanocomposites

ISOLATION AND MOLECULAR IDENTIFICATION OF A NOVEL PICHIA KUDRIAVZEVII FOL-27

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A novel yeast strain from fermented turnip beverage called shalgam produced in southern Anatolia region of Turkey was isolated upon incubation in YPD agar for 2 days at 30 °C. The DNA extraction of this yeast organism was performed using Machery-Nagel microbial genomic DNA isolation kit according to the protocols described in the manual. To quantify and DNA quality check, extracted DNA sample was loaded to NanoDrop spectrophotometer. Further, the DNA was amplified using the yeast specific primers for highly conserved regions of chromosome. The primers used to run PCR amplifications were as follows ITS1: TCCGTAGGTGAACCTGCGG, ITS4: TCCTCCGCTTATTGATATGC, NL1: GCATATCAATAAGCGGAGGAAAAG, NL4: GGTCCGTGTTTCAAGACGG, and GACA4: GACAGACAGACAGACA. Among 3 different primers used, only NL1/NL4 primer pair provided specific binding to DNA which became evident in gel electrophoresis bands. Thus, NL1/NL4 amplified PCR products were first purified and sent for Sanger sequencing. The DNA fragment reads were further processed using BLAST function in NCBI. The closest hits achieved with NL1/NL4 Sanger reads were belong to Pichia kudriavzevii strains although no 100% nucleotide identity achieved. This indicates that yeast organism isolated from fermented plant material "shalgam" is a unique strain of Pichia kudriavzevii sp. To better understand the metabolic potentials of this novel strain, whole genome analysis by next generation sequencing should be explored.

Keywords: Pichia Kudriavzevii, Fermented Plant Material, DNA Isolation, ITS, Sanger Sequencing, Molecular Identification

INVESTIGATION OF THE CYTOTOXIC EFFECT OF SILVER NANOPARTICLES OBTAINED FROM BETULA PENDULA ROTH LEAF EXTRACT BY GREEN SYNTHESIS ON MCF-7 CANCER CELLS

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Breast cancer is the most common type of cancer in women and the side effects of the methods used in the treatment are quite high. There is still a need for new treatment methods with lower side effects and higher efficiency for higher success rates and improving the quality of life of patients. In this study, silver nanoparticles (AgNPs) were synthesized from B,pendula plant extract by green synthesis method. The plant extract and AgNPs were treated separately for MCF-7 and MCF-12A cell lines for 24, 48 and 72 for three different hours and their cytotoxicity success levels and cancer selectivity against cancer cells were compared.

After the leaves of B.pendula plant were dried and the aqueous extract was obtained, AgNPs were synthesized. Characterization of synthesized AgNPs was performed with UV-vis, FTIR, TEM, XRD, EDX and DLS-ZETA. AgNPs and the aqueous extract of B.pendula plant were treated at different concentrations to each cell line for 24, 48 and 72 for three different hours after that, their cytotoxic effects were examined with XTT.

According to the results obtained, the average size of the DLS data result of AgNPs was found to be 109.02 nm. Compared to the plant extract, AgNPs were found to be much more effective as a cytotoxic agent against cancer cells. In addition, AgNPs have a cytotoxic effect on MCF-7 breast cancer cells at much lower concentrations compared to MCF-12A, which is a healthy epithelial cell. This clearly demonstrates the selectivity of B.pendula extract and AgNPs against cancer cells.

It shows that AgNPs synthesized with B.pendula extract have the potential to be a promising and adjunctive anti-cancer therapy agent as an alternative or supportive to chemotherapy in the treatment of breast cancer.

Keywords: Breast Cancer, Green Synthesis, Silver Nanoparticles, Cytotoxicity

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INVESTIGATION OF SILVER ION EFFECT ON SSDNA AND DSDNA BY PGM

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DNA is a biological material and used as a biomarker in medical, clinical, environmental and food analyses. DNA based detection methods rely on the hybridization mechanisms. Colorimetric, fluorescent, spectroscopic and electrochemical measurement techniques are commonly used ones for DNA quantification. Recently, portable and rapid techniques for DNA detection are gaining increasing interest. Portable glucose meter (PGM) has a high potential for this purpose. In this study, magnetite cross linked invertase aggregates (MCLIA) was prepared as a signal platform. Then prob DNA (ssDNA) was immobilized to this platform before it is hybridized with its complementary DNA (target DNA). The PGM signal was obtained with the addition of sucrose before and after the DNA hybridization that leads to glucose production. Target DNA was detected depending on the inhibition of invertase enzyme because of less glucose production by silver ion since it has a feature to inhibit the invertase enzyme. Besides, DNA can bind the silver ion with different affinity against single strand DNA (ssDNA) and double strand DNA (dsDNA). This is the first time, the effect of silver ion on ssDNA and dsDNA was revealed by PGM in this study. The results depicted that after the 750 ng prob DNA immobilization the enzyme inhibition decreases from 99.4 % to 71.0 % due to the binding of silver ion to ssDNA. The hybridization of 538 ng target DNA also resulted in a decrease in enzyme inhibition from 71 % to 28.1 %. Since, dsDNA captured more silver ion with respect to ssDNA that decrease inhibition effect on enzyme. Therefore, it is possible to detect the target DNA by MCLIA integrated PGM based on the different binding capacity/mechanisms of silver ions to DNA sequences.

Keywords: Silver ion, Silver DNA interaction, Manyetit Cross Linked Invertase Agregates (MCLIA), PGM

Support / sponsor note: This study was supported financially by the Scientific and Technological Research Council of Turkey (TUBITAK grant Number 119Z38).

INVESTIGATION OF ANTIFUNGAL ACTIVITY MECHANISMS OF ALPHA-PINENE, EUGENOL, AND LIMONENE

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Plant essential oils are preferred in cosmetics, medicine, food, and beverage industries for various purposes. Terpenoids, one of the major components of essential oils, are known to exert antifungal activity. However, their mechanism of action remains unclear. α-Pinene is found mainly in eucalyptus oils, eugenol is the active ingredient in clove oil, and limonene is the major component in the oil of citrus fruit peels. In this study, we aimed to determine the antifungal activity of α-pinene, eugenol, and limonene against Saccharomyces cerevisiae yeast cells. Besides, we focused on revealing the target side of the compounds on the yeast cells. Firstly, the antifungal activity of compounds was tested via minimum inhibitory concentration (MIC) measurement. After determining the MIC values, we performed a sorbitol effect assay to understand whether it acts on the cell wall or not. With sorbitol, the MIC values were not changed. It means that they are not effective on the yeast cell wall. Then, we measured the extracellular conductivity increase upon treatment with the compounds to understand the effect on the cell membrane. Eugenol and limonene were not changed the extracellular conductivity, and there was no ion leakage from the cell membrane. It can be said that the target side of the compounds is not the cell membrane. On the other hand, α-pinene damaged the yeast cell membrane causing a sudden increase in conductivity due to ion leakage. Moreover, an ergosterol effect assay with α-pinene was performed to detect cell membrane disruption via ergosterol or not. With ergosterol, the MIC value was not changed. α-Pinene must have another target than the ergosterol in the yeast cell membrane. Finally, revealing the mode of action of compounds against yeast cells will provide new insights into their usage in various fields.

Keywords: eugenol, limonene, α-pinene, antifungal activity, mode of action, Saccharomyces cerevisiae.

CHAOTIC SOLUTIONS IN GENE NETWORK MODELS

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Systems Biology is a holistic approach to understanding biological mechanisms by combining experimental results, mathematical modeling, and computational methods. One of the prominent issues in this field is the investigation of Gene Regulatory Networks(GRNs), which is the primary control step of proteins that play a role in many vital functions in biological systems. Although chaotic behavior in GRN models has been reported before, questions remain about the existence, advantages or potential functions of chaos in biological systems. In this study, we modeled a GRN motif with four genes and six interactions between these genes (activation or repression) and investigated the parameter space of the model that generates chaotic solutions. Lyapunov exponents were calculated with the Benettin algorithm to determine the system's dynamic properties. We followed the Unscented Kalman Filter(UKF) method to search for chaotic dynamics in the parameter space. However, the UKF approach failed to determine the parameter sets that resulted in a chaotic solution in the GRN motif. Then, we searched for values in the parameter space that would produce chaotic dynamics on three orthogonally selected 2-D planes having a limit of [0, 0.3] for each axis. We went through the parameter sets that provide positive values for the maximal Lyapunov exponent in a methodical manner. With this method, some parameter sets resulting in chaotic dynamics were obtained. The fact that the chaotic solutions found are rare in the parameter space and isolated from each other explained the failure of the UKF approach in this study. Interestingly, it was determined that chaotic solutions are localized between fixed point and oscillation dynamics. Although these results support the suggestion that chaos in GRNs may play a role in the transition from one oscillatory regime to another, cell differentiation, or robustness to cellular variants, further studies are needed to evaluate.

Keywords: Dynamical Systems, Gene Regulatory Networks, Systems Biology, Mathematical Modeling, Chaos

POSTER PRESENTATIONS

ENTRAPMENT OF MULTI-ENZYMES FOR CONVERSION OF STARCH TO MALTO-OLIGOSACCHARIDES

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Conversion of starch to value-added products requires several enzymes to efficiently break the complex structure of the polysaccharide. A specific combination of maltogenic amylase (MAG1) and cyclodextrin glucanotransferase (CGTase) provides a novel pathway of producing malto-oligosaccharides (MOS) from starch. However, the use of free enzyme is hampered by low enzyme recovery and low enzyme stability. Enzyme entrapment strategy was used in this study to improve enzyme recovery and stability for efficient conversion reaction. Both enzymes are entrapped individually in calcium alginate (CA) beads. Optimization of the entrapment process was performed using Response Surface Methodology by manipulating the calcium chloride, alginate and enzyme concentrations. The entrapped MAG1 (CA-MAG1) and CGTase (CA-CGTase) exhibited 88.06% and 89.45% of activity recovery, respectively. The thermal stability of both enzymes was enhanced greatly at 45 °C by retaining at least 50% of their activities after 50 minutes of incubation compared to that of free enzymes (10 minutes). The entrapped enzymes retained more than 50% of its activity after eight cycles of usage, showing high reusability. Hydrolysis of starch using the CA-MAG1 and CA-CGTase showed improvement of 1.25-fold compared to free enzyme with total MOS production of 183.82 mg/g during step-by-step reaction. The entrapment strategy used in this study was successful to develop a highly stable and reusable enzymes for MOS production.

Keywords: Enzyme Immobilization, Entrapment, Oligosaccharides
Support / sponsor note: This study is supported by Fundamental Research Grant Scheme, Ministry of Higher Education, Malaysia

THE CONSTRUCTION OF AFFINITY AGENT PRODUCTION PLATFORM BY USING PYRG AUXOTROPH ASPERGILLUS ORYZAE AND THE PRODUCTION OF THE NANOBODIES (VHH)

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The expression of monoclonal antibodies (mAbs) consisting of large (150 kDa) and complex structures is limited because of solubility, folding, effectiveness, and cost problems in heterologous microorganisms. Nanobody (VHH) is an affinity reagent that was discovered in camelids. VHH consists only of a heavy (H)-chain; nevertheless, hydrophilic amino acids are replaced with hydrophobic amino acids within the VL domain of mAbs to compensate for lacking a VL domain in VHH. The small size (4 nm and 2.5 nm in diameter), high specificity and affinity to bind antigens, low immunogenicity, thermal resistance, high solubility, and increased tissue penetration capacity of VHH are prominent features. Aspergillus oryzae is a filamentous fungus in GRAS (Generally Recognized as Safe) statute. It has been safely used in industrial biotechnology in producing various biomolecules for centuries. Our study aims to produce VHH in (orotidine-5'-monophosphate decarboxylase gene) pyrG auxotroph A. oryzae RIB40, taking advantage of its powerful secretory capacity. Additionally, it is aimed to describe that A. oryzae is a practical platform for expressing affinity reagents at a low cost. The auxotrophy was generated by gene replacement through plasmid carrying upstream and downstream sequences of pyrG but lacking the open reading frame of pyrG. The codon-optimized encoding sequence of VHH (PDB 1QBZ) with a 6xHistag fused to C-terminus was synthesized and inserted into an expression vector under the control of the native amylase gene promoter. Transformations were achieved through a protoplast-mediated transformation. Expressions studies showed that VHH was successfully expressed and secreted at 14 kDa in the soluble form. Metal affinity chromatography was used to purify secreted VHH. The binding of VHH to its antigen, RNAse A, was verified by gel filtration following incubation with RNAse A. The results demonstrate that A. oryzae can be used as a useful biotechnological platform to obtain functional VHH affinity reagents.

Keywords: Nanobodies, Aspergillus Oryzae, Pyrg Auxotrophy, Biotechnological Platform
Support / sponsor note: This study is supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) 1004 Grant No 20AG044.

FULL TEXTS

Evaluation of the apoptotic effect of tannic acid on canine mammary carcinosarcoma cells

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ABSTRACT

Mammary tumors are pathological cases that originate from the mammary gland and canine mammary tumors have an important role in in the treatment of human mammary tumors due to the similarities with human mammary tumors. Tannic acid (TA) has drawn attention due to its anticancer properties specifically inducing apoptosis in some types of cancers. In the veterinary field, there is no study yet on the effectiveness of TA in canine mammary tumors. For this purpose, we aimed to evaluate the apoptotic effect of TA on canine mammary carcinosarcoma (CMCS) cells which were obtained from an 11-year-old female with the complaint of a mass (3-5 cm). We determined the potential therapeutic effect of TA on CMCS cells through the viability WST-1 assay, Annexin-V analysis and Acridine Orange (AO) staining. Our findings demonstrated that TA significantly inhibited cell viability and caused apoptotic cell death with characteristic apoptotic morphology. Therefore, our results suggest that TA-based therapy could be a promising strategy for the treatment of CMCS cells. However, more studies are needed to elucidate the therapeutic effects of TA on different subtypes of canine mammary tumors with advanced molecular analysis.

Key words: Tannic acid, Apoptosis, Canine mammary tumor.

INTRODUCTION

Mammary tumors are pathological cases that originate from the mammary gland. As in humans, the incidence of mammary tumors in dogs is quite high (25-42%) (Klopfleisch et al., 2011). Because of the similarities in both species, studies on canine mammary tumors have an important place in the treatment of human mammary tumors. (Kumaraguruparan et al., 2006; Li et al., 2013). Approximately 40-50% of mammary tumors in dogs are malignant (Sorenmo et al., 2003). These are divided into different subtypes as sarcoma, carcinoma and carcinosarcoma (Sleeckx et al., 2011). Carcinosarcomas originate from both

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epithelial and mesenchymal breast tissue, with a very aggressive biological feature (Misdorp et al., 1999; Nunes et al., 2018). Although the first treatment approach in canine mammary tumors is surgical intervention, various postoperative complications have been reported (Sorenmo, 2003, Sorenmo et al., 2013, Al-Asadi et al., 2010). Adjuvant chemotherapy, which is another treatment option, is definitely preferred in patients after operative intervention since surgery alone is not sufficient in cases of carcinosarcoma. However, complications and side effects that may occur in all these treatment modalities are detected in most of patients. Therefore, there is a need for innovative treatment options in cases of carcinosarcoma.

The powerful drugs used in cancer treatments mostly originate from natural products (Aboul-Enein et al., 2012; Desai et al., 2008; Taneja et al., 2007; Greenwell et al., 2015; Mitra et al.. 2010). Among these antioxidants, tannic acid (TA) has drawn attention due to its anticancer and antibacterial properties (Yemmen et al., 2017; Cai et al., 2017; Kaczmarek et al., 2020; Yu et al., 2016; Ninan et al., 2016). TA is a naturally astringent hydrolyzable polyphenol (Baldwin et al., 2022). In *in vivo* study, TA shows a protective effect against the tumorogenic effect of benzo[a]pyrenediolepoxide (BPDE) on the skin (Greenwell et a., 2015; Khan et al., 1988). In another study, TA inhibits BPDE-induced liver mutagenesis (Bance et al., 1989). Furthermore, TA specifically induce apoptosis in breast, prostate, liver and colon human cancer cell lines (Jordan et al., 2018; Booth et al., 2013, Nie et al., 2016; Karakurt et al., 2016; Baer-Dubowska et al., 2020; Darwin et al., 2017). It is also known that TA induces apoptosis in cases of triple negative breast cancer (TNBC) and estrogen receptor positive (ER+) breast carcinoma (Darvin et al., 2017). Therefore, the use of TA in breast cancer is promising strategy. In the veterinary field, there has been no study yet about evaluating the effectiveness of TA in canine mammary tumors. In this context, the present study aimed to evaluate the apoptotic effect of TA on canine mammary carcinosarcoma (CMCS) cells.

MATERIAL and METHOD

Tissue sampling

The material of the study consists of a mixed breed intact bitch who presented with the complaint of a mass (3-5 cm) in the mammary gland. Mastectomy operation was performed on the bitch who was tentatively diagnosed with mammary tumor. A part of the tissue was referred to the pathology laboratory for histopathological examination. For this purpose, the collected tissue sample was fixed with 10% neutral buffered formalin solution. Paraffin-embedded samples were prepared to be 4-5 µm with the help of a microtome and stained with hematoxylin-eosin (Slaoui et al., 2017). The section obtained after staining was examined under the light microscope and evaluated according to Goldschmidt's classification (Goldschmidt et al., 2011). According to the histopathological examination, the tissue was diagnosed as carcinosarcoma.

Cell culture conditions

To obtain cells from tissues, the tissued were divided into 3-4 mm pieces and then washed with PBS several times. After incubation with collagenase enzyme at 37°C for 1 hour, the cells were centrifuged at 1500 × x g for 5 minutes. After centrifugation, the cells were washed with PBS and incubated in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% fetal bovine serum 1% penicillin-streptomycin in 5% CO_2 at 37°C.

Cell viability assay

To determine the cytotoxic effect of TA on CMCS cells, WST-1 analysis was performed. For this purpose an equal number of cells ($2x10^4$) was seeded into 96 well plate and incubated 24h. After incubation, the cells were treated with different concentrations (0.5, 1, 5 and 10 µM) of TA for 24 h. Afterwards, WST-1 dye was added into each well and incubated for 45 minutes at 37 °C in the dark. According to the WST-1 results, the most effective dose was selected for further experiments.

Annexin-V analysis

To determine the apoptotic effect of TA on CMCS cells. Annexin-V analysis was performed. For this purpose, and equal number of cells ($2x10^5$) was seeded into 6 well plate and treated with 10 µM TA for 24 h. Then, the cells were stained with Annexin-V dye and incubated 30 min in the dark. Then the stained cells were analyzed with Muse Cell Analyzer (Millipore).

Acridine Orange (AO) staining

To determine the changes on CMCS cell morphology after TA treatment, AO staining was performed. For this purpose, an equal number of cells ($4x10^5$) was seeded into 6 well plate and treated with 10 µM TA for 24 h. Then, the cells were stained with AO dye and incubated 30 min in the dark. Finally, the stained cells were analyzed with EVOS FL Cell Imaging System (Thermo Fisher Scientific).

Statistical analysis

Statistical analysis was performed by the GraphPad software package version 8. One-way ANOVA analysis of variance Post-Hoc Tukey's test was used for multiple comparisons. $p<0.05$ was considered statistically significant.

RESULTS

The effects of TA on cell viability of CMCS cells

We determined the cytotoxic effects of TA on CMCS cells by WST-1 analysis. According to our results, TA significantly inhibited the cell viability in a dose dependent manner (Figure 1, $p<0.05$). After 24 h incubation with 0.5, 1, 5 and 10 µM TA, the growth of CMCS cells was reduced to 96.33 ± 2.29%, 91.21 ± 1.72%, 90.88± 2.95%, and 65.63 ± 1.79%, respectively

(**p<0.05). Therefore, 10 µM TA treatment was selected as the most effective concentration of TA for further analysis.

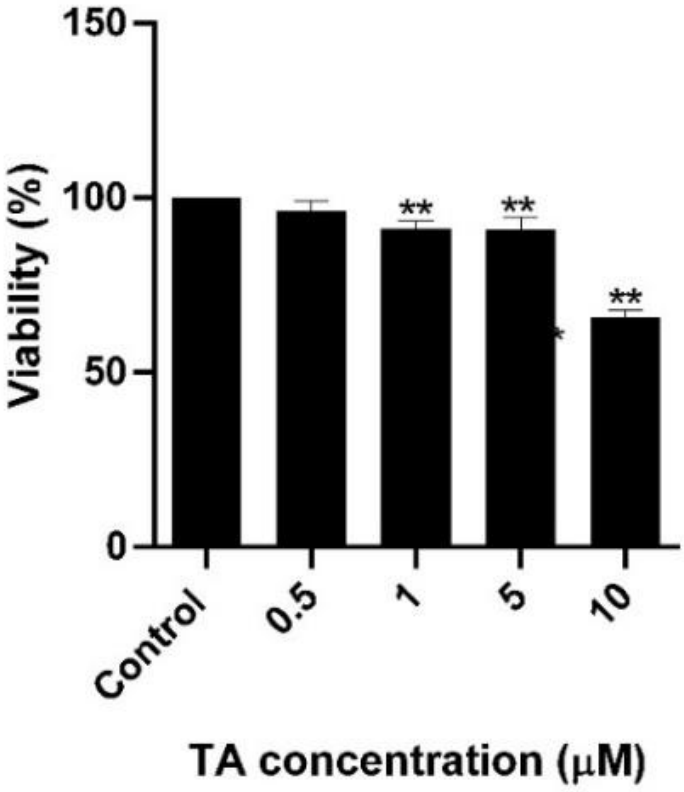

Figure 1. The cytotoxic effect of TA on CMCS cells. The cells were treated with different concentrations (0.5, 1, 5 and 10 µM) of TA for 24 h (**p<0.05)

The effects of TA on apoptotic cell death in CMCS cells

To determine the apoptotic effects of TA on CMCS cells, we performed the Annexin V assay (Figure 2). According to our results, TA significantly induced apoptotic cell number for 10 µM concentration (Figure 2A, *p<0.05). Following the treatment of 10 µM TA, a significant increase (7.03 ± 0.08% to 31.37 ± 0.99%) was detected in the number of total apoptotic cells in CMCS cells for 24 h (Figure 2B, *p<0.05).

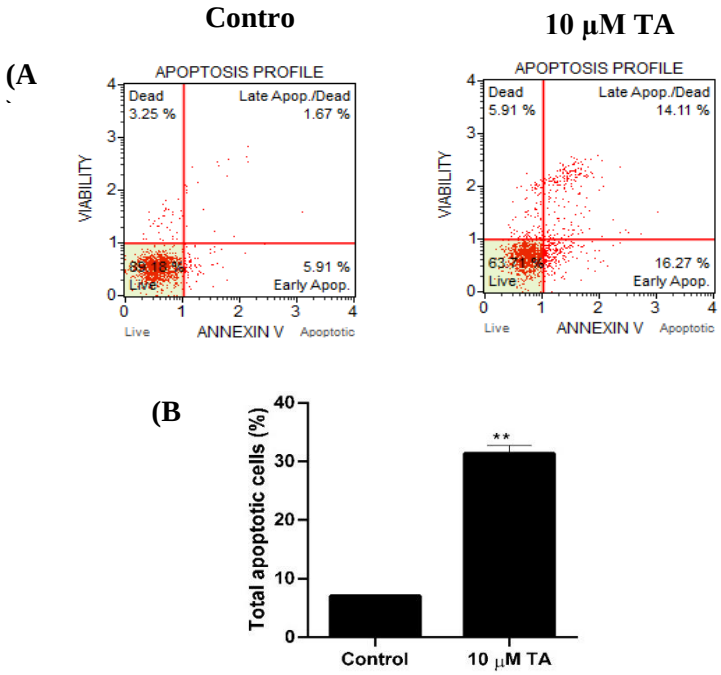

Figure 2. The apoptotic effect of TA on CMCS cells. (A) The cells treated with control, and 10 µM TA for 24h. (B) Statistical comparisons of the percentage of TA induced total apoptotic cell death in CMCS cells (**p<0.05).

The effects of TA on cell morphology in CMCS cells

To observe TA-induced apoptosis in CMCS cells, AO staining was performed (Figure 3). Compared to control cells, after 10 µM TA treatment, nuclear fragmentation, cell contraction and some vacuolar formation were occurred in CMCS cells.

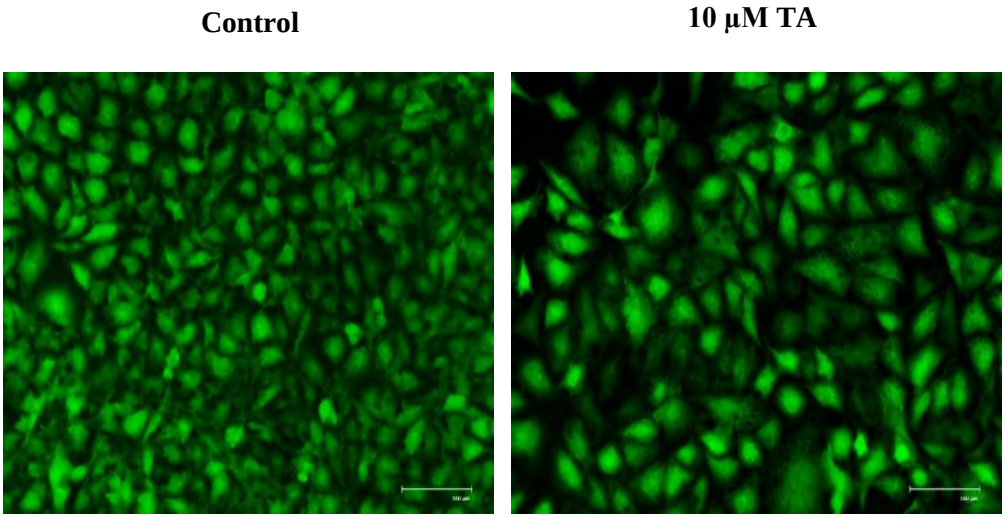

Figure 3. The effects of TA on the morphology of CMCS cells. The cells were treated with 10 µM TA for 24h.

DISCUSSION

In this study, we for the first time showed the potential therapeutic effects of TA on CMCS cells and our findings demonstrated that TA inhibited cell viability and caused apoptotic cell death. Therefore, our results suggest that TA-based therapy could be a promising strategy for treatment of CMCS cells.

The potential anticancer effects of TA have been extensively investigated in the literature. In one study, TA suppress the viability of A549 cells through induction of the intrinsic pathways of apoptosis as well as invasion, migration, and stemness (Sp et al., 2020). Darvin et al (2017) show that TA leads to caspase-mediated apoptosis in MCF-7 and MDA-MB-231 human breast cancer cells. Additionally, MCF-7 cells are more sensitive to the pro-apoptotic effect of TA than MDA-MB-231 due to estrogen receptor expression (Darvin et al., 2017). Moreover, TA modulates the EGFR/Jak2/STAT3 pathway inducing mitochondrial apoptosis in breast cancer cells (Darvin et al., 2017). On the other hand, Karakurt and Adali (2016), state that TA inhibits the proliferation, migration and invasion of PC-3 and LNCaP prostate cancer cells. Furthermore, the anti-cancer effects of TA on gingival squamous cell carcinoma are investigated by Ta et al., (2019) and they note that TA inhibits Jak2/STAT3 pathway by inhibiting G1 phase. Krajka-Kuźniak et al. (2015) shows that 10 µM TA activates the Nrf2/ARE pathway in HepG2 hepatoma cells. Canine mammary tumors are the most frequent types of cancer in bitches and proposed as a model of human breast cancer (Sorenmo, 2003). In our previous study, we show that curcumin which is another natural product that can act as an anticancer agent is an alternative treatment strategy on two subtypes (adenocarcinoma and inflammatory squamous cell carcinoma) of canine mammary tumors (Turna et al., 2022). Additionally, curcumin induces total apoptotic cell death and this effect is more profound in adenocarcinoma cells. Therefore, natural compounds may be the alternative treatment option in some cases with less invasive and should be extensively studied in canine mammary tumors.

Therefore, TA may inhibit proliferation of many cancer types and affects different mechanisms in cancer cells. Additionally, TA induces the apoptotic cell death in some types of cancer cells in consistent with our results.

CONCLUSION

In conclusion, there is no study in the literature that shows the effectiveness of TA in canine mammary tumors. In this context, we for the first time evaluated the cytotoxic and apoptotic effects of TA on CMCS cells. However, more studies are needed to elucidate the therapeutic effects of TA on different subtypes of canine mammary tumors with advanced molecular analysis.

ACKNOWLEDGMENT

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Ethical approval: The dog included in the study was operated for the treatment of mammary tumor and no experimental application was made. Postoperative waste tumor tissue constitutes the material of the study. The said practice is in compliance with the "Regulation on the Working Procedures and Principles of Animal Experimental Ethics Committees, prepared by the Ministry of Environment and Protection, published in the Official Gazette dated February 2014 and numbered 28914".

Conflict of interest: The author declared no conflict of interest.

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Antibacterial Efficacy of Alternative and Conventional Endodontic Irrigants on *S.mutans* and *E.faecalis*

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Abstract

Objectives: Effective removal of microorganisms from the root canal system plays an important role in the success of endodontic treatment. Sodium hypochlorite and chlorhexidine are commonly used irrigation solutions in endodontics, but due to their disadvantages, the search for alternative irrigation solutions continues. This study aimed to evaluate the in vitro antibacterial activities of alternative and conventional endodontic irrigation solutions against *Enterococcus faecalis* and *Streptococcus mutans.*

Methods: The following solutions were evaluated: 5.25% sodium hypochlorite (NaOCl); 2% chlorhexidine (CHX); 0.02% (200 ppm) hypochlorous acid (HOCl); 0.1% Polyhexanide (PHMB). Sterile saline was used as a negative control. Antiseptics were impregnated on disk-shaped filter papers. Then, these discs were placed first separately and then in pairs to investigate the synergistic effects of antiseptics when used alone or together, and the zone diameters were measured by the Kirby Bauer disc diffusion method.

Results: In *S.mutans,* 20mm inhibition zone diameter was measured for both CHX and HOCl when used alone, and the highest antimicrobial activity was observed. Approximately the same efficacy was observed in NaOCl (6mm) and PHMB (5mm). The highest efficacy was observed in CHX+HOCl (20mm) in combined use. In *E.faecalis*, the highest antimicrobial activity was observed in CHX as a 5mm inhibition zone diameter when used alone, and in CHX+HOCl (12mm) and CHX+PHMB (10mm) when used in combination.

Conclusions: Alternative endodontic irrigation solutions had different antimicrobial effects on test microorganisms. The use of hypochlorous acid, which is an alternative irrigation solution, together with chlorhexidine may provide microbiological advantages in clinical use.

Keywords: Antimicrobials, chlorhexidine, endodontics, irrigation, microbiology, sodium hypochlorite

INTRODUCTION

Microorganisms are the main factor in the etiology of periapical pathology (Kakehashi et al., 1965). Therefore, endodontic treatment aims to chemomechanically minimize bacteria in the root canal system and optimally seal the root canal space to prevent recontamination (Bystrom & Sundqvist, 1981). The persistence of microorganisms after endodontic treatment

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causes failure, therefore irrigation solutions with bactericidal properties are used during treatment (Pace et al., 2020; Siqueira, 2001). There are many root canal irrigation solutions on the market with different contents and properties.

Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are widely used in endodontic treatment and there are many studies in the literature. NaOCl has an effective organic tissue solvent and disinfection effect at high concentrations (Ayhan et al., 1999). CHX is an endodontic irrigation solution with broad spectrum antimicrobial activity, substantivity, and strong antiseptic properties. Although there are in-vitro cytotoxicity studies indicating that CHX has a higher cytotoxic effect than NaOCl (Trevino et al., 2011), there are also studies stating the opposite (Mollashahi et al., 2016). Due to the toxic effects of NaOCl and CHX on vital tissues, alternative irrigation solutions are being investigated.

Hypochlorous Acid (HOCl) has been suggested as an alternative irrigation solution to NaOCl because it provides effective cleaning of the root canal walls (Solovyeva & Dummer, 2000). There are also studies recommended for the disinfection of endoscopes, water systems of dental units, and dental impression materials (Rossi-Fedele et al., 2010). In a study, HOCl was compared with NaOCl and it was reported that they showed similar antibacterial activity on *E.faecalis,* however, HOCl showed much lower toxicity than NaOCl (Hsieh et al., 2020).

Polyhexamethylene biguanide or polyhexanide (PHMB) is an alternative to CHX (Santos et al., 2021). It has a wide spectrum of antimicrobial activity, the ability to adhere to the organic matrix, good cell and tissue tolerance, low-grade contact sensitivity risk, and wound healing stimulating effect (Arican et al., 2020; Eberlein & Assadian, 2010; Kramer et al., 2018).

In the literature, there is no study examining and comparing the antibacterial effects of conventional solutions and relatively new alternative root canal irrigation solutions on cariogenic bacteria. This study aimed to evaluate the *in-vitro* antibacterial activities of alternative and conventional endodontic irrigation solutions against *Enterococcus faecalis* and *Streptococcus mutans* when used alone or in combination.

MATERIAL and METHOD

The following commercially available irrigation solutions were evaluated: 5.25% sodium hypochlorite (NaOCl; Endosolve HP, Imicryl, Turkiye); 2% chlorhexidine (CHX; Ceraxidin-C, Imicryl, Turkiye); 0.02% (200 ppm) hypochlorous acid (HOCl; Crystalin, Natural Health Products-NHP, Turkiye); 0.1% Polyhexanide (PHMB; Actolind® w Solution, ACTO Pharma, Germany). Sterile saline was used as a negative control. Standard strains of *Streptococcus mutans* (ATCC 25175) and *Enterococcus faecalis* (ATCC 29212) were used in the study, and the strains were incubated in Columbia Agar with 5% sheep blood agar (Becton

Dickinson, GmbH) at 37°C for 24-48 hours. Antiseptics were impregnated on disk-shaped filter papers. Then, these discs were placed first separately and then in pairs to investigate the synergistic effects of antiseptics when used alone or together, and the zone diameters were measured by the Kirby Bauer disc diffusion method.

RESULTS

In *S.mutans,* 20mm inhibition zone diameter was measured for both CHX and HOCl when used alone, and the highest antimicrobial activity was observed. Approximately the same efficacy was observed in NaOCl (6mm) and PHMB (5mm). The highest efficacy was observed in CHX+HOCl (20mm) in combined use. Inhibition diameters of irrigation solutions on *S.mutans* as a result of the single- and double-disc synergy method are shown in Table 1.

Table 1. Inhibition diameters of irrigation solutions on *S.mutans*

Irrigation Solution	Inhibition zone diameter (mm)
CHX	20 mm
NaOCl	6 mm
HOCl	20 mm
PHMB	5 mm
CHX +NaOCl	15 mm
CHX + HOCl	20 mm
PHMB+ NaOCl	6 mm
PHMB + CHX	12 mm
PHMB + HOCl	10 mm
Sterile Saline	0 mm

In *E.faecalis*, the highest antimicrobial activity was observed in CHX as a 5mm inhibition zone diameter when used alone, and in CHX+HOCl (12mm) and CHX+PHMB (10mm) when used in combination. Inhibition diameters of irrigation solutions on *E.faecalis* as a result of the single- and double-disc synergy method are shown in Table 2.

Table 2. Inhibition diameters of irrigation solutions on *E.feacalis*

Irrigation Solution	Inhibition zone diameter (mm)
CHX	5 mm
NaOCl	0 mm
HOCl	2 mm
PHMB	1 mm
CHX +NaOCl	5 mm
CHX + HOCl	12 mm
PHMB+ NaOCl	1 mm
PHMB + CHX	10 mm
PHMB + HOCl	1 mm
Sterile Saline	0 mm

DISCUSSION

Microbial elimination is important in the success of endodontic treatment. *E.faecalis* is the major bacterial species isolated from root canals in endodontic failure. It has been reported that *E.faecalis* is mostly monoculture in 30-89% of teeth with post-endodontic failures (George et al., 2010). Also, it can cause life-threatening infections such as infective endocarditis (Madsen et al., 2017). *E.faecalis* is a Gram-positive, facultatively anaerobic, non-spore-forming, non-motile, cocciform bacterium (Kayaoğlu et al.). It is a microorganism resistant to harsh conditions. The virulence factors of *E.faecalis* associated with endodontic infection are lipoteichoic acid, sex pheromones, surface adhesins, extracellular superoxide, aggregation substances, lytic enzymes (gelatinase and hyaluronidase), and the cytolysin toxin, furthermore, new factors continue to be explored (Kayaoglu & Ørstavik, 2004). *E.faecalis* is related to endodontic infections, while *S.mutans* play the main role in the etiology of dental caries (Blancas et al., 2021). *S.mutans* can cause oral infections as well as induce systemic infections such as cardiovascular disorders (Lucchese, 2017). The importance of microbiological control in root canals increases when the relationship between focal oral infections and human systemic diseases is regarded. Considering all these conditions, *E.faecalis* and *S.mutans* species, which are the main factors in the pathology of oral and endodontic diseases, were used in this study.

According to the results of the current study, when used alone in *S.mutans,* the highest antimicrobial activity was observed in CHX and HOCl (20 mm inhibition zone diameter), while approximately the same activity was observed in NaOCl (6 mm) and PHMB (5 mm). In combined use, the highest efficiency was determined in CHX+HOCl (20mm). In *E.faecalis*, the highest antimicrobial activity was observed in CHX (5 mm) when used alone, while the efficacy of HOCl and PHMB was higher than NaOCl. In combination, the highest efficacy

was observed in CHX+HOCl (12 mm) and CHX+PHMB (10 mm). It was observed that the antibacterial activity of HOCl and PHMB, which are alternative irrigation solutions, were higher than NaOCl. Especially in *E.faecalis*, when chlorhexidine was used in combination with alternative solutions, it was determined that the antibacterial activity increased by 2 times.

In a study evaluating the antibacterial activity of root canal irrigation solutions based on NaOCl and electrolyzed oxidizing (EO) water (HOCl), it was concluded that EO waters containing HOCl had a bactericidal effect like that of conventional NaOCl against *E.faecalis* and *S.mutans*. It has been stated that low-concentration HOCl is equally antibacterial as 1.5% and 5.25% NaOCl and considering its low toxicity, it has the potential to replace NaOCl as an alternative irrigation solution for vital pulp therapy (Hsieh et al., 2020).

HOCl, naturally produced by the human immune system to fight infection, has antimicrobial activity (Ateş; Hsieh et al., 2020). Like HOCl produced by human immunity by the myeloperoxidase-H2O2-Cl system of phagocytic cells (Pullar et al., 2000), HOCl artificially produced for disinfection also can fight invading pathogens and infections (Ateş; Hsieh et al., 2020; Lapenna & Cuccurullo, 1996). HOCl has been reported to have a toxic effect on bacteria by causing complete disruption of bacterial ATP production (Barrette et al., 1989). Compared with NaOCl in the current study, it showed greater antibacterial activity against *E.faecalis* and *S.mutans*. There are similar results in the use of HOCl as an irrigation solution for cleaning root canals and removing the smear layer in endodontic treatment (Garcia et al., 2010; Hsieh et al., 2020).

PHMB is a powerful antimicrobial solution effective against Gram-positive and Gram-negative bacteria (Messick et al., 1999), yeasts (Larkin et al., 1992), and viruses (Medvedec Mikić et al., 2018). In one study, it was reported that 0.2% PHMB and 2.5% NaOCl solutions both successfully eliminated *E.faecalis* from mature dentin biofilm, but 0.2% CHX was not effective enough (Medvedec Mikić et al., 2018). Another study showed that 0.2% PHMB exerted significantly greater persistence on human dentin than 2% CHX (Chandki et al., 2020). PHMB is recommended as a suitable alternative to CHX as it reduces oral biofilm and has no reported side effects (Santos et al., 2021). In the current study, it was observed that 0.1% PHMB has antibacterial activity that can be an alternative to NaOCl. There is limited data in the literature on the use of PMHB as an irrigation solution in the endodontic literature, so more studies are needed on it.

This study showed that 5.25% NaOCl was not efficient enough to eliminate *E.faecalis* like the observations of other studies (Liu et al., 2010; Peciuliene et al., 2001; Portenier et al., 2003). It has been suggested that the resistance of *E.faecalis* to NaOCl may be due to its binding affinity to collagen fibers and hydroxyapatite (Chivatxaranukul et al., 2008;

Kayaoglu et al., 2008). Although similar results were seen in different studies, there was no host factor such as dentin in the current study.

In this study, the most effective antimicrobial solution was 2% CHX in eliminating *E.faecalis* and *S.mutans,* and these findings are consistent with the literature (Önçağ et al., 2003; Vianna et al., 2006). Few studies have tested *E.faecalis* against disinfectants without including host factors (Kayaoğlu et al., 2008). In a study, *E.faecalis* suspensions were treated with 2% CHX for 1 hour, and negative culture was observed (Fouad & Barry, 2005). In another study, it was found to be eliminated in 1 minute with 2% CHX gel (Gomes et al., 2006). It has been reported that 0.5% CHX has a good antibacterial effect after 1 and 24 hours of application (Kayaoğlu et al., 2008).

A greater antibacterial effect can be achieved in the root canal microflora with the combination of solutions compared to the single solution, because of the synergistic and/or additive effects of irrigants with different antimicrobial activities (Ozkan et al., 2020; Sundqvist, 1992). In the current study, it was observed that the antibacterial activity of CHX in combination with HOCl and PHMB was 2 times higher than the use of CHX alone on *E.faecalis*. Since there is no study in the literature on the combined use of relatively new alternative irrigation solutions, the findings cannot be compared with other studies. In addition, NaOCl + CHX showed less antibacterial activity compared to the use of CHX with alternative solutions, like in another study (Ozkan et al., 2020). It has been stated that the reason for this may be the orange-colored precipitate formed by parachlorophenol (PCU) or chlorophenylguanidyl-1,6-diguanidyl-hexane (PCGH) when CHX and NaOCl are used together (Basrani et al., 2007; Nowicki & Sem, 2011). To prevent the interaction of different solutions, it is recommended to irrigate the root canals with saline, sterile distilled water, or alcohol between solutions, and aspirate the remaining irrigant in the canal with a needle and dry it with paper cones or perform ultrasonic activation with EDTA (Bui et al., 2008; Keles et al., 2020; Prado et al., 2013). However, in this study, the above-mentioned methods were not applied to prevent the interaction of the solutions due to the difference in the experimental design.

In the present study, the absence of host factors such as blood, serum, dentin, and collagen in the experimental design prevents the results from being reflected in the *in-vivo* environment and constitutes the limitation of the study. In this technique, the absence of dentinal tubules that protect bacteria can be advantageous for disinfectants. However, this study evaluated the protection mechanisms of bacteria against different irrigation solutions in a standard *in-vitro* environment without host factors. There are many studies in the literature with different techniques and materials. Further studies are needed on endodontic alternative irrigation solutions, such as cleaning, antimicrobial, biocompatibility, effects on tooth structure, etc.

CONCLUSION

Within the limitations of this study, HOCl and PHMB irrigation solutions were found to have sufficient antimicrobial activity to be an alternative to conventional solutions for *S.mutans* and *E.faecalis.* It was determined that the combination of CHX and alternative irrigants increased the antibacterial activity. In particular, the combined use of HOCl with CHX may provide microbiological advantages in clinical use.

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Determination of the effects of Traf2 and Nck-interacting protein kinase (TNIK) inhibitor on PI3K/Akt/mTOR signaling in canine lipid-rich carcinoma cells

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ABSTRACT

Mammary gland tumors have the highest incidence among dogs. Lipid-rich carcinoma is very rare histological types of mammary tumor. However, it is generally observed in young female dogs and the success of current treatment option is limited. Therefore, innovative approaches are required for the treatment of canine mammary tumors. Traf2 and Nck-Interacting (TNIK) is a member of the mitogen-activated serine/threonine protein kinase family regulates Wnt signaling pathway. Additionally, the phosphatidylinositol-3-kinase (PI3K)/Akt and mammalian targeting of rapamycin (mTOR) signaling pathways is crucial for many aspects of cancer cell growth. Therefore, we aimed to determine the effect of NCB-0684 as a TNIK inhibitor on PI3K/Akt/mTOR signaling in canine lipid-rich carcinoma cells via determination of *Akt* and *mTOR* mRNA levels with RT-PCR analysis. Our findings demonstrated that NCB-0684 inhibited *Akt* and *mTOR* gene expression levels at higher concentration. However, the effectiveness of TNIK inhibitor is changed dependently its concentration. Thus, our results claim that NCB-0684 could suppress PI3K/Akt/mTOR signaling. However, further investigations need to clarify the inhibitory activity of TNIK inhibitor on PI3K/Akt/mTOR signaling in canine mammary tumors.

Key words: Canine mammary tumor cells, TNIK inhibitor, PI3K/Akt/mTOR signaling.

INTRODUCTION

Canine mammary gland tumors are the most common neoplasia among dogs due to spreading from the tumor into the mammary tissue. Among pets, dogs have the highest incidence of mammary tumors. Mammary tumor cases in dogs are divided into different subtypes as carcinoma, sarcoma and carcinosarcoma (Sleeckx et al., 2011). Approximately 50% of these mammary tumors in dogs exhibit malignant characteristics (Sorenmo et al., 2003). Lipid-rich carcinoma is very rare however it is generally observed in young female dogs. It has lymphatic invasion and distant metastasis (Monteros et al., 2003; Goldschmidt et al., 2011; Zappuli et al., 2019). The main method in the treatment of canine mammary tumors is surgical intervention. However, some postoperative complications such as edema formation in the hind legs, opening of the wound line due to necrosis, hematoma, bleeding,

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and seroma have been reported (Al-Asadi et al., 2010). After chemotherapy applications, many side effects such as allergic reactions, cardiotoxicity, neutropenia, nephrotoxicity and hepatotoxicity are observed (Santos et al., 2015). Therefore, innovative approaches are required in the treatment of canine mammary gland tumors.

Traf2 and Nck-Interacting (TNIK) is a member of the mitogen-activated serine/threonine protein kinase family and is a key regulator of Wnt signaling (Fu et al., 1999; Larhammar et al., 2017; Taira et al., 2004). Inhibition of Wnt-β-catenin in healthy breast tissue plays a role in the prevention of developmental disorders that occur in the fetus during pregnancy and in the reduction of cell proliferation (Angeloni et al., 2014). Wnt signal is responsible for proliferation, invasion and metastasis in breast tumor cases (Wen et al., 2020). High expression of TNIK and abnormal activation of Wnt-β-catenin signaling pathway play a role in breast tumor development (Jin et al., 2014; Shitashige et al., 2010; Mahmoudi et al., 2009). Particularly, higher expression of TNIK are detected in in cases of pancreatic, colorectal, and hepatocellular carcinoma (Jin et al., 2014, Zhang et al., 2016; Takahashi et al., 2015)

The phosphatidylinositol-3-kinase (PI3K)/Akt and mammalian targeting of rapamycin (mTOR) signaling pathways is crucial for many aspects of cell growth and survival (Alzahrani, 2019) and aberrant activation of PI3K/Akt/mTOR pathway induces uncontrolled Wnt-β-catenin signaling pathway. NCB-0846 is a small molecule as a TNIK inhibitor. Thus, TNIK inhibition may have an effect on PI3K/Akt/mTOR signaling. Therefore, in the presented study, the effect of NCB-0684, a TNIK inhibitor, on PI3K/Akt/mTOR signaling in canine lipid-rich carcinoma cells was investigated.

MATERIAL and METHOD

Tissue sampling

The material of the study consists of a French bulldog intact bitch who presented with the complaint of a mass (>5 cm) in the caudoabdominal mammary gland. Mastectomy operation was performed on the bitch who was tentatively diagnosed with mammary tumor. A part of the tissue was referred to the pathology laboratory for histopathological examination. For this purpose, the collected tissue sample was fixed with 10% neutral buffered formalin solution. Paraffin-embedded samples were prepared to be 4-5 µm with the help of a microtome and stained with hematoxylin-eosin (Slaoui et al., 2017). The section obtained after staining was examined under the light microscope and evaluated according to Goldschmidt's classification (Goldschmidt et al., 2011). As a result of the histopathological examination, it was determined that the tissue sent was lipid-rich carcinoma.

Cell culture conditions

The obtained tissues were divided into 3-4 mm pieces with the help of sterile scissors. After washing the tissue pieces with PBS several times, they were incubated with 10µl of collagenase enzyme at 37°C for 1 hour. After incubation, the supernatant was removed and the pellet was washed with PBS. Obtained tumor cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% fetal bovine serum 1% penicillin-streptomycin in 5% CO_2 at 37°C.

RT-PCR

We determined the most effective concentrations of NCB-0684 on canine mammary tumor cells in previous study as (2.5 and 5 µM) for 48h (Deveci Ozkan et al., 2022). Therefore the canine lipid-rich carcinoma cells ($1x10^6$) were cultured in 6 well plate and incubated with the most effective concentrations of NCB-0846. Total RNA was extracted with TRIGent (Total RNA Isolation Reagent, Biomatik). cDNA synthesis was conducted with High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific) and RT-PCR was performed with TaqMan™ Gene Expression Assay (Thermo Scientific) with StepOnePlus™ Real-Time PCR System (Thermo Scientific). ACTB was used for the reference gene.

Statistical analysis

Statistical analysis was performed by the GraphPad software package version 8. The fold change of the mRNA levels was statistically analyzed by software (https://www.qiagen.com/tr/shop/genes-and-pathways/data-analysis-centeroverview-page/otherreal-time-pcr-probes-or-primers-data-analysis-center/). $p<0.05$ was considered statistically significant.

RESULTS

The effects of NCB-0684 on Akt and mTOR mRNA levels in canine lipid-rich carcinoma cells

To explore the effects of NCB-0684 on *Akt* and *mTOR* expression levels on canine lipid-rich carcinoma cells, RT-PCR was conducted (Figure 1). The mRNA level of *Akt* and *mTOR* was 3.77 ± 0.03 and 5.95 ± 0.01, respectively at 2.5 µM of NCB-0684 ($p<0.05$). However, the treatment of 5 µM of NCB-0684 decreased the expression level of *Akt* (0.36 ± 0.05) and *mTOR* (0.97± 0.01) in canine lipid-rich carcinoma cells ($p<0.05$).

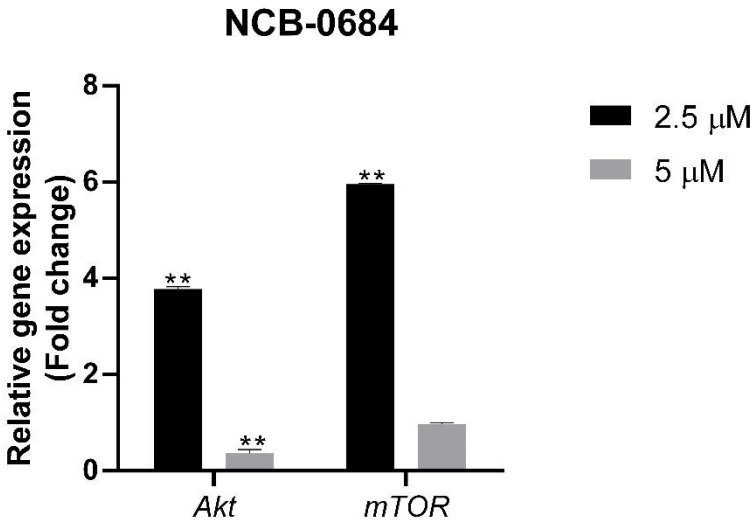

Figure 1. *Akt* and *mTOR* mRNA expressions in canine lipid-rich carcinoma cells following incubation with 2.5 and 5 μM of NCB-0684 for 48h (**p<0.05)

DISCUSSION

In this study, we for the first time showed the effects of TNIK inhibitor NCB-0684 on PI3K/Akt/mTOR signaling on canine lipid-rich carcinoma cells and our findings demonstrated that NCB-0684 inhibited *Akt* and *mTOR* gene expression levels at higher concentration despite of increasing lower concentration. Therefore, our results suggest that the effectiveness of TNIK inhibitor NCB-0684 is changed dependently its concentration and NCB-0684-dependent therapy could be a promising strategy for the treatment of canine mammary tumors.

Signaling in cancer cells often involves activation of receptor tyrosine kinases (RTK), which trigger cytoplasmic kinases (such as serine/tyrosine kinases). mTOR is an intracellular serine/threonine protein kinase centrally located in intracellular signaling cascades. Three major signaling pathways are important in cancers including the (PI3K)/AKT kinase chain, the protein kinase C family (PKC), and the mitogen-activated protein kinase (MAPK)/Ras signaling chains. The functional process of the PI3K/AKT/mTOR protein, which is an important signaling pathway, is closely related to receptor tyrosine kinases. Various RTKs such as vascular endothelial growth factor (VEGF) receptor (VEGFR), platelet-derived growth factor (PDGF) receptor-a, epidermal growth factor (EGFR), c-Met can be released from cancer cells (Faivre et al., 2006). These RTKs mediate PI3K/AKT/mTOR signaling pathway for gaining aggressive behaviors of cancer cells. Therefore, mTOR is an important antitumor target (Alzahrani, 2019). As anticancer agents, mTOR inhibitors are an important target in many cancer types (Hua et al., 2019). With the further development of molecular biology, the development of mTOR inhibitors and molecular drugs targeting the disrupted pathways in cancer cells will provide new

therapeutic approaches in cancer treatment (Murugan et al., 2019). In this context, the obtained results from our study showed the NCB-0486 could potentially inhibited *Akt* and *mTOR* gene expression and thus, the inhibition of Wnt signaling could mediate the suppression of PI3K/Akt/mTOR signaling pathway.

In our previous study, we show that NCB-0846 inhibits cell proliferation and enhances apoptosis in canine mammary tumor cells (Deveci Ozkan et al., 2022). Additionally, the potential effects of TNIK inhibitors have been investigated in the literature. In one study Masuda et al., (2016) NCB-0846 inhibits Wnt signaling and abrogated colorectal cancer stemness. In another study, NCB-0005 which is another molecule with inhibitory activity against TNIK suppresses TGFβ1-induced activation of Wnt signaling in A549 lung adenocarcinoma cells. Therefore, TNIK inhibition is associated with cell proliferation-related signaling pathways. In this context, our results claim that NCB-0684 as a TNIK inhibitor could be suppresses the aberrant activation of PI3K/Akt/mTOR signaling pathway.

CONCLUSION

In conclusion, as a new TNIK inhibitor, NCB-0846 has an inhibitory potential agent on PI3K/Akt/mTOR signaling in canine lipid-rich carcinoma cells. However, further investigations need to clarify and demonstrate the inhibitory activity of TNIK inhibitor NCB-0846 on PI3K/Akt/mTOR signaling in canine mammary tumors.

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Ethical approval: The dog included in the study was operated for the treatment of mammary tumor and no experimental application was made. Postoperative waste tumor tissue constitutes the material of the study. The said practice is in compliance with the "Regulation on the Working Procedures and Principles of Animal Experimental Ethics Committees, prepared by the Ministry of Environment and Protection, published in the Official Gazette dated February 2014 and numbered 28914".

Conflict of interest: The author declared no conflict of interest.

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Yüksek Şiddetli İnterval Yüzme Antrenmanı ve Beta-alanin Desteğinin Ratlarda Laktik Asit ve ACTH Hormon Düzeyine Etkisi: İlk rapor

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ÖZET

Bu çalışmanın amacı, beta-alanin desteği ve yüzme antrenmanının ratlarda laktik asit ve adrenokortikotropik hormon (ACTH) düzeyine etkisini göstermektir. Çalışmaya denek olarak yirmi dokuz (yaş: 12 hf) albino erkek rat dahil edilmiştir. Ratlar beta-alanin (n=7), antrenman (n=8), beta-alanin+antrenman (n=8) ve kontrol grubu (n=6) olmak üzere dört gruba ayrılmıştır. Ratlara beş hafta (bir hafta adaptasyon, dört hafta yüzme egzersizi, 5 gün/hf) boyunca yüksek şiddetli aralıklı yüzme antrenmanı yaptırılmıştır. Sıçanlara antrenman periyodu boyunca haftada 5 gün her hafta ölçülen vücut ağırlığına göre Beta-alanin takviyesi verilmiştir. Sıçanlarda yüzme periyodunun sonunda yapılan yüksek şiddetli egzersiz testinden sonra alınan kan örneklerinden laktik asit ve adrenokortikotropik hormon (ACTH) düzeyleri tespit edilmiştir. Gruplar arası farkı belirlemek için tek yönlü varyans analizi (ANOVA) kullanılmıştır. Bulgular: Grupların (Beta-alanin: 32,45±6,81 pg/ml, antrenman: 34,53±7,75 pg/ml, beta-alanin+antrenman: 30,10±7,91 pg/ml ve kontrol grubu: 22,33±3,43 pg/ml) ACTH düzeyleri arasında anlamlı bir fark bulunmuştur ($F(3,28)= 3,881$, $p=0,021$). Beta-alanin, antrenman ve beta-alanin+ antrenman gruplarında ACTH düzeyinin kontrol grubundan daha yüksek olduğu görülmüştür ($p<0,05$). Grupların (Beta-alanin: 146.05±44.06 U/L, antrenman: 144.76±33.11 U/L, beta-alanin+antrenman: 180,83±26,69 U/L ve kontrol grubu: 109,11±11,38 U/L) laktik asit düzeyleri arasında anlamlı bir fark bulunmuştur ($F(3,28)= 5,943$, $p=0,003$). Beta-alanin, antrenman ve beta-alanin+antrenman gruplarında laktik asit düzeyinin kontrol grubundan daha yüksek olduğu görülmüştür ($p<0,05$). Ayrıca, beta-alanin ve beta-alanin+antrenman gruplarında laktik asit düzeyinin egzersiz grubundan daha düşük olduğu bulunmuştur ($p<0,05$). Sonuç: Beta-alanin takviyesi, yüzme egzersizi ve egzersiz ile birlikte beta-alanin takviyesi ratların ACTH düzeyini artırırken beta-alanin takviyesinin laktik asit düzeyini düşürdüğü görülmüştür.

Anahtar Kelimeler: Rat, Yüzme, Beta-alanin, Laktik asit, ACTH hormonu

The Effect of Supplementation of Beta-Alanine and High Intensity Interval Swimming Training on Lactic Acid and ACTH Hormone in Rats: A Preliminary Report

Abstract

The purpose of this study was to determine the effect of beta-alanine supplementation and high intensity interval swimming training on lactic acid and adrenocorticotropic hormone (ACTH) levels in rats. Twenty-nine albino male rats (age: 12 weeks) were included as subjects in this study. Rats were divided into four groups as beta-alanine (n=7), training (n=8), beta-alanine+training (n=8), and control group (n=6). Rats performed high-intensity interval swimming training for 5-week (adaptation for one week, swimming training for four weeks, 5 days/week). During the training period, rats received Beta-alanine supplementation according to body weight measured every week for 5 days a week. Lactic acid and adrenocorticotropic hormone (ACTH) levels in rats were determined from blood samples taken after high-

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intensity swimming test performed at the end of the swimming exercise period. One-way ANOVA was used to determine difference between groups.

Results: A significant difference was found between ACTH levels of the groups (Beta-alanine: 32,45±6,81 pg/ml, training: 34,53±7,75 pg/ml, beta-alanine+training: 30,10±7,91 pg/ml and control group: 22,33±3,43 pg/ml) (F(3,28)= 3,881, p=0,021). ACTH levels were significantly higher in beta-alanine, training and beta-alanine+ training groups than in the control group (p<0,05). A significant difference was found between lactic acid levels of the groups (Beta-alanine: 146,05±44,06 U/L, training: 144,76±33,11 U/L, beta-alanine+training: 180,83±26,69 U/L and control group: 109,11±11,38 U/L) (F(3,28)= 5,943, p=0,003). It was found that lactic acid level was higher in beta-alanine, training and beta-alanine+ training groups than the control group (p<0,05). In addition, lactic acid levels were significantly lower in beta-alanine and beta-alanine+training groups than in the training group (p<0,05). Conclusion: It was revealed that beta-alanine supplementation, swimming training and exercise together with beta-alanine supplementation increased the ACTH level, while beta-alanine supplementation decreased the lactic acid level in the rats.

Keywords: Rat, Swimming, Beta-alanine, Lactic Acid, ACTH hormone

GİRİŞ

Şiddetli anaerobik egzersiz sırasında glikolitik bileşenler ile ortaya çıkan yorgunluğun başlıca nedeni olarak; kas liflerinde bulunan yüksek hidrojen (H+) iyonlarının asidoza neden olması gösterilmektedir. Kas fibrillerinde hidrojen iyonlarındaki (H+) artış, kas ve kan pH'ında bir düşüşü yani asidozun ortaya çıktığını ifade etmektedir. Asidozun ortaya çıktığı durumda ise laktik asit birikmeye başlamakta ve yorgunluk artışı ortaya çıkmaktadır. Kas asidozu, yüksek şiddetli aralıklı egzersizler sırasında yorgunluğun önemli bir nedeni olarak kabul edilmiştir. Düşük pH değeri glikolizi yavaşlatarak, endoplazmik retikulumdan kalsiyum salınımına ve kalsiyum iyonu bağlanmasına müdahale ederek kasılma süreçlerine bazı egzersizlerde yüksek yorgunluk algısını ortaya çıkarabileceği belirtilir. Ek olarak çalışmalarda, hidrojen (H+) birikiminin, fosfokreatinin yeniden sentezi ve fosfofruktokinaz aktivitesi gibi anaerobik metabolizmanın temel adımlarını inhibe ettiği gösterilmiştir. Başarı için, teknik mükemmelliğin belirleyici olduğu spor branşlarında yorgunluk; teknik becerilere ve karar verme sürecine müdahale edebileceğinden performans için zararlı olabileceği bilinmektedir. Vücut tarafından birçok tampon kullanıldığı belirtilmektedir. Ancak son yıllarda kas asidozunu azaltmayı amaçlayan beslenme stratejileri spor performansını artırma potansiyeline sahip görülmektedir. Bu doğrultuda son yıllarda dikkat çeken, asidozu tamponlamak için ergojenik yardımcı olarak kullanılan Beta alanin takviyesi öne çıkmaktadır (Mero ve ark 2013, Kratz ve ark 2017).

Beta alanin, karaciğerde sentezlenen esansiyel olmayan bir aminoasit ve insan iskelet kasında sitoplazmik bir dipeptittir. Tek başına beta alaninin ergojenik özelliklerinin çok sınırlı olduğu görülmektedir, ancak iskelet kaslarında karnosin oluşturmak üzere L-histidin ile birleştirildiğinde ergojenik etkileri ortaya çıkmaktadır (Harris ve ark 2006). Temel olarak, karnozinin öncelikli rolü, kas içi artmış hidrojen iyonunu tamponlama kapasitesi ile asit baz dengesinin korunmasına yardım etmesi olarak bilinir. Karnozinin de dahil olduğu kastaki fizikokimyasal tamponlar ile pH'daki değişikliklere karşı ilk savunma hattı

oluşturulmaktadır. Yani karnosinin, yüksek şiddetli egzersiz sırasında hücre içi bir tampon olarak görev gördüğü ve kas karnozin konsantrasyonundaki artışların döngü kapasitesini, ventilasyon eşiğini artırdığı ve yorgunluğu geciktirdiği gösterilmiştir (Mero ve ark 2010). Bu nedenle sporcular için kasın yüksek şiddetli egzersiz sırasında üretilen hidrojen iyonunu tamponlama yeteneğini artırmanın bir yolunun da Beta alanin olduğu düşünülmektedir (Hobson ve ark 2012). Beta alanin son yıllarda dayanıklılık antrenmanlarında oldukça etkili olan bir takviye olarak bilinmektedir. Beta alanin yüksek şiddetli egzersizleri daha uzun süre devam ettirebilmek için, antrenmana olan adaptasyonu geliştirdiği ve sporcunun daha uzun süre yorulmadan antrenmana devam edebilmesine katkı sağladığı belirtilmektedir (Rothschild ve Bishop 2019).

Hipotalamusun, adrenokortikotropik hormonunun (ACTH), ön hipofiz bezinden salgılanmasını kontrol ettiği ve bunun da insanlarda başta kortizol olmak üzere glukokortikoid hormonların adrenal korteks tarafından salgılanmasını uyardığı belirtilmektedir (Tsigosa ve Chrousos 2002). Kısacası, Adrenokortikotropik hormonun (ACTH), adrenal korteksten glukokortikoidlerin salınmasını sağlamakla görevli olduğu bilinmektedir. ACTH'ın salınımı, insanlarda kortizol olarak, sıçan ve farelerde ise kortikosteron olarak ifade edilmektedir (Torner ve ark 2017). ACTH çeşitli peptitleri vermek için parçalanmaktadır. ACTH'nin hipofiz bezinden kana salınımı CRH ve AVT tarafından düzenlenmektedir (Contarteze ve ark 2008). Bazı araştırmacılar, ACTH salınımını artıran ve bunun sonucunda glukokortikoid sentezini artıran uyaranları stresör olarak tanımlamışlardır (Tsigosa ve Chrousos 2002). ACTH salgısında artışa neden olan uyaranlar dikkate alındığında, yüzme egzersizi serum ACTH ve kortikosteron konsantrasyonlarını artırarak adrenal korteks ekseninin strese karşı negatif geribildirim verdiği belirtilmektedir. Egzersiz şiddeti ne kadar yükselirse ACTH ve kortizol seviyeleri o kadar yükselmektedir (Roper ve ark 2010). ACTH'ın akut strese cevap olarak eş zamanlı olarak salgılandığı belirtilmektedir. Bu sayede ACTH'ın, glukoneogenezi ve egzersiz sırasında gerekli olan glikoz oranını artırmada önemli rol oynadığı bilinmektedir (Harber ve Sutton 1984). ACTH' ın artması glikoz oluşumunu sağlarken, yüksek şiddetli interval egzersiz anında sporcunun yakıt olarak glikoza ihtiyaç duyması ve bunu yakıt olarak kullanması nedeniyle Beta alaninin ACTH'yi dolayısıyla glikoz artışına olan etkisi belirlendi.

MATERYAL VE METOD

Çalışmaya denek olarak yirmi dokuz (yaş: 12 hf) albino erkek rat dahil edilmiştir. Ratlar beta-alanin (n=7), antrenman (n=8), beta-alanin+antrenman (n=8) ve kontrol grubu (n=6) olmak üzere dört gruba ayrılmıştır. Ratlara beş hafta (bir hafta adaptasyon, dört hafta yüzme egzersizi, 5 gün/hf) boyunca yüksek şiddetli aralıklı yüzme antrenmanı yaptırılmıştır. Sıçanlara antrenman periyodu boyunca haftada 5 gün her hafta ölçülen vücut ağırlığına göre gavaj yöntemiyle Beta-alanin takviyesi verilmiştir. Sıçanlarda yüzme

periyodunun sonunda yapılan yüksek şiddetli egzersiz testinden sonra alınan kan örneklerinden laktik asit ve adrenokortikotropik hormon (ACTH) düzeyleri tespit edilmiştir. Gruplar arası farkı belirlemek için tek yönlü varyans analizi (ANOVA) kullanılmıştır. İkili karşılaştırmalarda Tukey testi kullanılarak anlamlılık düzeyi 0.05 kabul edilmiştir.

BULGULAR

Grupların ACTH düzeyleri arasında anlamlı bir fark bulunmuştur ($F(3,28)= 3,881$, $p=0,021$). Beta-alanin, antrenman ve beta-alanin+antrenman gruplarında ACTH düzeyinin kontrol grubundan daha yüksek olduğu görülmüştür ($p<0,05$). Grupların laktik asit düzeyleri arasında anlamlı bir fark bulunmuştur ($F(3,28)= 5,943$, $p=0,003$). Beta-alanin, antrenman ve beta-alanin+antrenman gruplarında laktik asit düzeyinin kontrol grubundan daha yüksek olduğu görülmüştür ($p<0,05$). Ayrıca, beta-alanin ve beta-alanin+antrenman gruplarında laktik asit düzeyinin antrenman grubundan daha düşük olduğu tespit edilmiştir ($p<0,05$).

Tablo 1. Adrenokortikotropik hormon (ACTH) ve laktik asit düzeyleri

	Beta-alanine grubu	Antrenman grubu	Beta-alanine+ antrenman grubu	Kontrol grubu	F değeri	P değeri
ACTH (pg/ml)	32.45±6.81*	34.53±7.75*	30.10±7.91*	22.33±3.43	3,881	0,021
Laktik asit (U/L)	146.05±44.06*,§	180.83±26.69*	144.76±33.11*,§	109.11±11.38	5.943	0,003

*p<0,05 kontrol grubundan anlamlı fark § p<0,05 antrenman grubundan anlamlı fark

TARTIŞMA VE SONUÇ

Beta-alanin takviyesi, yüzme antrenmanı ve antrenmanla birlikte verilen beta-alanin takviyesinin ratlarda ACTH düzeyini artırırken beta-alanin takviyesinin laktik asit düzeyinde düşüşe neden olduğu görülmüştür. Elimize geçen verilerde, Beta alanin alan grupların laktik asit düzeyi kontrol grubundan daha yüksek bulunmasının nedeni ise, kontrol grubunun 4 hafta boyunca egzersize tabi tutulmamasından kaynaklı olduğu düşünülmektedir. Kontrol grubundaki ratların son testte yüzme performansları beta alanin ve egzersiz gruplarına göre düşük bulunmuştur. Egzersiz ve beta alanin grubunda son testte en iyi yüzme süresi 2.50 sn iken kontrol grubunda en iyi derce 55-60 sn arasında kalmıştır. Bu değerler bize düzenli yapılan yüksek şiddetli interval egzersizin ve beta alanin takviyesinin performans üzerinde ne denli etkili olduğunu göstermektedir.

Gao ve ark (1988)'nın ve Siegler ve ark (2010)'nın yaptıkları çalışmalarda, yüzücülere kilogram başına verilen 0,3 gr Beta alanin takviyesinin yüzücülerin laktik asiti tamponlama kapasitesini artırabileceğini ve aralıklı yüzme performansını olumlu yönde

etkileyebileceğini göstermiştir (Mero ve ark 2010). Elde edilen sonuçlara göre; Beta alanin bir ergojenik yardımcı olarak kullanıldığında yüksek şiddetli interval antrenmanlarda ACTH'ı yükselterek metabolizmanın ekstra bir yakıt kaynağı oluşturmasını sağlarken yorgunluğa neden olan hidrojen iyonlarını azaltarak pH'ı yükseltip laktik asiti düşürerek performansı artırır bu da antrenmana olan adaptasyonu geliştirerek sporcunun daha uzun süre yorulmadan antrenmana devam edebilmesine katkı sağladığı düşünülmektedir.

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Stability of the housekeeping gene β-actin to temperature increase in rainbow trout

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Abstract

Housekeping genes are used as internal standards in gene expression studies. These genes guide the interpretation of the expression of target genes since these genes show stable and specific level of expression. However, deviations in the stability of housekeeping genes were observed depending on the differences of genes or organisms that leads to misinterpretation of the results of the study. In the present study, the response of β-actin, the control gene most commonly used in expression studies, to heat stress in rainbow trout species were studied. According to the findings, heat stress did not cause a significant change on the expressions of β-actin gene in this species ($P>0.05$). β-actin can be used as a housekeeping gene in thermal stress in rainbow trout.

Key words: Gene expression, rainbow trout, beta (β)-actin, thermal stress

INTRODUCTION

Polymerase chain reaction (PCR) is a molecular method that is used in the analysis of genes which serve as biomarkers. Also, real-time PCR is the most sensitive method that is used to detect the differences in the expression of messenger RNAs (mRNA) (Filby and Tyler, 2007; Bustin, 2000). Real-time PCR is mostly used to confirm the gene expression values (Filby and Tyler, 2007, Rajeevan et al., 2001). The principle of real-time PCR is based on increasing fluorescent signal proportional to the amount of the target gene.

To interpret the expression data of target genes correctly, we need housekeeping genes that are unaffected by experimental conditions and maintain their stabilities. Thus, expression levels of housekeeping genes never change much (Küçük, 2022). Many genes such as β-actin, glycealdehyde-3-phosphate dehydrogenase (GAPDH), elangation factor 1 alpha (ef1α) are used as housekeeping genes in fish (McCurley and Callard, 2008, Zheng and Sun, 2011; Hibbeler et al., 2008). However, the housekeeping genes do not always

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maintain their stability. Their use in experiments that are not suitable for their origin, may affects study and lead to erroneous results.

In the present study, the response of β-actin gene to thermal stress was analyzed. In accordance with this purpose, liver tissues of rainbow trout were used.

MATERIAL AND METHOD

Experimental Design

Healthy rainbow trouts (≃ 2 gr.) were taken from a fish farm and brought to the Dr. Nazmi Tekelioğlu Research Station (Cukurova University). Fish were fed with commercial feed (Optiline, Skretting, Norway) for 14 weeks and when they reached a weight of approximately 22.50 g (22.42 ± 2.85), they were transferred to fiber tanks in which the experiment would be conducted.

The experimental conditions were as follows: dissolved oxygen level: >7.0 mg/L, water hardness: 209.33 ± 7.03 ppm CaCO3, pH 7.0-8.5, ammonium nitrite and nitrate levels: NH 3 < 0.1 mg/L, NO2 < 0.1, NO3 < 0.5 and water temperatures were 17± 0.5°C. Fish were fed the commercial diets twice daily until apparent satiation for 60 days. The feed consumed was calculated daily. Photoperiod times were as 12:12 h.

To see the effect of temperature on the housekeeping gene β-actin, one group completed the trial period under the specified conditions and the other group was exposed to heat stress with a temperature increase of 1°C per day for seven days.

Fish sampling

The study was conducted in triplicate and three fish were taken from each experimental group (n=9). Fish were anaesthetized with clove oil (eugenol) (Botalife Co., Turkey). Liver tissue was taken to cryotubes (Cryovial, nuclease free, sterile) and dipped into liquid nitrogen. Liver tissues were stored at − 80 °C until they were used for total RNA isolation.

RNA extraction, cDNA synthesis and real-time PCR

Total RNA was isolated from fish liver with TRIzol-reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Then, total RNA quantity was checked up using the Qubit 2.0 (Thermo Fisher Scientific). The cDNAs were synthesized using OneScript Plus cDNA reverse transcription kit (Applied Biological Materials Inc., Canada). Reverse transcription was performed in 20 µl of final volume containing 4 µl 5X RT Buffer, 1 µl dNTP, 1 µl primers, 1 µl OneScript Plus RTase, 3 µl RNA (1000 ng), 10 µl depc water. The mixture was briefly centrifuged and put in thermal cycler according to the manufacturer's instructions. The resulting cDNAs were stored at − 20 °C until they were used for real-time quantitative PCR

2 µL of cDNAs were used for real-time PCR. The reactions were applied. Reactions were performed using SYBR green master Mix (Applied Biosystem) according to the manufacturer's instructions. The total volume was 25 ml and consisted of 12.5 µL of SYBR Green Master Mix, 1.25 µL of 10 pmol forward primer, 1.25 µL of 10 pmol reverse primer, 2 µL of cDNA template and 8 µL of water. RT-PCR device was Applied Biosystem 7500. Cycling conditions of device were 50 ◦C for 2 min, 95 ◦C for 10 min, followed by 40 cycles of 95 ◦C for 15 s and 60 ◦C for 1 min.

Primers of β-actin were designed on the sequence of rainbow trout beta actin gene available from Genbank and given in Table 1.

Statistical Analysis

SPSS 20.0 software was used for statistical analyses. Homogeneity of variance of data were checked. Gene expression data were evaluated using one-way ANOVA. The results were reported as mean ± standard error. The significance level was $p < 0.05$.

RESULTS

The results of β-actin mRNA expression were given in Fig 1. Group variances were homogeneous. The average cycle threshold (CT) value of standard group was 23.42 while it was 23.83 in the heat-stressed group. There was no significant difference between the groups ($p>0.05$).

DISCUSSION

The β-actin gene encodes the beta isoform protein of actin and plays a role in the cytoskeleton structure (Filby and Tyler, 2007; Küçük, 2022). The cytoskeleton can be defined as a complex and dynamic network of cells performing many vital functions such as cell division, phagocytosis, cytoplasmic flow, cell wall formation, and signal transmission (Anonymous, 2022).

In the present study, one group was exposed to heat stress for seven days with a daily increase of 1°C. As it is known, rainbow trout is a cold climate fish and adversely affected by high temperatures. The results show that there was no significant difference between the CT (cycle threshold) values which means the stability of the β-actin gene was not impaired.

Similarly, β-actin gene in *Channa striata* species was reported to be stable against temperature changes in the liver, gill and muscle. (Purohit et al., 2016). It was reported that β-actin is the most stable reference gene during development in *Siniperca chuasti* (Zhou et al., 2010). β-actin was reported to show small fluctuations and ef1α gene, another housekeeping gene, was more stable than β-actin (Olsvik et al., 2005). Similar to previous

study, the ef1α gene was indicated to be more stable than β-actin in *Solea senegalensis* and *Hippoglossus hippoglossus* larvae (Infante et al., 2008).

The expressions of genes can show some changes depending on the species of living things and even gender, due to the system in which they work. The genes selected as housekeeping gene stand out with their stability. However, it was revealed in the above studies that genes are selected as housekeeping may show slight changes according to the species, tissue and even gender. Our study is compatible with the literature in terms of showing the effect of thermal temperature on β-actin. β-actin can be used as a housekeeping gene in temperature studies in rainbow trout.

Table 1. Primers of β-actin gene.

Gene	Sequence 5′-3′	Amplification length (bp)	GenBank accession number
β-actin	F: CAGGGAGAAGATGACCCAGATTAT R: GCCCTCGTAGATGGGTACTG	160	NM_001124235.1

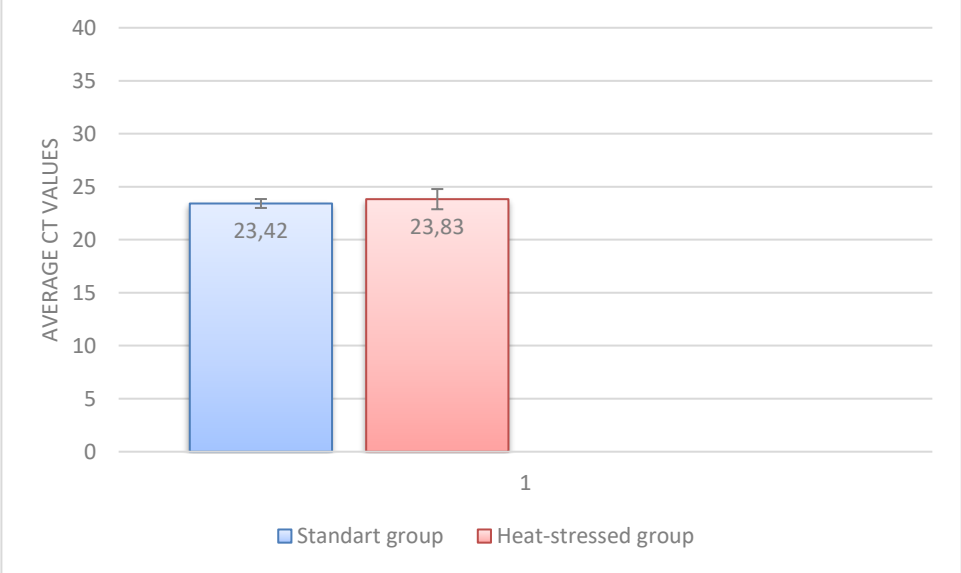

Figure 1. The average CT values of β-actin

CONCLUSION

The interpretation of target gene expression is based on the expressions of housekeeping genes. Thus, the choice of the housekeeping gene is very critical. According to the results of the present study, beta actin is stable and applicable for thermal stress studies.

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This study was carried out as a preliminary study aiming to determine 'the housekeeping primers' before the main study that would be conducted.

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