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INTERNATIONAL CONGRESS ON BIOLOGICAL AND HEALTH SCIENCES PROCEEDİNGS BOOK

All authors are responsible for the contents of their abstracts.

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Editor

Ulaş ACARÖZ

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Editor's Note

The first 'International Congress on Biological and Health Sciences' was organized online and free of charge. We are very happy and proud that various health science-related fields attended the congress. By this event, the distinguished and respected scientists came together to exchange ideas, develop and implement new researches and joint projects.

There were 15 invited speakers from 10 different countries and also approximately 400 submissions were accepted from more than 20 countries. We would like to thank all participants and supporters. Hope to see you at our next congress.

Best wishes from Turkey

Assoc. Prof. Dr. Ulaş ACARÖZ

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FULLTEXT

The Effects of Red Hot Pepper on Pancreatic and Ovarian Cancers

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Abstract:

Pancreatic ductal adenocarcinoma accounts for more than 90% of all pancreatic cancers and has a poor prognosis and a high mortality rate. Ovarian cancer is one of the most lethal gynecological cancers and affects 1 out of every 75 women in the world. The presence of metastasis in 75% of cases negatively affects the prognosis. Capsaicin (CAP) (trans -8- methyl-N-vanillyl-6-nonendamide) belongs to the Capsicum family and pungent ingredient in red hot peppers. In addition to showing the protective properties of CAP against different types of cancer, recent studies have proved that CAP also has an effect preventing tumor formation. The purpose of this study is to determine proliferative and/or apoptotic effects of low and high doses of CAP on PANC-1 cells a model of pancreatic ductal adenocarcinoma and SKOV-3 cells a model of ovarian adenocarcinoma. For this purpose, the cells were treated with basal medium (control group), 50 μM CAP (low dose group) and 250 μM CAP (high dose group). After 48 hours of treatment, the cells were fixed and immunocytochemistry procedure was performed with Ki-67 for determination of proliferative effects and cleaved-cappase-3 for determination of apoptotic effects. As a result of the study, Ki-67 expression decrease, and cleaved-caspase-3 expression increase with increased doses of CAP in both groups of cells. In consequence of the present study, the apoptotic effects of CAP on pancreatic and ovarian cancers can be lead of future chemoprevention studies and CAP can be combined with chemotherapeutic agents and used in treatment of pancreatic adenocarcinoma and ovarian adenocarcinoma.

Keywords: Capsaicin, Pancreatic Cancer, Ovarian Cancer, Apoptosis

1. Introduction

Cancer is defined as abnormal cell growth that occurs in any tissue or organ and can subsequently invade other tissues of the body and grow uncontrollably (Hausman, 2019). According to the World Health Organization (WHO), cancer ranks second in the causes of death worldwide, and one out of every six deaths is caused by cancer (WHO, 2021). Pancreatic ductal adenocarcinoma accounts for more than 90% of all pancreatic cancers and has a poor prognosis and a high mortality rate (Orth et al. 2019). Tumor localization generally limits surgical intervention (Ryan et

al. 2014). The probability of recurrence within 1 year is a high probability in patients with 10-20% early-stage operation (Gradiz et al. 2016). The use of chemotherapy as a treatment modality is quite common. Ovarian cancer is one of the most lethal gynecological cancers and affects 1 out of every 75 women in the world. The presence of metastasis in 75% of cases negatively affects the prognosis. Surgical procedure is required after chemotherapy for definitive treatment (Hu et al. 2010). Platinum-based chemotherapeutic agents are the first-line treatment for ovarian cancer as well as for lung cancer. However, resistance to cisplatin, which is one of the important platinum-based chemotherapeutics, creates an important obstacle for the treatment procedure (Ma et al. 2016). While chemotherapeutics used in the treatment of both types of cancer cause different dose-dependent toxicities, drug resistance that develops over time may reduce or eliminate the targeted benefit to the drug (Amrutkar 2017). To overcome this, studies in recent years have focused on the use of different drug combinations, as well as alternative therapies such as the use of biological substances (such as food additives, plant extracts, etc.), small target molecules (Palmer et al. 2017).

Capsaicin (CAP) (trans-8-methyl-N-vanillyl-6-nonendamide) belongs to the Capsicum family of plants and is the active ingredient that gives pepper its bitterness (Yang et al. 2006). For many years, it has been used as a spice, food additive, and medicine for its anti-inflammatory, anti-fungal, and anti-analgesic effects (Fattori et al. 2016). It is widely used clinically in obesity, rheumatoid arthritis, diabetic neuropathy, and various gastrointestinal and cardiovascular problems (Jose et al. 2010, Sharma et al. 2013). CAP shows its effect generally through transient receptor potential vanilloid 1 (TRPV1) (Minke et al. 1975). However, the antitumor effect occurs independently of TRPV1 by inhibiting cell growth and proliferation, such as suppression of the mitosis pathway, blockade of the mitochondrial respiratory tract and angiogenesis (Chapa-Oliver and Mejia-Teniente 2016). In addition to showing the protective properties of CAP against different types of cancer (lung, prostate, pancreas, colon and skin) in many previous studies, recent studies have proved that CAP also has an effect preventing tumor formation (Basith et al. 2016, Srinivasanet al. 2016). It was observed that CAP applied at different doses on pancreatic cancer cells significantly reduced the survival rate of cells after 100 μM dose (Lin et al. 2013). Zhang et al. (2008) applied different doses of CAP to pancreatic cancer cell lines up to a concentration of 250 μM and it was shown that normal acinar cells preserved their viability, but cancer cells entered apoptosis with increasing doses. In the same study, an in vivo xenograft model was applied, and it was reported that 2.5 mg / kg CAP administered orally reduced the tumor size 2.4 times (Zhang et al. 2008). In this study, it was aimed to determine proliferative and/or apoptotic effects of low and high doses of CAP on pancreatic adenocarcinoma and ovarian adenocarcinoma.

2. Materials and Methods

Reagents:

Capsaicin (M2028, analytical standard grade, ≥99%) was purchased from Sigma; DMSO (N182) was purchased from Ambresco, for CAP vehicle solution; ImmPRESS® HRP Anti-Rabbit IgG (Peroxidase) Polymer Detection Kit (MP7401) was purchased from Vector Lab. for immunocytochemistry; primary antibodies Ki-67 (abcam, 16667) for cell proliferation marker and cleaved Caspase-3 (sc-56053) for apoptosis marker were purchased from Santa Cruz Biotech.

Cell Culture:

The pancreatic adenocarcinoma cell line PANC-1 and ovarian adenocarcinoma cell line SKOV-3 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were plated at a density4X104 cells/well in 24-well plates and grown on coverslips precoated with 10 % FBS and maintained in DMEM for PANC-1 cells and McCoy5a for SKOV-3 containing 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in an atmosphere containing 5% CO_2. The following day (day 0), the media were removed and replaced with fresh media for cell control (CC); low and high doses (50, and 250 µM) of CAP. At 48 hour after treatment, the experiments were terminated.

Assessment of Cell Proliferation and Apoptosis Using Immunocytochemistry:

The cells on the cover slips were washed three times in PBS. The cells then were fixed in 4% paraformaldehyde at room temperature for 15 min and permeabilized with 0.1% Triton X-100 for 10 min. Then the cells were blocked for 20 min and followed by incubation with Ki-67 (1:200), and c-Caspase3 (1:200) antibody at 4°C overnight, then incubation with seconder antibody for 30 min. Cells then treated with DAP for 5 min and counterstained with Harris Hematoxylin for 2 min and the images were observed under the Nikon Eclipse 80i microscope. Randomly five microscopic areas were counted and the % value of staining cells was calculated for each experiment groups.

Statistical Analysis:

The data were analyzed using the IBM SPSS Statistics 22. Statistical significance between the groups was analyzed by the Kruskal-Wallis Test and followed by Mann-Whitney U posthoc test. The level of significance was defined as $p \leq 0.05$.

3. Results

Capsaicin decreases of the viability of A549 cells at high concentration.

When the PANC-1 and SKOV-3 cells were observed in the light microscope, the morphology of the cells was dramatically changed especially in PANC-1 cells (Fig. 1). The cell-cell interactions were disrupted in low dose of CAP in PANC-1 cells and high dose CAP group in SKOV-3 cells and regular epitheloid structure was deformed (Fig 1D). Ki-67 immunoreaction in cells' nuclei disappeared with the high dose treatment (Fig 1). The statistical differences were observed in all groups (Fig 2).

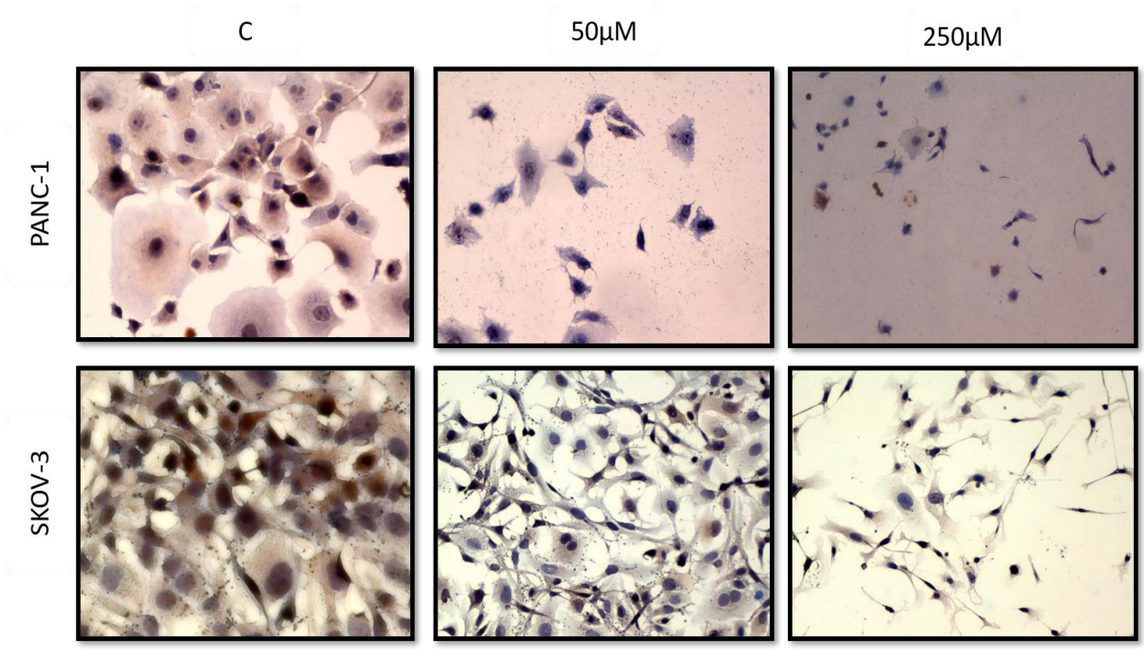

Figure 1. Ki-67 expression in PANC-1 and SKOW-3 cells.

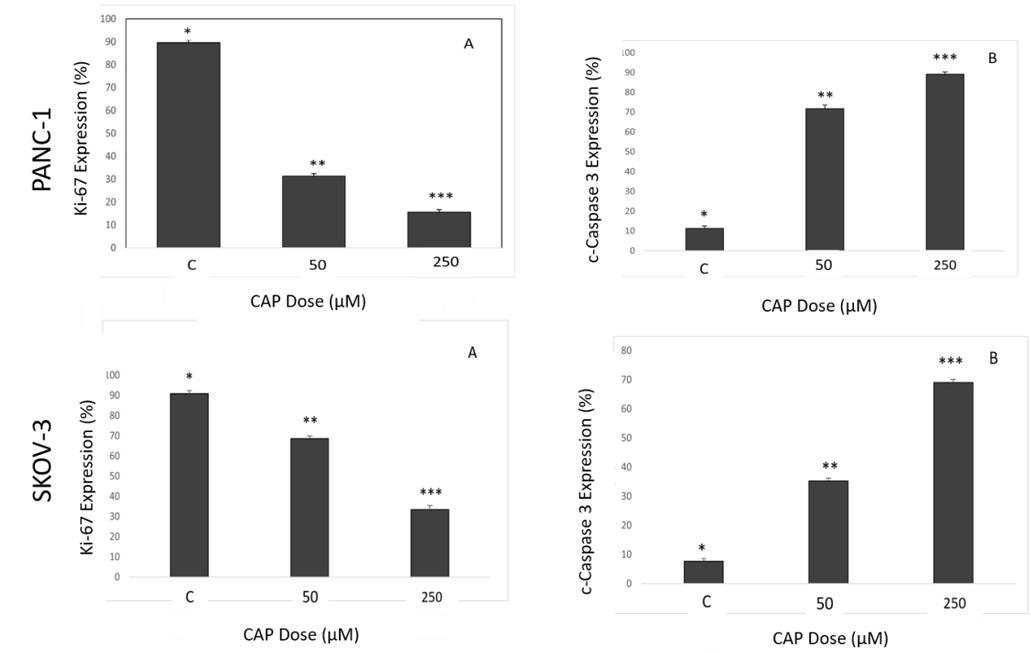

Figure 2. Ki-67 and c-Caspase 3 expression in PANC-1 and SKOW-3 cells.

Capsaicin induces apoptosis in A 549 cells at high concentration.

Apoptosis was indicated by morphological results (Fig. 3D). c-Caspase 3 immunoreactive cells observed when the CAP treatment increase (Fig 3). The increase in dose groups was significant compared to the control groups ($p \leq 0.05$) (Fig. 4.).

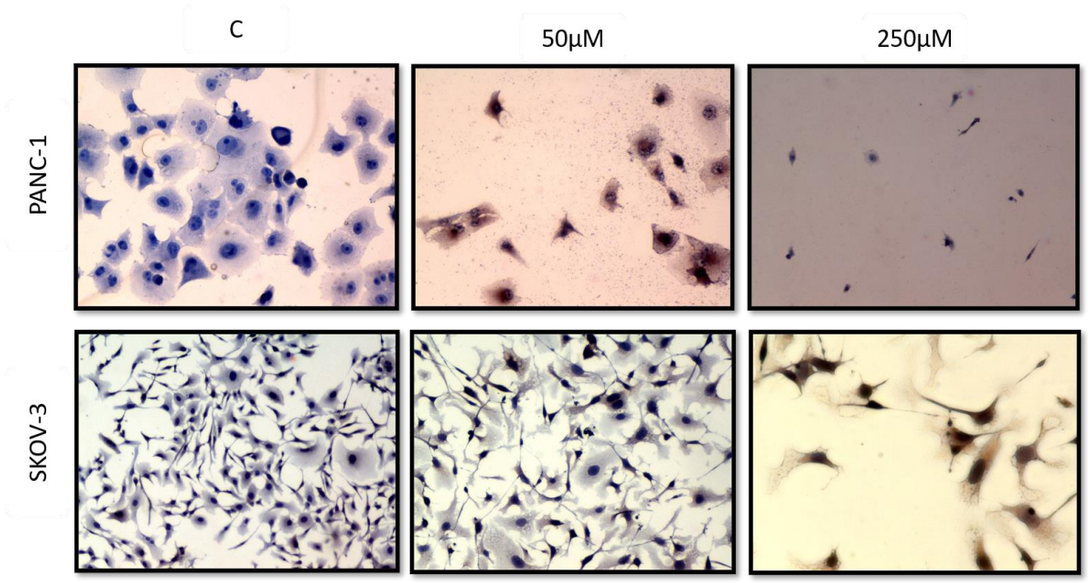

Figure 3. c-Caspase 3 expression in PANC-1 and SKOW-3 cells.

4. Discussion

Cancer is an abnormal cell growth that occurs in almost any tissue or organ and can then invade other tissues of the body and grow uncontrollably (Hausman 2019). Pancreatic cancers are quite common all over the world, generally not suitable for operative intervention and with poor prognosis; ovarian cancer, is an important problem due to the high rate of metastasis (Ryan et al. 2014). While chemotherapeutics used in the treatment cause different toxicities depending on the dose, drug resistance that develops over time may reduce or eliminate the targeted benefit against the drug. In order to overcome this, studies in recent years have focused on the use of different drug combinations, as well as alternative therapies such as the use of biological substances (such as food additives, plant extracts, etc.), small target molecules (Palmer et al. 2017). The anticarcinogenic and anti-tumor effect of capsaicin, the active ingredient of red hot pepper, is known (Yang et al. 2006). This study has determined the effects of different doses of CAP on pancreatic and ovarian cancer firstly.

Studies examining the effect of CAP on different cancer types have shown that high doses cause apoptosis due to sensory denervation (Schneider et al. 2014, Thoennissen et al. 2010). Zhang (2008), treated normal acinar cells and pancreatic cancer cell lines with different doses of CAP, and they found that, low doses of CAP induce proliferation in normal acinar cell, but not cancer cells. But in the same study, with the increasing doses of CAP increase the apoptosis rates in both cell types (Zhang et al. 2008). In the present study, apoptosis was induced with the increasing doses of CAP. Especially, the 3-fold reduction in PANC-1 cells is significant. Cell death caused by increasing CAP dose in SKOV-3 cells is statistically significant, but not as much as in PANC-1 cells. This indicates that ovarian cancer cells are more sensitive to CAP. In another study PANC-1 cells were treated with 200µM CAP, and capsaicin-induced apoptosis may correlate with downregulation of the PI3K/Akt pathway (Zhang et al. 2013). Lin et al, different doses of CAP

(0.1-50 μg/ml) with folic acid-conjugated lipid nanoparticle on SKOV-3 cells and they observed the decrease in cell viability (Lin et al. 2017).

5. Conclusion

In summary, low and high doses of CAP induce apoptosis and reduce the cell proliferation in pancreatic adenocarcinoma and ovarian adenocarcinoma cell lines. The increase in apoptosis was observed in PANC-1 and SKOV-3 cells with increasing capsaicin dose. This study can be lead of future chemoprevention studies and CAP can be combined with chemotherapeutic agents and used in treatment of pancreatic and ovarian adenocarcinomas.

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The Proliferative Effects of PRP on Cattle Ovarian Culture

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Abstract:

Platelet rich plasma (PRP) is currently used in human and veterinary medicine. It is a blood product with features such as injecting damaged area or applying it topically. The main purpose of using PRP is to secrete alpha granules rich in growth factors and cytokines for therapeutic purposes. PRP contains several growth factors that induce cytokines that could stimulate follicular growth and steroidogenesis. In the ovary, apoptosis is the cellular mechanism removing all but few growing follicles during their way to ovulation. The aim of this study is to determine the proliferative and / or apoptotic effects of low and high PRP doses on ovarian tissue. For this purpose, ovaries were collected from slaughterhouse. Ovarian tissue was sliced (5 slices/ovary) and placed into the culture medium for 3 days. Ovaries were divided into 3 groups; control group, low dose group (700 x 10^3 platelets/ml) and high dose group (2100 x 10^3 platelets/ml). After 3 days, the slices were fixed and routine immunhistochemistry method were performed with PCNA (proliferating cell nuclear antigens) to determine proliferating effects and active PARP-1 to determine apoptotic effects. As a result , it was observed that, PRP protects the follicles against apoptosis and atresia and stimulates follicular development. This report is the first known study of PRP in ovarian cultures and has a potential innovation for future studies, which will ty to define follicular proliferation and apoptosis in cow's ovaries.

Keywords: PRP, ovarian culture, proliferation

1. Introduction

The mammalian ovary is responsible for the development, maturation, and release of mature oocytes for fertilization, as well as for the synthesis and secretion of hormones that are essential for follicular development, menstrual/estrous cyclicity, and maintenance of the reproductive system and its function. In cattle, from mid-pregnancy to reproductive senescence many follicles are activated to enter the growth phase, which is characterized by both proliferation of the granulosa cells and an increase in the oocyte size (Goegeon et al. 2003). Granulosa cells secretes autocrine and paracrine factors and play an crucial role in oocyte maturation, follicle growth, oocyte survival, and ovulation. Therefore, a disruption of their functional activities could have a significant impact on reproductive efficiency (Hussein et al. 2005). A lot of in vitro cultures have been designed with the aim of growing individual follicles, or whole ovaries, at varying stages of development. These cultures involves short- and long-term methods, individual pre-antral follicle cultures, granulosa cell–oocyte complexes, co-cultures and whole ovary cultures, depending on the endpoints required for the study (Eppig and Schroeder 1989),(Murray and Spears 2000). Platelet rich plasma (PRP) is a cellular part of plasma derived from whole blood centrifuge and comprises a higher proportion and greater number of growth factors than normal blood (Yılmaz and Kesikburun 2013). Platelet derivatives include a variety of cytokines, chemokines, and growth

factors (e.g., hepatocyte growth factor, stromal-derived growth factor-1). Platelet-derived mediators cause and modulate fibroblast activation as well as leukocyte, neutrophil, and macrophage recruitment, resulting in the removal of dead cells and cellular waste (Gurtner et al. 2008). Platelet-released factors also regulate the proliferation and migration of other cells involved in tissue mend (Crowley et al. 1994). The positive effect of PRP is probably due to the high concentration of growth factors stored within platelet -granules, which include platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-ß), vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor (FGF) and insulin like growth factor (IGF) (Wang and Avila, 2007). In this study, we used low and high doses of PRP to determine the proliferative effects of the ovary applied to organ cultures of similar cells and subjected to certain conditions.

2. Material and Methods

Ovarian Tissue Culture:

Ovaries were collected from adult cows killed at a slaughterhouse located 180 km away from the laboratory. Ovaries were held at 4 °C and brought to the laboratory within three hours. After washing in cold (4 °C) sterile HBSS (Hanks' Balanced Salt Solution), ovaries (n = 10) were brought to laboratory in cold HBSS with 200 U/ml penicillin and 200 µg/ml streptomycin. In laboratory, corpus luteum were selected and cortical slices (3-4 mm3) were cut in cold HBSS. 3 slices were randomly assigned to each 3 group (control group, low dose PRP group, high dose PRP group). Ovaries were cultured with MEM alpha containing 4% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in an atmosphere containing 5% CO2. Under short-term culture conditions (4 days), the treatment was repeated in fresh medium every day.

PRP Preperation:

Blood was taken from the cattle into 2 injectors at a dose of 46 ml. Before taking blood, sodium citrate, an anticoagulant, was added to the injectors 4ml. It was brought to the laboratory for centrifugation. Centrifugation was performed initially in low-speed high time (400 G 20 minutes), then in high-speed low time (1000 G 10 minutes). After the first centrifugation, 3 layers are formed. The upper layer is plasma, the middle layer is buffy coat, and the lower layer is erythrocytes. After the first centrifugation, the buffy coat was removed and transferred to a new falcon tube. Afterwards, the second centrifuge process was applied and the remaining third part was PRP that we obtained. In order to prevent the PRP from coagulating, 10% calcium gluconate was added to bind ionized calcium and inhibit the coagulation cascade. PRP examination was divided into two groups; low dose group (700 x 103 platelets/ml) and high dose group (2100 x 103 platelets/ml).

Morphological Examination:

The ovaries were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. The largest five µm thick sections cross-sections were cut from paraffin blocks, mounted on slides, and

dried overnight. After dewaxing and rehydration, sections were used for Crossman's triple staining to determine ovarium morphology (Crossman 1977).

Immunhistochemical Examination:

Standard streptavidin biotin peroxidase complex technique was carried out using Histostain Plus Kit (Zymed, South San Francisco, CA) and ImPRESS reagent kit (MP 7451). Antigen retrieval was carried out by boiling sections in microwave oven at 750 W in sodium citrate buffer (1 M, pH 6.1) for PCNA and cleaved PARP-1 staining. After cooling, slides were rinsed with PBS and endogenous peroxidase activity was blocked by 10 min incubation at room temperature in 3% H2O2 solution in distilled water. After blocking with ImPRESS reagent kit for 20 min to reduce nonspecific antibody binding, sections were incubated with primary antibodies, a rabbit monoclonal antibody to PCNA diluted to 1:200 and a rabbit polyclonal antibody to c-PARP1 diluted to 1:200 for overnight at 4°C. Sections were then incubated with secondary antibody for 30 min at room temperature. Finally, 3.3'-diaminobenzidine (DAB) was used for colour development and counterstaining was performed with haematoxylin. Slides processed without primary antibodies were included for each staining as negative control. Ovarian slides were visualized under Nikon Eclipse 80i microscope for morphological and immunhistochemical evaluation.

3. Results

When ovaries were examined morphologically, healthy growing follicles (primordial, primary, secondary, tertiary and graff follicles), atretic follicles and cortex medulla distinction were observed (Figure 1). In ovarian section cell proliferation was detected with PCNA and PCNA immunoreaction was observed in growing follicles granulosa cells, theca cells and interstitial cells nuclei (Figure 2, Table 1). Apoptosis was examined with c-PARP1 and the immunoreaction of antibody was seen in regression follicles (Figure 3, Table 1).

Table 1. PCNA and Cleaved PARP1 expressions on follicles.

Follicules/Ab	Primordial	Primer	Seconder	Antral
PCNA	++	++	+++	+++
Cleaved PARP - 1	+	+	+	+

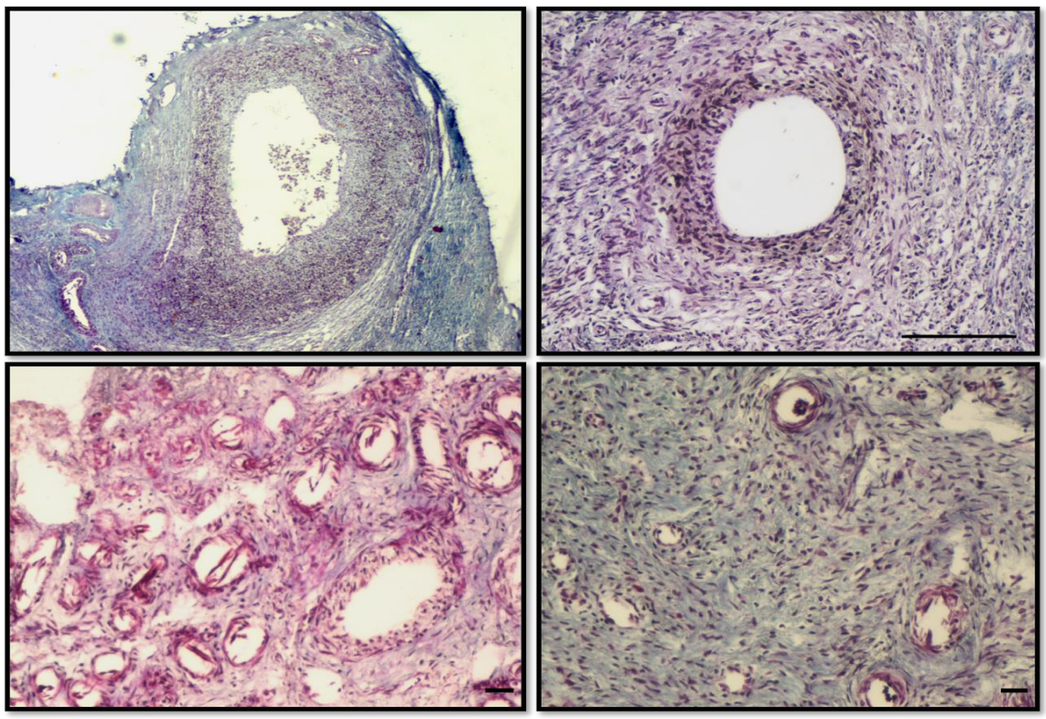

Figure 1. Morphological examination of ovaries.

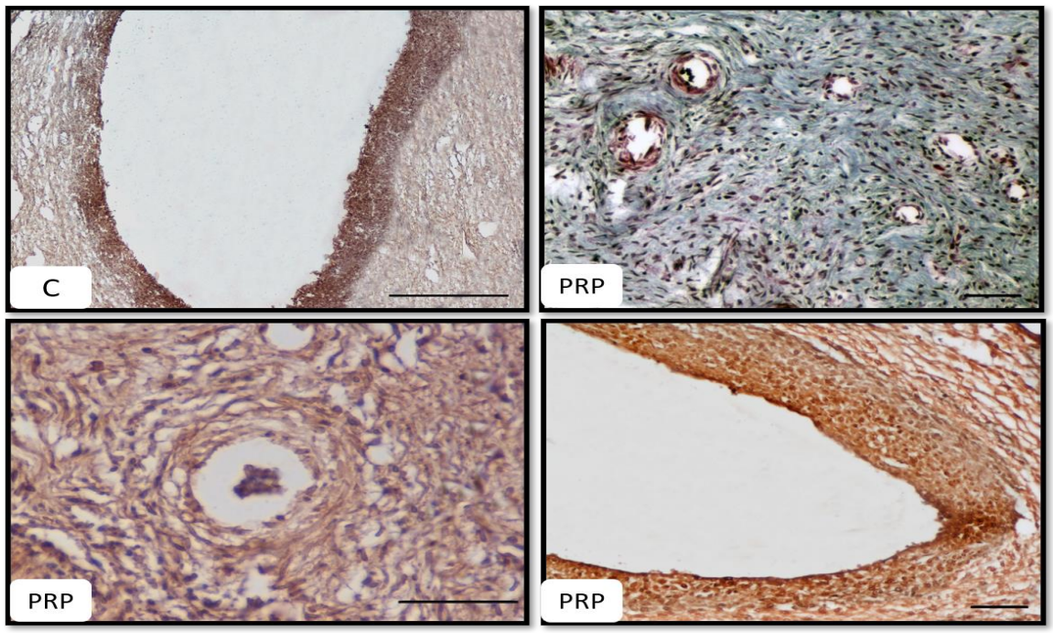

Figure 2. Proliferation results of control and PRP treatment groups.

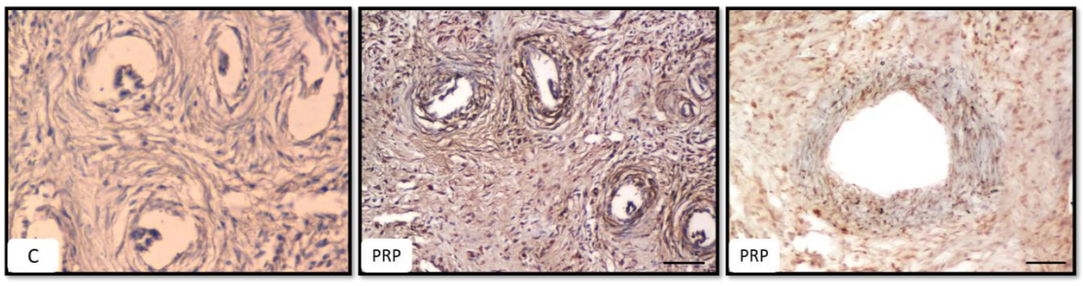

Figure 3. Apoptosis results of control and PRP treatment groups.

4. Discussion

Previous studies have shown that PRP induces cell proliferation (Hosseini et al. 2017). There are many autonomous and environmental factors that prevent the granulosa cells, which are responsible for oocyte maturation and hormone synthesis in the ovary, from being healthy and their proliferative characteristics (Stefansdottir et al. 2014). In this study, the proliferative effects of PRP on short term bovine ovary culture were tested for the first time.

For bovine and other farm domestic species, the development of culture systems capable of supporting the growth of immature follicles to a stage where they could be matured and the oocyte fertilized would ensure a large supply of oocytes for manipulation (Stefansdottir et al. 2014). Beck et al. (2018) aimed to assess the avian chorioallantoic membrane (CAM) for short-term culture of adult bovine ovarian tissues compared with a traditional in vitro culture system and It has been stated that in both culture methods, angiogenesis is satistified and can be used as a model in many infertility studies with the development of healthy and growing follicles (Beck et al. 2018). In the current study, we performed a traditional method, 4-day short-term bovine ovary culture, and as a result of the morphological examinations, regular ovarian cortex medulla structure, healthy primordial follicles, as well as growing and atresia follicles at different stages were observed. With the presence of high rates of growth factors in PRP content, the ovary serves both in protecting the follicle structure and inducing the proliferation of granulosa cells. Hosseini et al. (2017) Added PRP to the three-dimensional ovary culture instead of FBS, and showed that conclusion, our results showed that PRP supports in vitro viability and growth of encapsulated/isolated human primordial and primary follicles (Hosseini et al. 2017). In similar, in previous study, while we applied only 4% FBS to the culture in the control group, the addition of PRP to the medium in the experimental groups caused an increase in the proliferation of the ovarian follicles granulosa and theca cells. On the other hand, Otala et al. (2002) found an increase in caspase 3 expression after 48 short term ovarium culture application (Otala et al. 2002). However, in our study, we did not observe a similar increase in cleaved PARP-1 expression, which is an apoptosis marker and It suggests that PRP may prevent apoptosis.

5. Conclusion

In conclusion, previous study determined that PRP has proliferative effect on ovarian granulosa, theca and interstitial cells and protects the follicles against apoptosis and atresia. It can be emphasized that PRP applications can be beneficial and should be supported with further studies in order to improve conditions that develop due to individual or environmental reasons and affect ovarian follicular development.

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The Proliferative and Apoptotic Effects of Capsaicin on Cattle Ovarian Culture

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Abstract:

Ovaries are responsible for fertility and the basic functional unit of the ovary is the follicle. The structure of the follicle consists of an oocyte and surrounding granulosa and theca cells. Apoptosis and proliferation are essential for ovarian functions and development, and a mechanism that regulates biological timing in females. Capsaicin (CAP) (N-vanillyl-8-methyl-alpha nonenamide) is the active ingredient in red hot pepper. CAP has been shown to stimulate the release several neuropeptides, so it has an important role in ovarian steroidogenesis, follicular development, and ovulation. The aim of this study is to determine the proliferative and / or apoptotic effects of low and high CAP doses on ovary. For this purpose, ovaries were collected from cattles. Ovarian tissue were sliced (5 slices/ovary) and placed into culture medium for 3 days. Ovaries were divided into 3 groups; control group, 100 μM CAP (low dose group) and 500 μM CAP (high dose group). After 3 days, ovarian slices were fixed and routine immunhistochemistry method were performed with PCNA (proliferating cell nuclear antigens) to determine proliferating effects and active PARP-1 to determine apoptotic effects. As a result of the study, it was observed that, low-dose CAP protects the follicles from apoptosis and atresia and stimulates follicular development. On the contrary, high doses of CAP causes apoptosis in the follicles. Ovarian culture method is very important specially on cattles. It is thought that our study will make a significant contribution to the literature, since it is the first study in this field to examine the direct effects of CAP on the ovary in cattles.

Keywords: Capsaicin, ovarian culture, apoptosis, proliferation

1. Introduction

The ovary is central to female reproductive function, and responsible for the development, maturation, and release of mature oocytes for fertilization, as well as for the synthesis and secretion of hormones that are essential for follicular development, menstrual/estrous cyclicity, and maintenance of the reproductive tract and its function (Kaipia, 1997). The interaction between oocytes, granulosa and theca cells has important role in ovarian functions (Baillet and Mandon-Pepin 2012). The fate of the growing oocyte, granulosa and theca cell layers were determined with the apoptosis. Ovulation was observed selected developing follicles. Most of the follicles undergo in atresia, occurs with apoptosis, which is programmed cell death of cells (Kaipia 1997). Ovarian functions and folliculogenesis develop in response to the central nervous system with induction of gonadotropin releasing hormone (GnRH) and thus follicle stimulating hormone (FSH) and it stimulates the production and release of the stimulating hormone (LH) from the pituitary (Guler and Zik, 2018). Morphological and biochemical changes in the ovary start with the effect of various hormones, autocrine and paracrine factors during the fetal period occur simultaneously with folliculogenesis and this follicle reserve gradually decreases with natural progression and

causes infertility Zik et al. 2019). There are numerous studies on ovarian physiology, especially folliculogenesis, hormone production and ovulation. However, organ culture studies about the development process of the ovary is inadequate. Among all the species studied, cows have become a good source of models in *in vitro* studies due to the similarity of the physiology of their follicles to humans and the loss of productivity of dairy breeds (Swensson 2001). These cultures have become a widely used tool to study the development of follicles in reproductive biology and toxicology and have been successfully established in mouse, rat, cattle, sheep, pig, primates and humans (McLaughlin and Telfer 2010).

Capsaicin (CAP) (8-methyl-N-vanillyl-6-nonenamide) is the active ingredient of all hot peppers, including red hot peppers, jalapeno and habarenos, and has an alkaloid structure (Govindarajan 1986). TRPV1 (transient receptor potential vanilloid 1), a sub-family member known as vanilloid receptor 1 was initially identified as the receptor for capsaicin (Holzer 1991). Previously, there have been studies about capsaicin effects in many different clinical conditions such as pain relief, diabetes, gastrointestinal and cancer (Sharma et al. 2013; Pramanik et al. 2011; Josse et al. 2010), There are some studies examining the effects of CAP on the genital system (Ozer et al. 2005). Ozer et al. examined the genital systems of chickens by adding non-toxic doses of red-hot pepper to their daily chick diets until the age of five months, and at the end of the study, they stated that the genital system organs developed faster, and it was shown that CAP was a biological activator substance. In previous studies in our department, it has been observed that low doses of CAP have a proliferative effect on the ovarian granulosa cells and contribute the follicle development (Zik et al. 2012; Zik et al. 2010).

In this study, it was aimed that the proliferative and / or apoptotic effects of low and high doses of CAP on bovine cultured ovaries.

2. Materials and Methods

The ovaries collected from the cows in the slaughterhouse were brought to the laboratory in medium containing Hanks' Balanced Salt Solution (HBSS) 2% 100 U / ml penicillin, 0.1 mg / ml streptomycin (Pan-Biotech; P06-07300), maintaining the cold chain at 4 ° C. Ovarian tissue was sliced (5 slices / ovary), and three groups (control group, low dose 100 µM CAP and high dose 500 µM CAP) were randomly generated. The sliced ovaries were kept for three days at 37°C in an atmosphere culture medium containing 5% CO 2. ALFA-MEM, the basal medium for organ culture; 4% FBS; Penicillin 1% 100 U / ml, streptomycin 0.1 mg / ml; 1% Amphotericin was included. The control group was treated with only basal medium. 100 µM CAP was added to the treatment group, which included low dose Capsaicin (M2028, analytical standard grade, - 99%, obtained from Sigma.) and 500 µM CAP was added to the high dose CAP group. Capsaicin was dissolved in ethanol.

The following two days (day 1 and 2), treatment procedures were repeated. On the third day, ovarian slices were fixed and routine immunohistochemistry was performed with primary antibody PCNA (proliferative cell nuclear antigens) (Santa Cruz Biotech, 7907) to determine cell

proliferation effects and cleaved-PARP1 (Santa Cruz Biotech, h215) to determine apoptotic effects.

Morphological Examination:

Tissue samples were fixed in 10% buffered formaldehyde-saline solution and embedded in paraffin blocks. Tissue sections taken from the paraffin blocks with a thickness of 5 μm were dried in the oven overnight. After paraffin removal and rehydration, sections were stained for morphological examination by Crossman's Triple method (Crossman 1977).

Immunohistochemical Examination (Proliferation/Apoptosis):

Standard streptavidin biotin peroxidase complex technique was carried out using ImPRESS reagent kit (MP 7451). Antigen retrieval was carried out by boiling sections in microwave oven at 750 W in sodium citrate buffer (1 M, pH 6.1). After cooling, slides were rinsed with PBS and endogenous peroxidase activity was blocked by 10 min incubation at room temperature in 3% H2O2 solution in distilled water. After blocking with ImPRESS reagent kit for 20 min to reduce nonspecific antibody binding, sections were incubated with primary antibodies, a rabbit monoclonal antibody to PCNA diluted to 1:200 and a rabbit polyclonal antibody to c-PARP1 diluted to 1:200 for overnight at 4°C. Sections were then incubated with secondary antibody for 30 min at room temperature. Finally, 3.3'-diaminobenzidine (DAB) was used for color development and counterstaining was performed with hematoxylin. Slides processed without primary antibodies were included for each staining as negative control. Ovarian slides were visualized under Nikon Eclipse 80i microscope for morphological and immunohistochemical evaluation.

3. Results

Ovaries were examined morphologically, primordial, primary, secondary, tertiary and graff follicles, atretic follicles and cortex medulla distinction were observed in control group and low dose CAP group (Figure 1). However, granulosa cells were deformed, and oocyte-granulosa ell interaction was disrupted in high dose CAP group. In ovarian section, cell proliferation was detected with PCNA. In primordial follicles weak PCNA immunoreaction was observed and the reaction is increased as the follicles grow. Specially in antral granulosa cells, strong expression was seen in low dose CAP group (Figure 2, Table 1). PCNA reaction was decreased in high dose of CAP (Figure 3, Table 2). Apoptosis was examined with c-PARP1 and the immunoreaction of antibody was seen in regression follicles especial in high dose CAP groups (Figure 3, Table 2). c-PARP1 expression was strong in seconder and antral follicles granulosa and theca cells in high dose CAP group.

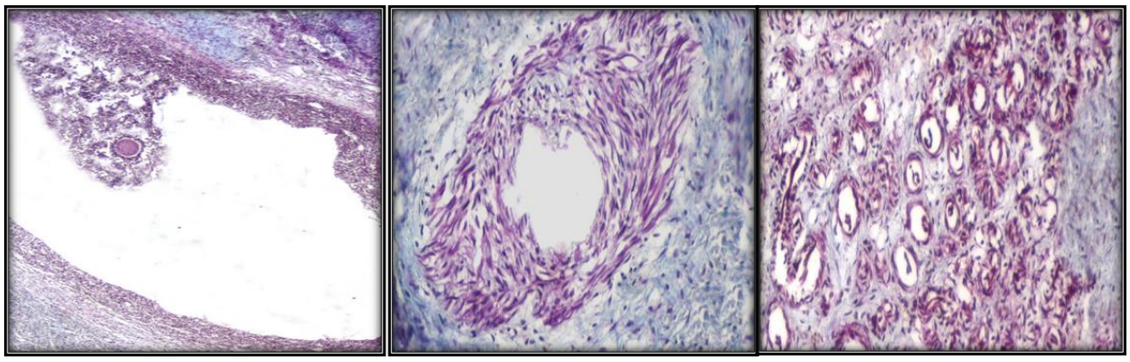

Figure 1. Morphological examination of ovaries.

Table 1. The proliferation and apoptosis effects of low dose CAP treatment on ovarian follicles.

Follicules/Ab	Primordial	Primer	Secondary	Antral
PCNA	+	++	+++	+++
Cleaved PARP-1	-	-	+	+

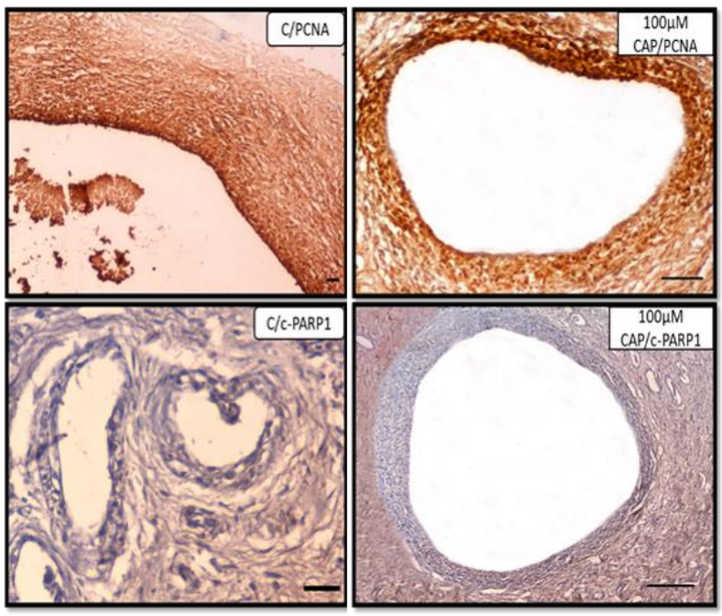

Figure 2. Proliferation and apoptosis results of control and low dose CAP treatment groups.

Table 2. The proliferation and apoptosis effects of high dose CAP treatment on ovarian follicles.

Follicules/Ab	Primordial	Primer	Seconder	Antral
PCNA	+	+	+	+
Cleaved PARP-1	++	++	+++	+++

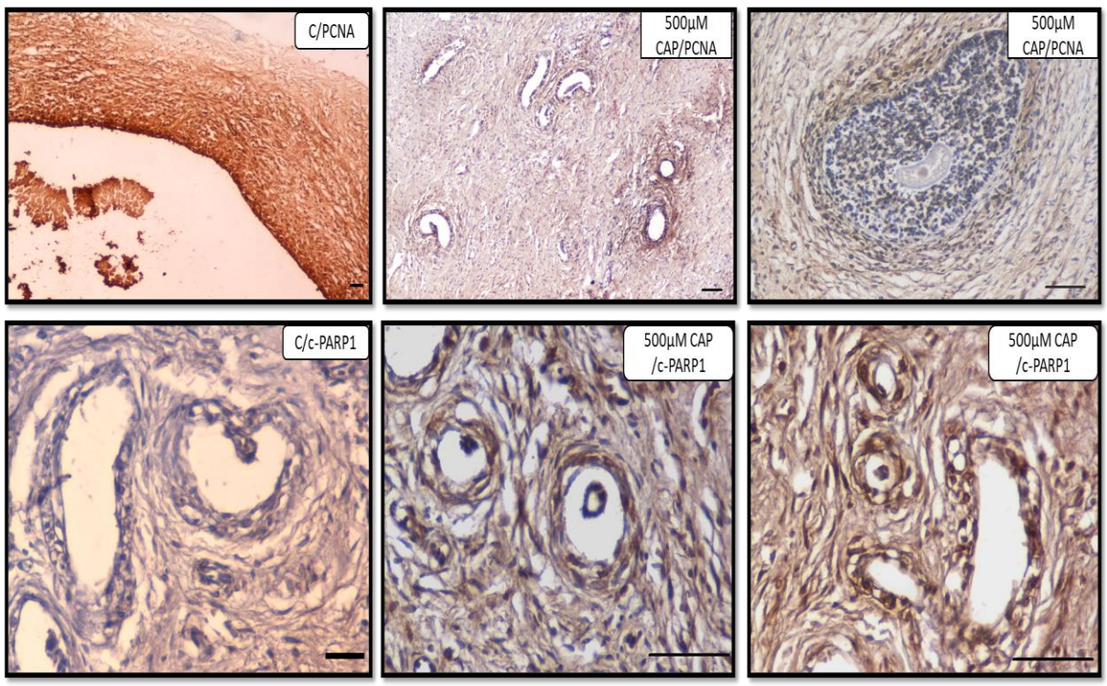

Figure 3. Proliferation and apoptosis results of control and high dose CAP treatment groups.

4. Discussion

Granulosa cells play an important role in the development, maturation and follicle formation of the ovary starting from the fetal life (Ciesiolka et al. 2016). By stimulating the central nervous system by gonadotropins, the ovarian nerve innervation was activated, through different pathways of the afferent and sympathetic branches of the peripheral nervous system. CAP plays a role in the regulation of proliferation and apoptosis mechanism in ovarian cells by stimulating the release of neuropeptides from afferent neurons. In the literature, there are in vivo studies investigating the neurotoxic effect of high doses of CAP on the female genital system (Quiroz et al. 2014; Alatriste et al. 2013; Morán et al. 2003; Pintado et al. 2003; Traurig et al. 1984). This is the first study that investigate the CAP effects on in vivo bovine ovarian organ culture.

In vitro studies using high-dose CAP show that it induces apoptosis in cells (Guler and Zık 2018). It was shown that apoptosis was triggered by activation of the mitochondrial pathway with increasing CAP dose in pancreatic acinar cells and pancreatic tumor cell lines (Pramanik et al. 2011). In an in vitro study, Mizrak et al. applied different doses of CAP to rat spermatogonial stem cells and observed that the use of high-dose CAP induced in vitro apoptosis and negatively affected spermatogonial survival (Mizrak et al. 2008).

In similar, the present study showed that, high dose of CAP stimulates the apoptosis in granulosa, theca and intercitital cells in ovaries. Zik et al. showed that the use of low CAP doses inhibits apoptosis and stimulates the development and proliferation of granulosa cells (Zik et al.2010a; Zik et al. 2010b). Guler and Zik treated granulosa cells with low dose CAP (10, 50µM), and observed that cell proliferation was induced. In the present study, we observed that, PCNA expression was diffuse and strong though low doses of CAP induce the proliferation in granulosa, theca and interstitial cells (Guler and Zik 2018).

Previous studies are consistent with our results that high doses of CAP cause apoptosis in granulosa cells at 24 and 48 hours.

5. Conclusion

We observed that low-dose CAP administration had proliferative effects on ovarian granulosa, theca and interstitial cells, while high-dose CAP administration had apoptotic effects on ovarian follicles.

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Protective Treatment and Poisoning in Pigeons in Konya Province Folklore

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Abstract:

The pigeon motif, which has a versatile and comprehensive place in Turkish folk culture, appears as a common and living motif that has a great place in Konya's ethnography.
In this study, it was aimed to compile folkloric information about poisoning and their treatment, and protective treatment of external and epidemic diseases of pigeons raised in Konya province, and contribution of the folklore of veterinary medicine in particular, and to the Turkish folklore in general. The material of the study was composed of written and audio folkloric information obtained from 23 resource persons, who were determined to have information about protective treatment and poisoning as a result of interviews with 28 resources dealing with pigeon breeding in Konya province, between 11.07.2020-14.08.2020 through the "*Information Collection Form*". The audio material obtained with the sound recorder was transcribed in the framework of the information collection form and evaluated with content analysis. The folkloric data on the subject were evaluated under the headlines of "*protective treatment in external diseases, protective treatment in epidemic diseases, poisoning and treatment*". It was determined that in Konya province, pigeons were fed with garlic and vinegar was added to their drinking water for protective treatment in external diseases. In order to protect the pigeons from epidemic diseases, in compulsory cases, the feet of the foreign pigeons are disinfected and a quarantine is applied for a month, vaccines are administered twice a year, especially to protect against plague and smallpox. In poisoning, ayran, kephir, yogurt, egg or honey syrup is drunk with the help of a syringe. As a result, it can be claimed that disinfection, vaccination, quarantine practices are performed in protective treatment in pigeons, and natural foods are used by preparing them with very rationalist and rational methods for the health of the digestive system and protective treatment, the methods used in poisoning from past to present are carried out with similar approaches in Konya Region and when all the data are taken into consideration, the rich folklore knowledge of Konya province contributes to the Turkish cultural heritage.

Keywords: folklore, konya, pigeon, poisoning, treatment.

1. Introduction

Protective medicine is a set of methods and strategies created with the aim of detecting and controlling the disease in advance. Although it is not known exactly when the practices related to protective medicine started in what period of history, it is stated that it coincided with a period after therapeutic medicine. At the same time, it is mentioned that there is a very long period in which protective medicine and therapeutic medicine are intertwined. It is reported that one of the first nations to use the concept of protective medicine in history is the Chinese, and the oldest known examples of the practices on this subject are found in the Mesopotamian nations (Doğan 2008).

Strengthening the immune system in protective treatment, ensuring disinfection of enterprises with diseases, isolation and vaccination practices, prevention of secondary infections (Avcı 2017) and,

paying attention to coop cleaning and disinfection especially to protect against infectious diseases (Garip 2017) are reported as issues that need to be emphasized.

With this study, it was aimed to compile folkloric information about poisoning and their treatment, and protective treatment of external and epidemic diseases of pigeons raised in Konya province, and contribution of the folklore of veterinary medicine in particular, and to the Turkish folklore in general.

2. Materials and Methods

The sample of the study and information about the data collection tool were presented in the paper entitled “Pigeon in Folklore of Konya Province” (Çelik et al. 2020). In this study, among the parameters of the “Information Compilation Form” developed as a data collection tool, those related to “protective treatment and poisoning” were used.

The material of the study was composed of written and audio folkloric information obtained from 23 resources, who were determined to have information about protective treatment and poisoning as a result of interviews with 28 resources dealing with pigeon breeding in Konya province, between 11.07.2020-14.08.2020 through the information collection form. The resources were coded according to the district and interview date (K1,…, K28) and indicated as superscript in the text. Since K2-3, K6-7 and K18 did not provide information on the subject, these were not used in the resources list.

The audio material obtained with the sound recorder was transcribed in the framework of the information collection form and evaluated with content analysis. The folkloric data on the subject were evaluated under the headlines of “protective treatment in external diseases, protective treatment in epidemic diseases, poisoning and treatment”.

The study was conducted with the approval of Selcuk University Faculty of Veterinary Medicine Experimental Animal Production and Research Center Ethics Committee (SÜVDAMEK), dated 11.06.2020 and numbered 2020/56.

3. Results

The folkloric results related to the protective treatment in external and epidemic diseases and poisoning for pigeons raised in Konya are presented below.

Protective Treatment in External Diseases:

In general, pigeons’ coops are frequently disinfected to protect them from external diseases.K4-5,15-17,19 Slaked limeK4-5 and ozone are used for disinfection. Vitamins are added to drinking water.K5 Foreign pigeons are not allowed in the coop.K15-17,19 It is essential that the coop floor is dry to prevent diarrhea.K27-28

In order to protect against infectious diseases, safflower, which is said to have an antibiotic effect, is fedK25 or garlic pickle, which is kept from daylight for 15 days, is drunk.K9-10 In addition, a mixture of two liters of water, three liters of apple cider vinegar, half kg of garlic, half a kg of lemon with shell, 100 grams of salt, a bunch of thyme and parsley, yeast powder is added to the water once a week for protective purposesK11; a mixture of five liters of water, a handful of garlic, five or six lemons which are kept in the heat for about 12 days, is drunk for antibiotic purposes.K27

Pigeons are fed with fresh yeast and yogurt mixture as probioticK11 and clay soil for crop digestion. For immunity protector and digestive health, pickled garlic-lemon is drunk (a mixture of 200 ml of vinegar, a lemon, four heads of garlic and five liters of water) that is rested in a cool place for 20 days. For immunity, a mixture of 10 liters of water and 250 ml of apple cider vinegarK23-26; five liters of water and 50 ml of vinegarK27-28 or 20 liters of tomato paste juice, two cloves of garlic and one or two tablespoons of salt are drunk after resting for about five or six months.K26 Bluestone is added to drinking water to clean the digestive system.K27-28

Protective Treatment in Epidemic Diseases:

In order to protect the pigeons from epidemic diseases, no foreign pigeons are taken into the coopK9-11,14, in compulsory cases, the feet of the foreign pigeons are disinfectedK20-21 and a quarantine is applied for a month.K9-11,13-14 Vaccines are administered twice a year, especially to protect against plagueK12-14,20-21,27-28 and smallpox.K15-17,19,23-26

To protect from bloody diarrhea, garlic is fed and vinegar is added to drinking water.K20-21 Apple cider vinegar is used to reduce fever.K1

Poisoning and Treatment:

A poisonous feed or food is called “ağılı”, and a deliberate poisoning is called “ağılama” among the people.K1,5,8,10,12-13,15-17,19-20,23-28 The animal that died by being poisoned is given names such as “öldü (dead)”K8,14,27-28, “leş (carrion)”K20-21 or “mundar (filthy)”.K21

Pigeons are often poisoned by eating medicated or moldy feed.K23-26 In determining whether the feed to be given to pigeons is medicated; The feed is thrown into a glass full of water and it is seen that the color of the water becomes cloudy at first step. If the water does not get its old color after waiting for a while, it is understood that the feed is medicated.K27

Poisoned animals experience loss of balanceK4-5 and sudden death.K12-13 In poisoning, ayranK1,11,15-17,19-23,25-28; kephirK4,8,20-21,25; yogurtK21; eggK15-17,19 or honey syrup (five liters of water, two tablespoons of honey)K26-28 is drunk with the help of a syringe. After the pigeons are vomiting by drinking plenty of water, ayran is drunk.K24

The meat of poisoned animals is not consumed. In general, animals whose meat is consumed are called “mısmıl” among the people.K23-26 Pigeons that die by poisoning are buried randomlyK1,20-21,23-26; fed to the animalsK4,14-15,27-28; thrown into the garbage dump so

that the animals do not get used to the areaK4,8,11-13,16-17,19-21,23-26 or buried in a suitable place if an emotional bond is established with the pigeon.K9-11,27-28

4. Discussion

In the disease of pigeons with diphtheria, half of lemon juice and olive oil were mixed and the mouth was washed (Özmen 1981); Prevention of secondary infections, strengthening the immune system, disinfection of enterprises with disease, isolation and vaccination are recommended practices in protective treatment (Avcı 2017); In Newcastle disease, which is very acute and causes epidemics in pigeons, most of the cases occur as a result of contact with infected chickens (Dakman et al. 2008); Coop cleaning and disinfection will protect from infectious diseases (Garip 2017); Garlic and apple cider vinegar, which are considered as natural antibiotics, are good for many diseases and play an important role in protective treatment (Ekinci 2017); In the study, it can be said that the data that foreign pigeons were not taken into the coop in order to protect the pigeons from epidemic diseases, in compulsory cases, the feet of the foreign pigeons were disinfected and quarantine was applied for a month, vaccines were applied twice a year to protect from plague and smallpox are parallel to the data of Avcı, Özmen, Dakman et al. and Garip. While it can be said that the data that garlic is fed and vinegar is added to drinking water for protective purposes are parallel to the statements of Ekinci, it can be stated that rational and alternative medicine methods are used to prevent secondary infections and contamination in order to protect pigeons from epidemic diseases in Konya province folklore.

The eriton was added to pigeons' water every fifteen days to purify them from microbes or vitamins were given to protect their health (Özmen 1981); Garlic, kephir, thyme, apple cider vinegar and honey can be used as natural antibiotics (Ekinci 2017); Many breeders fed the garlic and onion mixtures which they prepared themselves to accelerate the metabolism of pigeons, and the vegetables which they believed to have an intestinal cleansing effect such as carrots, celery and beets during the winter months (Alataş 2017); In a study carried out in the Central Anatolia Region, Konya Section, breeders used bluestone in foot disinfection (Yaşar et al. 2013); In the study, the data that in order to protect against infectious diseases which is stated to have an antibiotic effect safflower was fed, or garlic pickle was drunk; vitamins were added to drinking water; "a mixture of water, apple cider vinegar, garlic, lemon, salt, thyme, parsley, sourdough" was added to their water once a week; "a mixture of water, garlic, lemon" is drunk for antibiotic purposes; a mixture of fresh yeast and yogurt is used as probiotics; clay soil is fed for crop digestion; a mixture of water and vinegar is used for immunity; pickle juice consisting of "a mixture of vinegar, lemon, garlic and water" is drunk for immunity protection and digestive health, and bluestone is added to drinking water to clean the digestive system are generally similar to the study data given above. In the Konya province folklore, it can be evaluated that observational and rational methods are used for protective treatment, breeders frequently or

excessively use foods with natural ingredients for this purpose, and their practices are very close to today's protective rational treatment procedures.

In the studies conducted in the Central Anatolia Region, Konya Section and Bozlak culture, poisoning was called "ağulanma" and liquids such as garlic yogurt, egg yolk, ayran, sugar water, black molasses were drunk (Yaşar et al. 2013, Sinmez 2011), and "mısmıl" name was given to animals whose meat was eaten, the animals that were not slaughtered were called as "üleş, mundar (filthy)", and the animals that died filthy were thrown outside the village (Yaşar et al. 2013); In the study carried out in the Lower Euphrates River Valley, reported that a few chicken eggs were broken and fed with their shell (Yüksel 2012); In İbn-i Sina's translation work titled El Kanun Fi't-Tıbb, milk was drunk in poisoning (Özçelikay 2008). In the study, it was determined that the data of animals whose meat was consumed were called "mısmıl", poisonous feed or food "ağılı", deliberate poisoning "ağılama", the dead animal "leş (carrion)" or "mundar (filthy)"; drinking ayran, kephir, yogurt, egg or honey syrup with the help of syringe; drinking ayran after the pigeons were vomited by drinking plenty of water; dumping the dead pigeons or being buried somewhere were similar to the above study data. In addition, it can be stated that words such as "ağulanma, leş (carrion), mundar (filthy), mısmıl" are also used in common meanings in the Konya region, and the methods used in poisoning are applied with a similar approach similar to the above study data.

5. Conclusion

As a result, it can be said that disinfection, vaccination, quarantine practices are applied in protective treatment in pigeons, but isolation is never mentioned, and natural substances such as "clay soil, bluestone" are used for the health of the digestive system, and natural foods such as "garlic, apple cider vinegar, safflower, lemon, salt, thyme, parsley, yeast, yogurt" are used by preparing them with very rationalist and rational methods for protective purposes. It can be claimed that the methods used in poisoning from past to present are carried out with similar approaches in Konya Region and when all the data are taken into consideration, the rich folklore knowledge of Konya province contributes to the Turkish cultural heritage.

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Determination of Satisfaction Status of Inpatients with Hospital Nutrition Services and Examination with Various Variables

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Abstract:

In this study, it was aimed to determine the satisfaction level of inpatients from nutritional services and to examine the effects of different variables on satisfaction. The study data were obtained from 413 voluntary patients who were hospitalized for at least 3 days between March-April 2017 in hospitals in Konya. In order to conduct the study, written permission was obtained from Selcuk University Faculty of Medicine Non-Clinical Research Ethics Committee (Decision Number 2016/251) and the hospitals where the study will be conducted. Patient identification form containing patient information and 5-point Likert type expressions were used to evaluate the satisfaction of inpatients from nutritional services. The five point Likert type scale included responses coded between 1 and 5 from “never” to “always”. All statistical analysis were performed using the IBM SPSS Statistics 20 Software and statistically significant differences were determined using a p value <0.05. The average age of 413 inpatients (57.9% female and 42.1% male) participating in the study was found to be 42.3. According to the answers given to the satisfaction statements, the rate of those who were satisfied with the statement "The staff serving my meals are in uniform and clean" was found to be the highest with 98.5%, while the rate of those who were satisfied with the statement "Hot meals are served hot enough" was found to be the lowest with 30.7%. Between the genders of inpatients; their marital status, age groups, educational levels, their staying time in hospital and their diets total satisfaction scores, a statistically significant different was not found (p>0.05). But, clinic where they hospitalized satisfaction scores, a statistically significant difference was found (p<0.05). Since the nutritional needs of hospitalized patients must be met regularly, nutritional services are one of the most important components of patient satisfaction. For this reason, the satisfaction of the patients should be measured continuously and necessary improvement activities should be done.

Keywords: Nutrition service, patient satisfaction, quality in health

1. Introduction

One of the most important factors in hospital recovery is adequate food intake. Malnutrition can lead to muscle wasting and immune deficiency, leading to an increase in complication, infection and mortality rates (Johansen et al. 2004). Feeding food consumption from nutrition services is one of the most important factors (Hartwell et al 2006). It is necessary to measure and know the degree of satisfaction with the services provided to these patients.

In this study, satisfaction level of inpatients with nutritional services was evaluated and compared with various variables.

2. Materials and Methods

Research data were obtained from 413 patients hospitalized in two hospitals in Konya between February 2017 and April 2017.

In order to conduct the study, written permission was obtained from Selcuk University Faculty of Medicine Non-Clinical Research Ethics Committee and from the hospitals where the study will be conducted.

In order to make the satisfaction assessment more efficient, the study included patients who were volunteers aged 18 and over, hospitalized for at least three days, and who had no communication impairment.

Patients with cognitive and emotional disorders, inpatient treatment in psychiatry, oncology, pediatrics and intensive care units, only enteral and only parenteral nutrition and liquid diet were not included in the study.

The research data were collected by the researcher with a questionnaire method using the face to face interview technique. In this questionnaire, 25 questions were used to evaluate the patient descriptive socio-demographic information and satisfaction with nutrition services. The five point Likert type scale included responses coded between 1 and 5 from "never" to "always".

Qualitative variables are expressed as number (n) and percentage (%), while quantitative variables are expressed as mean ($\bar{X}$), and standard deviation (Sd). Whether the quantitative data were normally distributed or not was examined with the "Kolmogorov-Smirnov Test". Mann-Whitney U test was used for nonparametric test conditions in comparing the means of the two groups. The Kruskal Wallis H test, which are nonparametric test assumptions, was used to compare three or more independent groups. When the difference between the groups was determined, the groups that made the difference were determined using the POST-HOC test.Statistical significance level was evaluated by taking $p < 0.05$ in the evaluation of the data.

3. Results

The distribution of information on the socio-demographic characteristics of the patients included in the study by gender is given in Table 1.

It was determined that 57.9% of the patients were female and 42.1% were male. When the distribution of the age groups of the patients was examined, it was determined that 8% were 30 years and younger, and 36.3% were 61 years and over. It was determined that 73.1% of the patients included in the study were married, 38.7% were primary school graduates and 37.8% were housewives (Table 1).

Table 1. Socio-demographic data according to the gender of the inpatients (n=413).

Socio-demographic variables		Female (n=239)		Male (n=174)		Total (n=413)	
		n	%	n	%	n	%
Age groups (years)	30 and below	28	11,7	5	2,9	33	8,0
	31-45	39	16,3	40	23,0	79	19,1
	46-60	84	35,2	67	38,5	151	36,6
	61 and above	88	36,8	62	35,6	150	36,3

Martial status	Married	160	66,9	142	81,6	302	73,1
	Single	79	33,1	32	18,4	111	26,9
Education level	Illiterate	46	19,2	4	2,3	50	12,1
	Literate	40	16,7	15	8,6	55	13,3
	Primary school	98	41,0	63	36,2	160	38,7
	Middle school	15	6,3	39	22,4	54	13,1
	High school	20	8,4	40	23,0	61	14,8
	University	20	8,4	13	7,5	33	8,0

The characteristics of the inpatients regarding hospital stay information according to gender are given in Table 2.

When Table 2 was examined, it was found that half of the patients (49.6%) had a hospital stay between 3-7 days and 26.2% were hospitalized in the orthopedic service. Almost half of all patients (45.5%) take a normal diet.

Table 2. Hospital stay information data according to the gender of the inpatients (n: 413).

Hospital stay information data		**Female (n=239)**		**Male (n=174)**		**Total (n=413)**	
		n	**%**	**n**	**%**	**n**	**%**
Stay hospital (day)	3-7	134	56,1	71	40,8	205	49,6
	8-14	60	25,1	66	38,0	126	30,5
	15-21	18	7,5	27	15,5	45	10,9
	22 and above	27	11,3	10	5,7	37	9,0
Clinic	Chest diseases	22	9,2	17	9,9	39	9,4
	Orthopedics	57	23,8	51	29,4	108	26,2
	Neurology	31	13,0	30	17,2	61	14,8
	Cardiology	39	16,3	30	17,2	69	16,7
	Internal medicine	38	15,9	30	17,2	68	16,5
	Physiotheraphy	37	15,5	10	5,7	47	11,4
	General surgery	15	6,3	6	3,4	21	5,1
Diet therapy	Normal	106	44,4	80	46,0	186	45,0
	Diabetic or wasting	51	21,3	27	15,5	78	18,9
	Salt-free	70	29,3	47	27,0	117	28,3
	Low fat or low cholesterol	8	3,3	16	9,2	24	5,8
	Rich in protein	4	1,7	4	2,3	8	2,0

In this study, the mean scores and satisfaction rates of the responses to 25 statements were evaluated one by one.

According to the responses to the satisfaction statements, "The staff serving my meals are in uniform and clean." While the rate of those who were satisfied with their statement was found to be the highest with 98.5%, the rate of this statement was found to be 98.3% for women and 98.8% for men. "Hot meals are served hot enough." In his statement, the rate of those who were satisfied was found to be the lowest with 30.7%, while the rate of this statement was found to be 27.6% for women and 35.0% for men.

In this study, no significant difference was found between the satisfaction scores of the variables of gender, marital status, age, profession and education level ($p > 0.05$) (Table 3).

Table 3. Satisfaction score average according to the socio-demographic data of the inpatients.

Socio-demographic variables		Total (n=413)		Points	
		n	%	X̄±Sd	p
Age groups (years)	30 and below	33	8,0	4,25±0,07	0,462
	31-45	79	19,1	4,32±0,06	
	46-60	151	36,6	4,32±0,04	
	61 and above	150	36,3	4,26±0,02	
Gender	Female	239	57,9	4,26±0,02	0,080
	Male	174	42,1	4,33±0,03	
Martial status	Married	302	73,1	4,28±0,02	0,583
	Single	111	26,9	4,31±0,04	
Education level	Illiterate	50	12,1	4,35±0,06	0,079
	Literate	55	13,3	4,32±0,04	
	Primary school	160	38,7	4,27±0,03	
	Middle school	54	13,1	4,24±0,05	
	High school	61	14,8	4,39±0,05	
	University	33	8,0	4,14±0,07	

Mann-Whitney U Test, Kruskal Wallis Test

In this study, no significant difference was found between satisfaction scores of the duration of hospital stay and diet therapy variables. ($p > 0.05$). However, a significant difference was found between the satisfaction scores of giving treatment according to the clinic ($p < 0.05$) (Table 4).

Table 4. Satisfaction score average according to hospital stay information data of the inpatients.

Hospital stay information data		Total (n=413)		Points	
		n	%	X̄±Sd	p
Stay hospital (day)	3-7	205	49,6	4,30±0,03	0,269
	8-14	126	30,5	4,32±0,04	
	15-21	45	10,9	4,22±0,06	
	22 and above	37	9,0	4,22±0,06	
Clinic	Chest diseases	39	9,4	4,19±0,06	0,000
	Orthopedics	108	26,2	4,43±0,03[a]	
	Neurology	61	14,8	4,35±0,05[a]	
	Cardiology	69	16,7	4,21±0,06	
	Internal medicine	68	16,5	4,38±0,05[a]	
	Physiotheraphy	47	11,4	4,08±0,05[b]	
	General surgery	21	5,1	4,03±0,06[b]	
Diet therapy	Normal	186	45,0	4,32±0,03	0,098
	Diabetic or wasting	78	18,9	4,32±0,05	
	Salt-free	117	28,3	4,21±0,03	
	Low fat or low cholesterol	24	5,8	4,41±0,07	

Rich in protein	8	2,0	4,14±0,16	

Kruskal Wallis Test,
The difference between a-b and different superiors on the same line is significant. p<0,05

4. Discussion

In this study, the mean scores of the responses to 25 statements were also evaluated one by one. “The staff serving my meals are in uniform and clean." While the expression was found as the second expression with the highest average score with an average score of 4.73 ± 0.47, when evaluated according to the percentage of satisfaction, it was found that 98.5% of the patients participating in the study were the most satisfied. In a study conducted in Pakistan, the expression "hygiene of the serving personnel" was determined as the third statement with the highest score with an average score of 4.36 (Zahid et al. 2015). It can be said that the staff serving food in hospital nutrition services take care and pay attention to their cleanliness.

"Hot meals are served hot enough" in our study. The mean score given to the statement was found to be the expression with the lowest average score with 3.19 ± 0.88. When the satisfaction percentage of this expression was evaluated, it was found that only 30.7% of the patients participating in the study were satisfied with the food temperature. This result shows that the temperature cannot be maintained well in the food service of the hospital where the study was conducted. In a study, the expression "the appropriateness of the grades of hot dishes" was found as the expression with the lowest average score (3.7) (Zahid et al. 2015). In the study conducted in our country, 41.7% of the patients answered yes to the statement "Are you satisfied with the warmth of the food" and it was also found that the satisfaction rate was the lowest (Şahin et al. 2006). Although isothermic trays are used for food distribution in the hospital where the study is conducted, the low food service temperatures may be due to the length of the distribution time of the meals, the lack of proper closure of the lids of the trays, and the failure to routinely monitor food temperatures from the kitchen to the service.

In the literature, different results have been found regarding whether demographic characteristics of patients such as gender, age, education level and income level have an effect on satisfaction. These results may have different reasons. In this study, no significant difference was found between the satisfaction scores of the variables of gender, marital status, age, profession and education level (p>0.05). Similar results have been found in many studies (Abdelhafez et al 2012; Al-Torky et al. 2016).

Duration of hospital stay is one of the important factors affecting patients' satisfaction with nutritional services. In a study conducted in Iran, the satisfaction scores of those who stayed in hospital for more than 1 month were found to be significantly lower than the other groups (p<0.05) (Mirmasoudi et al. 2016). In some studies, no difference was found between duration of stay and satisfaction (p>0.05) (Wright et al 2003; Messina et al. 2013). In one of these studies, the menu alternatives offered at lunch and dinner to patients with long hospital stays (14 days) were increased and this was thought to be ultimately effective (Messina et al. 2013). In this study, the

satisfaction of patients with a hospital stay of 15-21 days and more than 22 days was lower than the patients who stayed in the hospital for 3-7 days and 8-14 days, but this difference was not significant ($p>0.05$).

Dietary treatments offered in hospitals are also one of the variables thought to have an impact on satisfaction. When the studies on this subject are examined, diets were evaluated by dividing the diets into two groups as normal and therapeutic diets in general, and no significant difference was found between these 2 diets on satisfaction (Zahid et al. 2015; Fallon et al 2008). In another study, 2 different hospital nutrition services satisfaction scales were applied to the same patients, and while a significant difference was found between diet types and satisfaction scores in one scale ($p<0.05$), no significant difference was found between diet types and satisfaction in the other scale ($p>0.05$) (Wright et al 2003). In this study, there was no significant relationship between diet types and satisfaction points ($p>0.05$).

The satisfaction scores of the patients hospitalized in the internal medicine, orthopedics and neurology clinics were significantly higher than the satisfaction scores of the patients hospitalized in the general surgery and physical therapy clinics ($p<0.05$). In a study conducted in Saudi Arabia, the satisfaction of the patients hospitalized in three clinics, including obstetrics, internal medicine and surgery, was examined and no difference was found between the clinics (Abdelhafez et al. 2012). One of the reasons for the lower satisfaction with nutrition services in general surgery and physical therapy clinics may be that the food intake of the patients in this clinic is negatively affected due to the various operations they have undergone.

5. Conclusion

One of the most important factors that reduce the satisfaction of inpatients from nutritional services is that the meals offered to the patients are not served at appropriate temperatures and the inadequate taste of the meals. In this direction, it is extremely important to choose the appropriate equipment in the follow-up of technological developments, to continuously monitor food temperatures within the scope of Food Safety Management Systems, and to carry out innovation studies in standard tariffs to increase the taste and variety in meals.

Despite the negative prejudices against hospital meals in the society, there are not enough comprehensive studies measuring the satisfaction level of hospitalized patients with nutritional services. There is a need for regular and larger studies on this issue.

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Determination of Specific Turkish Noodle's Glycemic Index Value

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Abstract:

Although the consumption of low glycemic index (GI) foods is of great importance in terms of healthy nutrition, the GI value of many foods is not known clearly. This study aimed to determine the GI value of noodles, which is one of the most commonly consumed foods in our country. Noodles were produced by the researcher according to the noodle standard and contain type 550 wheat flour, egg, salt, and water. In the GI test, noodles were used as test food while white bread and glucose as the reference food. Before the test, the available carbohydrate contents of the foods were determined by analyzing the protein, fat, moisture, ash and dietary fiber. Amounts of foods containing 25 g of available carbohydrates were used in the test. GI test was performed on 15 healthy female individuals who had undergone endocrinological examination. Participants were given reference and test foods after 10-12 hours of fasting once a week for 7 weeks. The test was applied once for noodles and three times for reference foods. Capillary blood glucose values were measured and recorded before consumption and at 15, 30, 45, 60, 90, and 120 minutes after the first bite. According to these values, the increasing area under the curve for each food was calculated and the GI value of noodles according to glucose and white bread was determined. The GI value of noodles was found 83.2 according to white bread and 54.8 according to glucose. According to the GI classification, foods with a GI below 55 have a low GI. These results showed that noodles specific to our country are classified as low GI foods. Noodles are a healthy source of carbohydrates as long as portion control is observed. GI studies for other foods specific to our country should be increased.

Keywords: noodle, glycemic index, glycemic response.

1. Introduction

One of the most important factors in maintaining health is a healthy diet. Choosing low glycemic index foods in the diet is one of the important components of a healthy diet. Diets based on low glycemic index foods proved to be effective in the prevention of many chronic diseases. Such diets ensured glycemic control in Type 2 diabetes patients and reduced LDL cholesterol level (Goff et al. 2013, Jenkins et al. 2002, Livesey et al. 2008). They are also known to balance insulin level and proinflammatory parameters such as C reactive protein (CRP) and reduce the risk of obesity and obesity-related diseases (Juanola-Falgarona et al. 2014, Schwingshackl & Hoffmann 2013). GI is the percentage of the blood glucose increase area formed over two hours by test food containing 25 g or 50 g available carbohydrates consumed by the same individual compared to the blood glucose increase area formed by the reference food containing an equal amount of available carbohydrates. In 1997, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) expert committee stated that GI was a good classification method that could be used to protect health and select appropriate carbohydrate sources for the treatment of certain

diseases (FAO, 1997). According to the glycemic index classification, foods are divided into three groups as low (GI <55), medium (GI = 55-70) and high (GI≥70) GI foods (Güler and Bilici, 2017).

Although the consumption of low glycemic index (GI) foods is of great importance in terms of healthy nutrition, the GI value of many foods is not known clearly. Studies in this area are limited due to the difficulty of GI testing procedures. There are several glycemic index tables used around the world. However, these tables do not include country-specific foods. This study aimed to determine the GI value of noodles, which is one of the most commonly consumed foods in our country.

2. Materials and Methods

The study consists of 4 steps. In the first stage of the study, noodles were produced in accordance with the noodle standard in the laboratory of the nutrition and dietetics department. Noodles are prepared on the basis of 100 g wheat flour (Selva, wheat flour for baklava pastry). And contain type 550 wheat flour, egg, salt, and water. Since it was suggested that the flour used in making noodles should be bright colored, low-bran, fine particle and not too hard, wheat flour for baklava and pastry was used in the study. In the GI test, noodles were used as test food while white bread and glucose as the reference food.

In the second stage, proximetry (moisture, ash, total protein and fat) and total dietary fiber analyzes of white bread, one of the reference foods, and noodles, which is the test food, were performed. Using the data obtained as a result of these analyzes, the amounts of white bread and noodles containing 25 g available carbohydrates were calculated. The amounts of available carbohydrates were determined by subtracting the sample's moisture, ash, protein, total fat and total dietary fiber values from 100 (ISO, 2010).

In the third step of the study, 15 healthy volunteers who met the study criteria were reached to calculate the in vivo SR value of noodles. The individuals, who were informed about the rules to be observed during the research process, were examined by the physician in endocrinological examination.

In the fourth phase of the study, 15 individuals were given reference and test foods after 10-12 hours of fasting for 7 weeks at an interval of one week. Reference foods were consumed three times and noodles, which were test foods, were consumed once. In order to prevent any differences regarding the content, white breads were bought freshly from the same producer at the same time on the morning of the test. In glycemic index studies, it is recommended to evaluate the amount of 25 g or 50 g available carbohydrate. In this study, in order to avoid difficulties due to the high amount of consumption, the participants consumed the amounts of foods containing 25 g available carbohydrates. Before consumption (0 minutes) and at 15, 30, 45, 60, 90 and 120 minutes after the first bite, duplicated capillary blood was taken from the finger and blood glucose

values were measured and recorded. Glycemic index calculations were made by creating glycemia curves with the recorded glucose values. GI values were calculated for both glucose and white bread. In calculating the glycemic index, the method of calculating the increasing area under the curve recommended by the FAO / WHO Experts Committee and also included in the ISO standard No. 26642 was used (ISO, 2010).

3. Results

The moisture (%), fat (%), protein (%), total dietary fiber (%), ash (%), available carbohydrate (%), total carbohydrate (%) values of the reference and test nutrients are shown in Table 1.

Table1. Moisture, fat, protein, total dietary fiber, available and total carbohydrate values (%) of reference and test foods.

Food	Moisture (%)	Total fat (%)	Protein (%)	Total dietary fiber(%)	Ash (%)	Available carbohydrate (%)	Total carbohydrate (%)
White bread	27,98	0,48	8,96	5,25	1,58	55,75	61
Noodle	7,23	2,9	11,51	5,05	1,39	71,92	76,97

The amounts of reference and test foods containing 25 g available carbohydrates are given in Table 2. In the GI test, the amounts of foods containing 25 g available carbohydrates were used, and this value was found as 25 g for glucose, 44.8 g for white bread and 34.7 g for noodles.

Table 2. Amounts of reference and test foods containing 25 g available carbohydrates.

Foods	Available carbohydrate (%)	25 g available carbohydrates amount containing (g)
Glucose	100	25
White bread	55,75	44,8
Noodle	71,92	34,7

The comparison of the two-hour blood glucose measurement values of the reference and test foods according to the foods and time is given in Table 3. Blood glucose values measured at minute zero are similar for all foods**.** After consuming glucose and noodles, blood glucose level reached its highest level in the 30th minute. After the consumption of white bread, the highest blood glucose level was observed in the 45th minute.

Table 3. Comparison of two-hour blood glucose measurement values of reference and test foods according to foods and time (mg / dL).

Time	**Glucose**	**White bread**	**Noodle**
(min.)	$\bar{X}$±SS	$\bar{X}$±SS	$\bar{X}$±SS

0.	86,5±4,0	87,8±4,5	87,6±3,2
15.	111,1±10,8	93,4±7,6	99,4±4,9
30.	139,2±11,0	114,5±10,3	116,9±8,0
45.	134,1±14,1	120,7±10,2	110,9±12,4
60.	119,5±17,6	111,1±10,6	101,9±13,8
90.	98,3±16,1	100,2±10,5	96,2±5,7
120.	84,7±6,4	92,7±6,9	93,1±3,0

The arithmetic mean, standard deviation and lower-upper values of the blood glucose increase areas formed by the reference and test foods consumed are given in Table 4. While the average area of blood glucose increase formed by glucose from reference foods was found to be 2979.4, that of white bread was found to be 1982.0. The noodle's was found to be 1606.5.

Table 4. Arithmetic mean, standard deviation and lower-upper values of the blood glucose increase areas formed by the reference and test foods consumed.

Foods	**Blood glucose increase areas (n=15)**	
	$\bar{X}$±SS	Min-max
Glucose	2979,4±874,32	2073,6-4312,6
White bread	1982,0±553,82	1527,1-4090,0
Noodle	1606,5±583,83	945,0-3802,5

The arithmetic mean, standard deviation and lower-upper values of GI values of noodles according to glucose and white bread are given in Table 5. The GI value of noodles was found 83.2 according to white bread and 54.8 according to glucose. It was determined that noodles were included in the low glycemic index food group.

Table 5. Arithmetic mean, standard deviation and lower-upper values of GI values of noodles according to glucose and white bread

Food	GI value according to glucose		GI value according to white bread	
	$\bar{X}$±SS	min-max	$\bar{X}$±SS	min-max
Noodle	54,8±11,5	27,5-69,0	83,2±18,11	45,2-110,1

4. Discussion

The flour used for making noodles should be brightly colored, low-bran and fine-particle. Wheat used for noodle flour should not be too hard and should be milled gradually to reduce starch damage (Dündar, 2014). In this study, wheat flour for baklava and pastry was used. There is no in vivo study in the literature to determine the glycemic index value of Turkish noodles. In the

international glycemic index table, the glycemic index value of Australian noodles was determined as 62 and that of instant noodles as 47 (Foster-Powell et al. 2002). In this study, the GI value of noodles according to glucose was found to be 54.8. Since egg noodles are frequently consumed in our country, egg noodles were used in our study. The glycemic index value of Australian noodles is higher than that of the noodles used in this study, and it was thought that this difference may be due to the absence of eggs in the formulation.

5. Conclusion

It has been determined that noodles, which are frequently consumed by the Turkish society, have a low GI value. Noodles are a healthy source of carbohydrates as long as portion control is observed. There is little research in this area, as tests to determine the glycemic index of foods have rigorous procedures. The standard glycemic index tables used worldwide do not include country-specific foods. Glycemic index studies for other foods specific to our country should be increased.

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Assessments of Diarylheptanoids from *Juglans regia* L. as potential inhibitors of SARS-CoV-2 Main Protease

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Abstract:

The disease was named COVID-19 when multiple cases of atypical pneumonia with symptoms similar to viral pneumonia emerged in China in December 2019 due to the SARS-CoV-2 virus. No effective therapy for COVID-19 is currently available. Although many different vaccine development studies against SARS-CoV-2 have reached the final stage, the protection that these vaccines will provide to society is not known for now, and therefore, effective antiviral drugs should be developed. In this study, the interactions of diarylheptanoids found in *Juglans regia* L. with the SARS-CoV-2 main protease were analyzed by molecular docking. 3D Structures of Juglenin A, Juglenin B and Juglenin C, which are diarylheptanoids were obtained from PubChem Database and the structure of SARS-CoV-2 Main Protease from the Protein Databank. The main protease was prepared for the molecular docking process by cleaning from heteroatoms, water and ligand molecules, adding polar hydrogen atoms and performing energy minimization. Potential energies of diarylheptanoids were reduced by minimizing energy. The MGL Tools software was used to create a grid box covering the active sites of the main protease, and Autodock Vina software was used for the molecular docking process. Ten poses were obtained as a result of the molecular docking process, and the interactions between amino acid residues and Juglenins. The pose showing the best binding affinity and the lowest RMSD value was analyzed with the Discovery Studio Client 2020 software. As a result of molecular docking, it was determined that Juglanin A, B and C showed high binding affinities with the SARS-CoV-2 main protease and formed hydrogen bonding interactions with amino acid residues. The results showed that the interactions of Juglanins (A, B and C) with residues in the active site of the main protease were limited, but their interactions with the His41 and Cys145 amino acid residues, which have a catalytic role, could still be effective. As a result of the *in silico* analyzes, it has been found that the use of Juglanin A, B, and C together and in combination with other effective compounds may be beneficial in antiviral drug development studies against SARS-CoV-2.

Keywords: SARS-CoV-2, main protease, *Juglans regia* L., diarylheptanoids, molecular docking.

1. Introduction

Multiple cases of atypical pneumonia occurred with symptoms similar to viral pneumonia that emerged in December 2019 in Wuhan, China. The reason was determined as a new coronavirus, later called the 2019 novel coronavirus (SARS-CoV-2), and this disease was called COVID-19. Coronavirus belongs to a large number of virus families known as coronaviridae (Song et al. 2019). Coronaviruses are classified into four subfamilies as alpha, beta, gamma and delta coronaviruses, according to their form and host. It is thought that the hosts of delta and gamma coronaviruses are birds and pigs, and the hosts of alpha and beta coronaviruses are bats (Banerjee et al. 2019, Paules et al. 2020). SARS-CoV-2 is a novel coronavirus that causes respiratory illness which has been called the COVID-19 infections. Respiratory disease deaths and inherent morbidity and mortality made SARS-CoV-2 a significant and recurring public health issue worldwide. Coronaviruses are enveloped as part of the Coronaviridae family, encoding more than

20 proteins with a single-stranded RNA genome. Analysis indicates that SARS-CoV-2 can bind high-affinity human ACE2 (Hoffmann et al. 2020). No effective therapy for COVID-19 is currently available, and there is a lack of literature on COVID-19 treatment. However, the steps taken are restricted to avoid future complications and organ injury through prevention and complementary therapies (Rodríguez-Morales et al. 2020). Main protease (MPro) is an important component of the viral process, and thus a possible target receptor to suppress the pandemic COVID-19 (Jin et al. 2020). Therefore, the main protease was preferred as the target structure in this study.

Juglens regia L. (common walnut) is a tree of economic importance which is highly valued for their timber and edible nuts. Almost anywhere in the world with a moderate climate, these species grow well (McGranahan and Leslie 1991). Walnut trees grow free-crowned and can be up to 25-30 meters tall. Its leaves have a long stem and consist of 5 to 9 leaflets arranged opposite each other (Şen, 2011). The oil obtained from its kernel is of high value, obtained from the walnut kernel, which is evaluated as food and pharmacological (Gao et al. 2019). *Juglans regia* L. is known to show antioxidant and antimicrobial, anti-inflammatory effects (Vieira et al. 2019). It is rich in essential oils, sterols, fatty acids (Rafaat 2018), flavonoids (Ghasemi et al. 2011) diarylheptanoids (Liu et al. 2008), and tannins (Fukuda et al. 2003).

Although many different vaccine development studies against SARS-CoV-2 have reached the final stage, the protection that these vaccines will provide to society is not known for now, and due to the mutations that the virus may pass in the future, it is also necessary to develop effective drugs for treatment. In addition to the design and chemical synthesis of protease inhibitors, the investigation of enzyme inhibitors among natural substances to obtain drugs with reduced side-effects is one of the modern therapeutic techniques for virus infection. In this study, the interactions and binding affinity of diarylheptanoids, which are known to be found in the walnut shell and which is a secondary metabolite, with SARS-CoV-2 main protease were investigated by molecular docking analysis.

2. Materials and Methods

Determination of Phytochemical Library:

The diarylheptanoids found in the *Juglans regia* L. shell were determined by literature review. The diarylheptanoids determined were Juglanin A, Juglanin B and Juglanin C (Liu et al. 2008).

Protein Receptors Preparation:

The structure of SARS-CoV-2 Main Protease (PDB ID: 7BQY) (Jin et al. 2020) was obtained from the https://www.rcsb.org/ website in PDB format. Protein was prepared using Biovia

Discovery Studio 2020 Client for docking. The active site of the protein molecule was determined, then loaded with polar hydrogen atoms leaving only the protein structure. Energy minimization of protein was performed with Gromos 43B1 using Swiss-PdbViewer (v.4.1.0) (Guex and Peitsch 2005) software and saved as PDB for analysis.

Ligand Preparation:

The 3D Juglenin A (CID: 70697839), Juglenin B (CID: 44155973) and Juglenin C (CID: 101868826) structures were downloaded in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov), and uff-force field energy minimization and PDB conversion were carried out with Open Babel v.2.4.0 software (O'Boyle et al. 2011).

Molecular Docking:

To build a grid box for the active areas, MGL Tools (Morris et al. 2009) were used to define and store the grid box size to cover the active areas. Autodock Vina (Trott and Olson 2010) software has been used for molecular docking. Confirmation was chosen with the lowest vina score and the lowerest mean RMSD values.

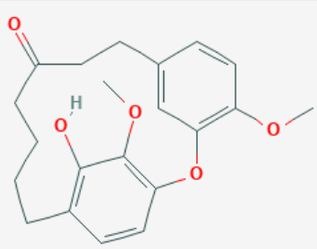
Visualization:

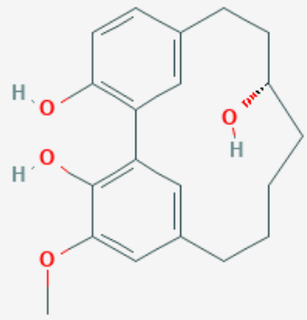
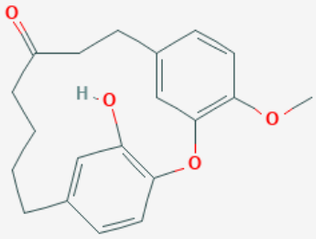
The Biovia Discovery Studio 2020 Client program was used to define the interactions between the receiving and ligand configurations of the complex to classify the interactions between amino acids of receptors and ligands. The 2D and 3D visualization processes were performed by this program.

3. Results

The findings indicate that the ligands have greatly docked to the target protein, after a successful docking of all the ligands used in these docking experiments. The best possible docking positions were preference for the lowest binding affinity and the lowest RMSD values. Table 1 offers substances with molecular docking analysis. The best binding affinity showed Juglanin B with -7.4 kcal/mol, while Juglanin A showed -6.6 kcal/mol and Juglanin C -6.3 kcal/mol.

Table 1: Phytochemicals used for binding with the target protein and binding affinities.

Compound	Structure	PubChem Compound ID	Binding affinity
Juglanin A		70697839	-6.6 kcal/mol

Juglanin B		44155973	-7.4 kcal/mol
Juglanin C		101868826	-6.3 kcal/mol

Juglanin A interacted with M pro at high affinity with a binding affinity of -6.6 kcal/mol (Figure 1). This is also evidenced by THR 198 and hydrogen bonding with the ligand. Hydrogen bond interactions occurred between Juglanin A and main protease with CYS145 and LEU141 residues, while hydrophobic interactions occurred between HIS41 (twice), MET49 and HIS163 residues.

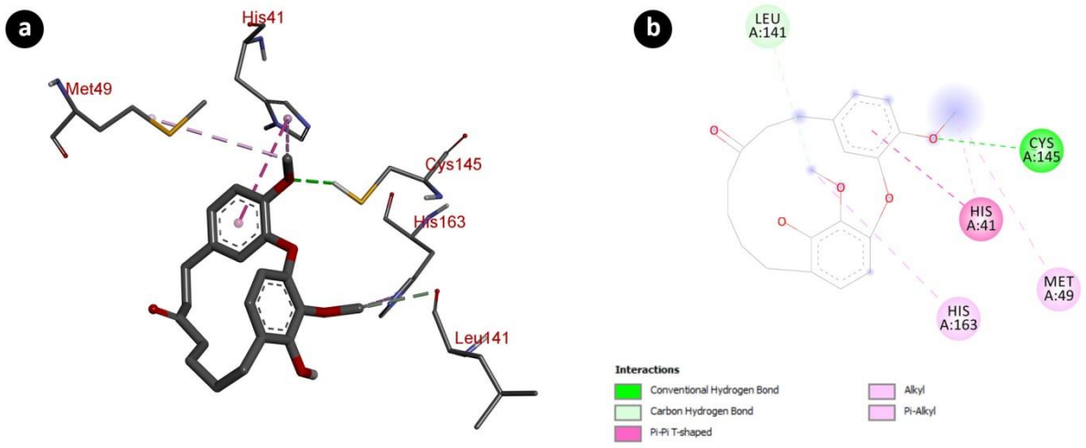


Figure 1. Juglanin A docked in SARS-CoV-2 main protease (PDB ID 7BQY) with (a) Amino acid residues involved in interaction and (b) Binding interaction of Juglanin A with amino acid residues.

Juglanin B interacted with the main protease, showing the best binding affinity of -7.4 kcal/mol among the compounds in this study. Juglanin B has established hydrogen bond interactions with HIS163 and HIS164 (twice) residues, hydrophobic interactions with HIS41 and MET165 residues (Figure 2)

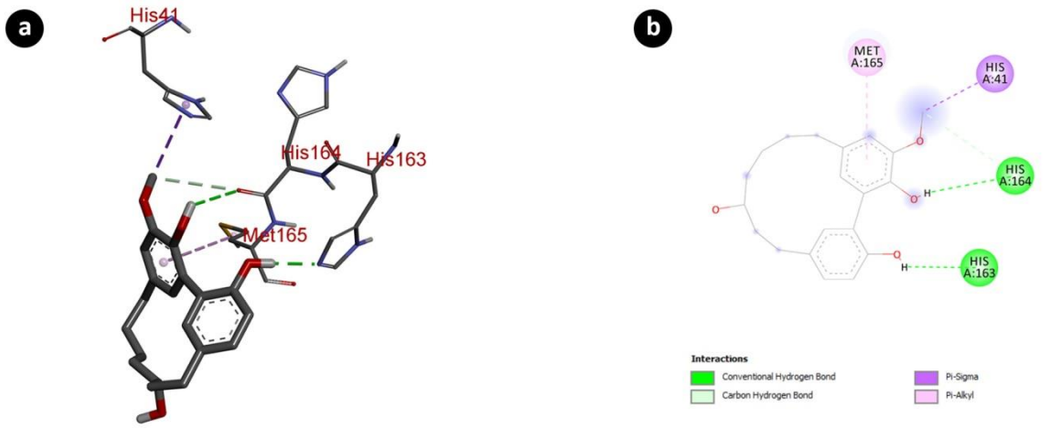


Figure 2. Juglanin B docked in SARS-CoV-2 main protease (PDB ID 7BQY) with (a) Amino acid residues involved in interaction and (b) Binding interaction of Juglanin B with amino acid residues.

Juglanin C interacted with the main protease of SARS-CoV-2, showing a binding affinity of -6.3 kcal/mol. The compound formed a hydrogen bond with residue GLU166, hydrophobic interactions with residues MET49 (twice) and HIS 41 (Figure 3).

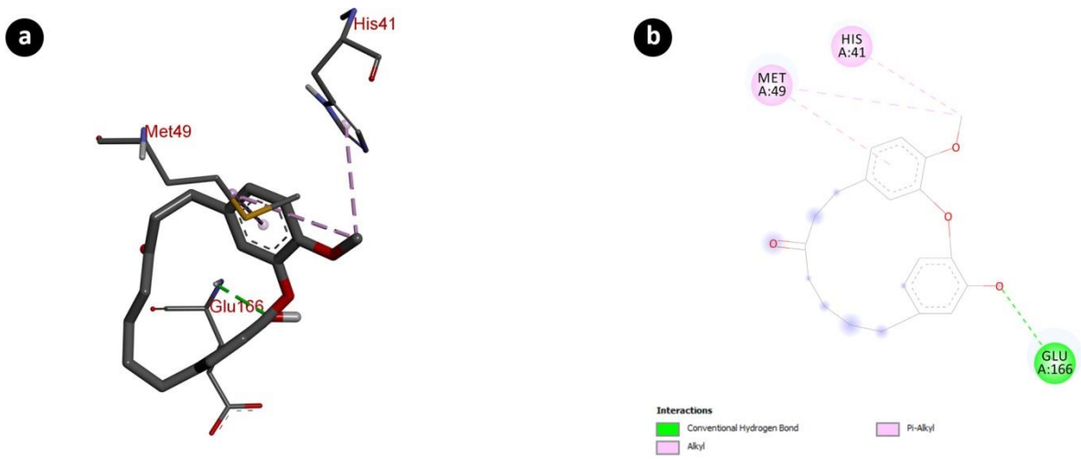


Figure 3. Juglanin C docked in SARS-CoV-2 main protease (PDB ID 7BQY) with (a) Amino acid residues involved in interaction and (b) Binding interaction of Juglanin C with amino acid residues.

4. Discussion

The findings of this study, which analyzed the effect of diarylheptanoids found in *Juglans regia* L. against SARS-CoV-2 Main protease, showed that Juglanin A, B and C had separate effects on Mpro. Juglanin B showed the best binding affinity with -7.4 kcal/mol, while Juglanin A simultaneously interacted with the HIS41 and CYS145 residues, which have catalytic roles. The interaction of Juglanin C was more limited only with HIS41 of the catalytic residues. However,

these three diarylheptanoids interacted with amino acid residues in the active site. This indicates that diarylheptanoids can act on the SARS-CoV-2 main protease.

5. Conclusion

Each of the phytochemicals in the study interacted with different amino acids and showed good binding affinities. They had few interactions with amino acid residues in the active site. However, interactions with HIS41 and CYS145 residues, located in the active site and having an important catalytic role, showed that these phytochemicals could still be effective in main protease inhibition. As a result of the interactions that occurred, it has been shown that the use of these compounds together and in combination with other compounds in drug development studies against SARS-CoV-2 may be beneficial. Where each of these compounds interacts with catalytic residues in the active site of SARS-CoV-2 main protease, these interactions can be used to design antiviral drugs effective against SARS-CoV-2 to inhibit min protease. Also, investigation of the binding activities of other phytochemicals will contribute to the drug development process for SARS-CoV-2.

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Removal of Methyl Orange From Environmental Wastewater using Coriander Seeds

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Abstract:

The spread of toxic effects in wastewater in various industries has detrimental effects on water resources, soil fertility, aquatic organisms, and ecosystem integrity. One of the biggest problems in the textile industry is the discharge of colored substances. Many organic and inorganic pollutants need to be eliminated in the wastewater of industries. Various color and dye wastes in industrial wastewater and dye content in textile wastewater disrupt the aesthetic appearance. Also, dyes can emit toxic and carcinogenic metabolites under anaerobic conditions. Dyes can be treated by physical and chemical methods to prevent pollution in the aquatic environment. However, the cost of these methods is extremely high. Sludge can be produced in some of these methods. To eliminate the color of the wastewater is a need for alternative methods. Adsorption is the most common method for the removal of dyes from wastewater. In addition to it is a well-known process to treatment pollutants from aqueous media. Researchers have recently opted for more economical and environmentally friendly adsorbents. As an example of these; Tea residues, sawdust, oily coffee beans, tree ferns, chitosan, olive oil waste, orange juice residues, orange peels, algae, dried plants, and olive stone residues, plant seeds can be given. This study: it is aimed to remove the methyl orange dye from the wastewater by grinding the seeds of coriander (Coriandrumsativum L.) and using it as an adsorbent. The effects of contact time, pH, temperature, and initial concentration of methyl orange on adsorption were investigated by the batch method and optimum conditions (45 minutes, pH:6, 50 °C, and 50 mg/L, respectively) were determined. Langmuir, Freundlich, Temkin, Dubinin-Radushkevich adsorption isotherm models and Pseudo first-order kinetic model, Pseudo second-order kinetic model, the suitability of intra-particle diffusion model adsorption process was investigated. The adsorbent process was found to be suitable for the Langmuir isotherm model and the Pseudo second-order kinetic model.

As a result of the adsorption process, q_{max} value was found as 28.86 mg/g; coriander seed particles have been identified as a cheap, convenient, and effective natural adsorbent to extract methyl orange from wastewater.

Keywords: adsorption, coriander seed, isotherm, kinetic, methyl orange.

1. Introduction

Environmental pollution is constantly increasing both in the world and in our country. This increase harms the planet we live in, the environment we interact with, and the life of all living things. Considering the run out of water resources in one day, water pollution and control are very important in recent years. Dyes are one of the most common pollutants in the world. The reform of technology has enabled many materials to have vivid colors. However, many problems also appear together with the extensive use of dyes. In the process of using dyes, up to 20% of them fade into dye wastewater, causing water pollution (Wang et. Al., 2010). Dye wastewater is considered a dangerous contaminant due to its diffusibility, difficulty in biodegradation, destruction of ecological balance, and enrichment in the human body through the food chain (Goswami and Phukan, 2017; Bao, 2013) Also, Dyes may spread toxic and carcinogenic metabolites in anaerobic

conditions. Dyes can be treated by physical and chemical methods to prevent pollution in the aquatic environment. However, the cost of these methods is extremely high. Sludge can be produced in some of these methods. To eliminate the color of the wastewater is a need for alternative methods (Savcı and Uysal, 2017).

Some traditional methods of dealing with aqueous pollution include precipitation, coagulation, reduction, membrane separation, ion exchange, adsorption, photocatalysis, and so forth (Shu et. Al., 2017; Yuan et. Al., 2016). Among these methods, adsorption technology is cost-effective and easy to operate, which is an effective method to handle water pollution (Hakami et. Al., 2012). Therefore, the quantity and quality requirements of adsorbents are increasing. At this moment, there are many kinds of commercially available adsorbents for different applications. The development of environmentally friendly, cheap, and more efficient adsorbents is a very active research topic. Therefore, alternative low-cost adsorbents such as chitin (Gurusamy and Jiunn-Fwu, 2008) coffee (Franca et. al., 2009), tea waste (Tamez, 2009), orange peel (Mokhtar, 2005), rice husk (Ola et. Al., 2005), bark (Tan et. Al., 2010) and coir pith (Kavitha and Namasivayam, 2007). have been studied.

In this study; for the adsorption of methyl orange dye from wastewater by grinding the seeds of coriander (Coriandrumsativum L.) and using it as an adsorbent; it is aimed to determine the optimum parameters and to evaluate the adsorption kinetics, isotherm models, and thermodynamic properties with the obtained data.

2. Materials and Methods

2.1. Chemicals and Devices

The coriander seed particles used were purchased from local markets. Methyl orange dyestuff, Hydrochloric acid (HCl), and Sodium hydroxide (NaOH) are of analytical purity and were obtained from Merck. A stock solution of methyl orange dyestuff was prepared (250 mg/L) and diluted to the desired concentrations.

In the experiments, WiseStir multiple mechanical stirrer heater, NÜVE FN 400 oven, Thermo Scientific ultrapure water device, 620 Lab pH Meter, Optizen POP UV spectrophotometer were used.

2.2. Adsorption Studies

Methyl orange adsorption studies on coriander seed particles were carried out using the batch method. Methyl orange at different concentrations and pH was contacted with the adsorbent at different temperatures for varying times. HCl and NaOH solutions were used in studies for pH adjustments. At the end of the adsorption process, the adsorbent and methyl orange solutions were separated by filtering with filter paper. The amount of methyl orange remaining in the solution medium was measured at 466 nm with a UV spectrophotometer.

The adsorption capacity of coriander seed particles at time t (q_t, mg/g) and equilibrium (q_e, mg/g), methyl orange removal efficiency (A %) was calculated from the following equations:

$$q_t = \frac{(C_o - C_t)}{m} \text{ x V} \quad (1)$$

$$q_e = \frac{(C_o - C_e)}{m} \text{ x V} \quad (2)$$

$$A(\%) = \frac{(C_o - C_t)}{C_o} \text{ x } 100 \quad (3)$$

In equations, q_t and q_e are the adsorption capacity (mg/g) at t and equilibrium, respectively; C_o, C_e and C_t, initial concentration at time t, methyl orange equilibrium concentration (mg/L) and liquid phase concentration, respectively; m is the amount of adsorbent (g); The volume (L) and A (%) of the solution are the percent adsorption (Yi et. al., 2016).

By using coriander seed particles, methyl orange adsorption from wastewater has been studied by batch method. Four different parameters affecting the adsorption were investigated. These parameters are contact time, pH, initial concentration and temperature effect.

3. Results

3.1. pH Effect

The most important parameters for the adsorption experiments are examined pH effects. The effect of pH on adsorption was determined by studying at different pH ranges (10 mL of 100 mg/L methyl orange solutions, 0.1 g of adsorbent, 45 min, 25 °C, 620 rpm). The results are shown in Figure 1 and the optimum pH value was found to be 6.0.

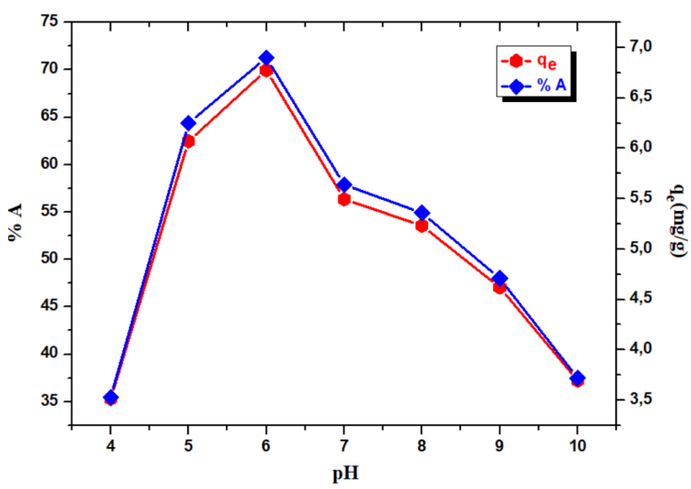

Figure 1. The effect of pH on Methyl Orange adsorption.

3.2. Effect of Contact Time and Kinetic Studies

The effect of contact time on methyl orange adsorption capacity was studied using a series of contact times (10 mL of 100 mg/L methyl orange solutions, 0.1 g adsorbent, pH 6.0, 25 °C, 620 rpm). The results are shown in Figure 2. the optimum contact time was found to be 45 minutes and this value was used in other parameter studies.

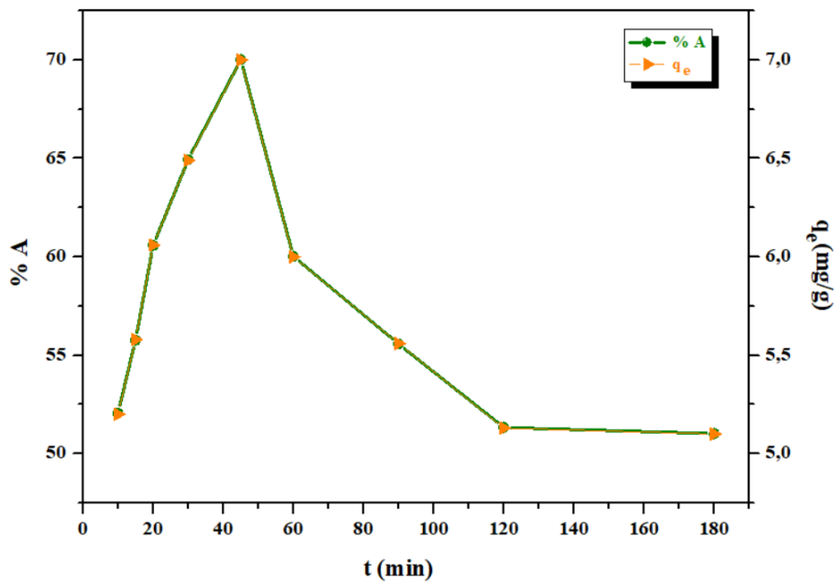

Figure 2. The effect of contact time on methyl orange adsorption.

The kinetics of methyl orange adsorption were investigated with Pseudo first order (Figure 3a) and Pseudo second order (Figure 3b) and intra-particle diffusion (Figure 3c) models according to the following equations and the calculated parameters are shown in Table 1.

$$\ln (q_e - q_t) = \ln q_e - k_1.t \quad (4)$$

$$\frac{t}{q_t} = \frac{1}{k_2\, q_e^2} + \frac{1}{q_e}.t \quad (5)$$

$$q_t = K_{id}.\, t^{1/2} + I \quad (6)$$

Here, qt and qe are the adsorption capacity and the amount of methyl orange adsorbed in equilibrium (mg/g), k_1 (1/min) and k_2 (g/mg.dk), the ratio constant of the pseudo-first-order and pseudo-second-order model, K_{id} particle inside diffusion rate constant (mg/g.dak$^{1/2}$). The value of I gives an idea of the thickness of the boundary layer, in other words, the greater the intersection degree, the greater the boundary layer effect. (Chen et al., 2018).

Table 1. Kinetic parameters for methyl orange adsorption.

	Pseudo-first order			**Pseudo-second order**			q_{exp} (mg/g)
	k_1 (dak^{-1})	q_{e1} (mg/g)	R_1^2	k_2 (gmg^{-1}dak^{-1})	q_{e2} (mg/g)	R_2^2	
MO	**0.06**	**3.53**	**0.9945**	**0.04**	**6.24**	**0.9986**	**7**

Intra-particle diffusion			
	I	***R^2***	***K_{id} (mg/g.dk$^{1/2}$)***
MO	**3.42**	**0.9855**	**0.57**

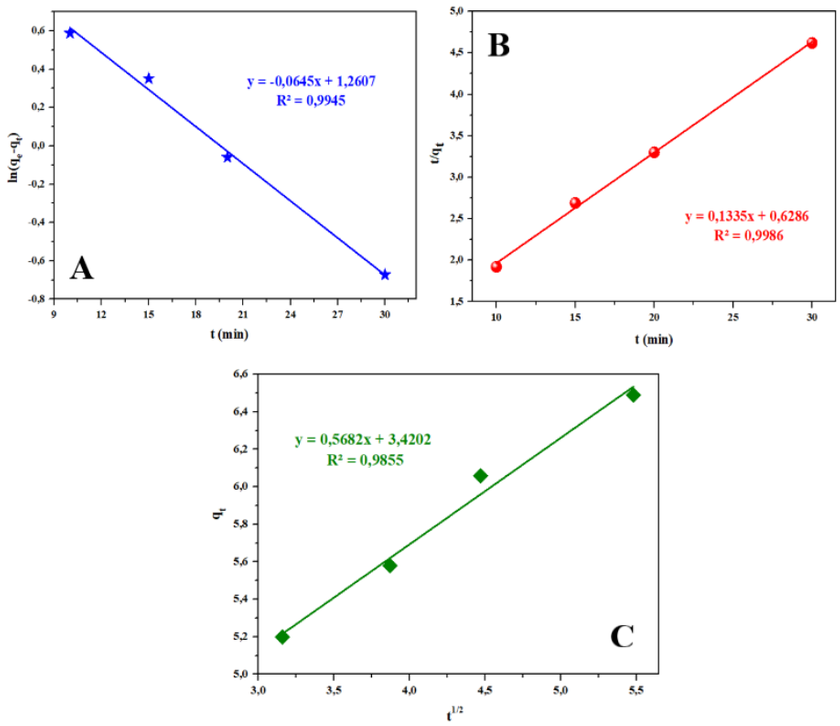

Figure 3. (a) Pseudo First Order (b) Pseudo Second Order Adsorption (c) Intra-Particle Diffusion Kinetics for methyl orange adsorption.

3.3. Initial Concentration Effect and Isotherm Studies

The effect of the initial concentration of methyl orange on the adsorption capacity was studied using (10-300 mg/L) initial ion concentrations (pH 6.0, 0.1 g adsorbent, 45 min, 25 °C, 620 rpm). The optimum initial concentration was found to be 50 mg/L. The results are shown in Figure 4.

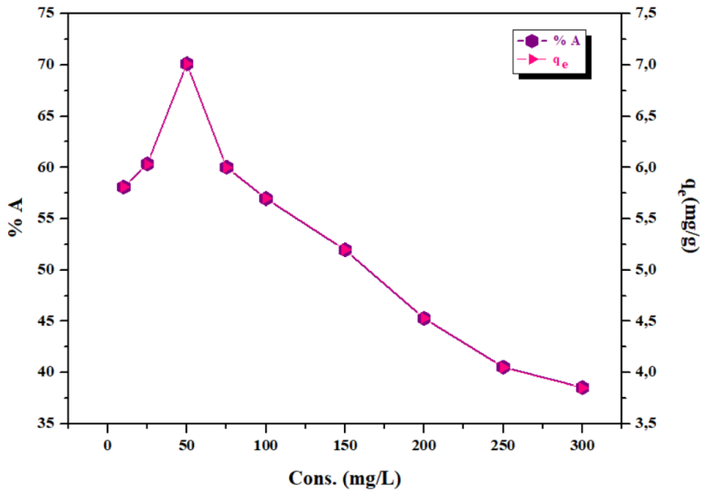

Figure 4. Effect of the initial concentration of methyl orange on adsorption.

Equilibrium data were analyzed with the help of Langmuir isotherm, Freundlich isotherm, Temkin isotherm and Dubinin-Radushkevich isotherm models to obtain the most suitable isotherm. These are the most common isotherms used to characterize the solid-liquid adsorption system.

The Langmuir isotherm describes a single layer adsorption on a uniform surface containing a limited number of adsorption sites. When a region is full, no other adsorption process takes place in that region. The equations of the Langmuir adsorption isotherm are as follows (Kong et al., 2016):

$$\frac{C_e}{q_e} = \frac{1}{q_{max}\, b} + \frac{1}{q_{max}} C_e \qquad (7)$$

$$R_L \ \frac{1}{1+b\,C_0} \qquad (8)$$

In the equation, q_e (mg/g) is the amount of methyl orange adsorbed on the adsorbent, C_e (mg/L) is the equilibrium concentration in solution, b (L/mg) and q_{max} (mg/g) are the constants related to the adsorption energy and adsorption capacity of Langmuir, respectively. The dimensionless separation factor R_L can be used to predict the affinity between sorbate and sorbent. The R_L value describes the absorption process as follows: $R_L > 1$, negative; $R_L = 1$, linear; $0 < R_L < 1$, convenient and $R_L = 0$, irreversible (Bulut et al., 2018).

Freundlich isotherm is applied for heterogeneous surfaces and multilayer adsorption; It is expressed by the following equation (El-Maghrabi et al., 2017):

$$\ln q_e = \ln K_F + (1/n) \ln C_e \qquad (9)$$

Here; n and K_F are Freundlich constants related to adsorption density and adsorption capacity, respectively.

The Dubinin-Radushkevich (D-R) isotherm model assumes that the properties of the sorption curves are related to the porous structure of the sorbent (Rahman-Sani et al., 2015). The model equations are as follows:

$$\ln q_e = \ln q_o - \beta\varepsilon^2 \qquad (10)$$

$$\varepsilon = RT \ln (1+(1/C_e)) \qquad (11)$$

Where; ε is Polanyi potential, R (8.314 J/mol.K) gas constant, T (K) temperature, Ce (mol/L) equilibrium concentration, qo (mol/g) is the maximum absorption capacity according to D-R model and β (mol/J^2) is constant depending on the sorption energy. Average absorption energy (E, J/mol) is calculated as follows:

$$E = \frac{1}{\sqrt{2\beta}} \quad (12)$$

The E value can give an idea of whether the adsorption process is physical or chemical.

The temkin isotherm assumes that the sorption heat of all sorbates in the sheet decreases linearly with the coverage due to sorbent-sorbate interactions, and that the sorption process is characterized by uniform distribution of some binding energy up to some maximum binding energy. This model is expressed with the following equation (Kaynar et al., 2016).

$$q_e = \mathrm{B} \ln (K_T) + \mathrm{B} \ln (C_e) \quad (13)$$

Here; KT (L/g) is the equilibrium binding constant corresponding to the maximum binding energy, and the B constant is related to the heat of absorption (B = RT/b).

The graphs of the Langmuir, Freundlich, Temkin and Dubinin-Radushkevich isotherms are shown in Figure 5, and isotherm parameters in Table 2.

Table 2. Isotherm parameters for methyl orange adsorption.

	Langmuir				**Freundlich**		
	q_{max} **(mg/g)**	R_L	**b(L/mg)**	R^2	K_f **(mg/g)**	**n**	R^2
MO	**28.86**	**0.053**	**0.36**	**0.9914**	**0.74**	**1.04**	**0.9895**
	Dubinin-Radushkevich				**Temkin**		
	q_m **(mg/g)**	β **(mol^{2}kJ^{-2})**	**E(kJ/mol)**	R^2	K_T **(L/g)**	**B(J/mol)**	R^2
MO	**12.66**	**6.85**	**0.35**	**0.8133**	**15.86**	**4.67**	**0.9443**

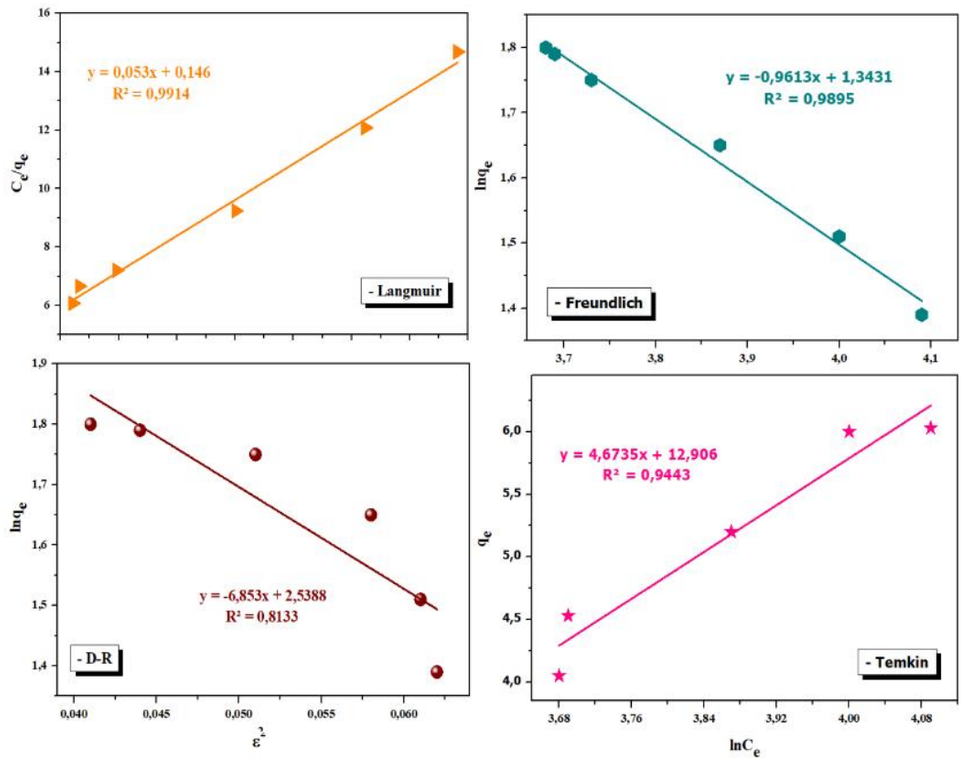

Figure 5. Isotherm plots for methyl orange adsorption on coriander seed particles

3.4. Temperature Effect and Thermodynamic Studies

Temperature change influences the adsorption process. In the experiments, a relative increase was observed in the adsorption capacity and adsorption rate when the temperature increased (pH 6.0, 0.1 g adsorbent, 45 min, 50 mg/L methyl orange solutions of 10 mL, 620 rpm). This indicates that methyl orange adsorption is an endothermic process. The effect of temperature on methyl orange adsorption is shown in Figure 6.

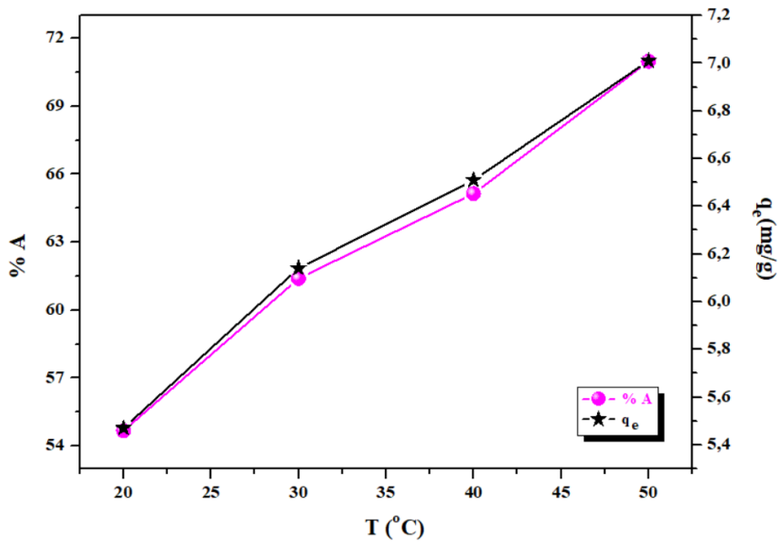

Figure 6. Effect of temperature on methyl orange adsorption.

The thermodynamic parameters enthalpy change (ΔH°), entropy change (ΔS°) and free energy (ΔG°) are obtained from the following equations (Sen et al., 2017).

$$\ln K_d = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT} \quad (14)$$

$$\Delta G° = \Delta H° - T\Delta S° \quad (15)$$

$$K_d = \frac{C_{ad}}{C_e} \quad (16)$$

In equality; C_e is the equilibrium concentration of methyl orange in the solution; K_d is the equilibrium constant; C_{ad}, the concentration of methyl orange adsorbed to the adsorbents at equilibrium; T (K) is temperature; R (8.314 (J/mol.K) is the gas constant. ΔH° and ΔS° are obtained from the slope and intersection of the Van't Hoff plot of $1nK_d$ with respect to 1/T (Figure 7). values are obtained from the slope and intersection of the graph and these results are shown in Table 3.

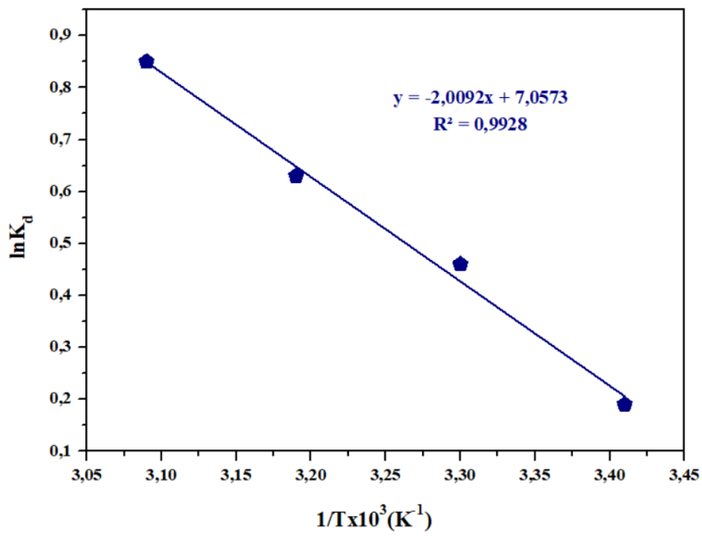

Figure 7. Change of lnK_d with 1/T in methyl orange adsorption.

Table 3. Thermodynamical parameters for methyl orange adsorption.

	Termodinamik parametreler				
	Sıcaklık (K)	***ΔH⁰ (J/mol)***	***ΔS⁰ (J/mol K)***	***TΔS⁰ (kJ/mol)***	***ΔG⁰ (kJ/mol)***
MO	**293**	**16.7**	**58.67**	**17.19**	**-0.46**
	303			**17.77**	**-1.16**
	313			**18.36**	**-1.64**
	323			**18.95**	**-2.07**

4. Discussion

The effects of parameters such as contact time, pH, temperature, and initial concentration of methyl orange on adsorption were investigated by batch method. Optimum conditions in this parameter study where coriander seed particles are used as an adsorbent; contact time was 45 min, pH 6.0, 50 °C, and initial methyl orange concentration was 50 mg/L.

Table 1 continues the correlation coefficients of the kinetic models and qe calculated from the equations. According to this result, it was seen that the pseudo quadratic kinetic model is more suitable for the adsorption program because the correlation coefficient is higher (0.9986) and the calculated q_e value (6.24) is close to the experimental q_{exp} value (7).

Correlation coefficients with Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich isotherm parameters are given in Table 2. When correlation coefficients were compared, it was seen that the Langmuir model was more suitable for adsorption studies than other isotherm models. The R_L value was calculated as 0.053 and the RL value indicates the suitability of coriander seed particles for methyl orange removal from aqueous solutions. Therefore, the adsorption process is explained more appropriately by the Langmuir isotherm model. Methyl orange adsorption is assumed to be a single layer and heterogeneous. Table 2 shows the E values of Dubinin-Radushkevich isotherm for uranium ions (0.35 kJ/mol), it is thought that methyl orange adsorption occurs by physical adsorption.

The positive value of ΔH° shows the endothermic nature of the adsorption, which is also consistent with the results obtained in Figure 6. The positive value of ΔS° indicates the increased randomness at the solid/interface during the adsorption of thorium ions. The negative values of ΔG° showed that methyl orange adsorption is applicable and is spontaneous. Also, the decreasing value of ΔG° with increasing temperature indicates that the adsorption of methyl orange onto coriander seed particles is more suitable at high temperatures.

5. Conclusion

The compatibility of the adsorption with kinetic models, isotherm models, and thermodynamic expressions was investigated with the data obtained because of the experiments for the adsorption of methyl orange from wastewater onto coriander seed particles.

Pseudo-first-order kinetic model, pseudo-second-order kinetic model, and intra-particle diffusion models were evaluated for methyl orange adsorption processes. The most suitable kinetic model was found to be the pseudo-second-order kinetic model. The isothermal models of Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich were examined in terms of their suitability for adsorption processes. Adsorption isotherms equilibrium data were found to be quite suitable for the Langmuir model in the concentration range studied. As a result of the analysis of thermodynamic parameters, it was determined that adsorption processes are spontaneous and endothermic. Also, the positive entropy indicates the level of interest of methyl orange to coriander seed particles.

According to the results obtained; shows that coriander seed grains are an inexpensive, effective, and easy adsorbent that can be used to remove methyl orange from wastewater.

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Changes in Vitamin C and E Levels of Van Fish Exposed to Fungicide Toxicity

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Abstract:

The widespread use of pesticides every day affects all living things negatively by disrupting the natural balance. Tebuconazole containing fungicides are mostly used for wheat and its derivatives. It is located near the agricultural areas where wheat is cultivated in Lake Van. The lake water is contaminated with pesticides as a result of the irrigation and rainwater of agricultural areas filtering and flowing into the lake. Therefore, in this study, it was aimed to investigate the effects of tebuconazole main substance fungicide, which is widely used worldwide, on Van fish. Antioxidants are very important defense systems for the immune system in metabolism. In this respect, antioxidant vitamins are of great importance. When intense oxidative stress occurs, the defense of antioxidant enzymes may be insufficient. In this case, antioxidant vitamins fight against oxidative stress. In the study, Van fish obtained from Lake Van were placed in water tanks and divided into concentration and control groups. Tebuconazole at concentrations (2.5 mg / L) was administered to each group at 24, 48, 72 and 96 hours. At the end of these periods, tissue sampling of the fish was done. Vitamins C and E were analyzed in the supernatants obtained by homogenization of fish tissues. In the study, the parameters were analyzed by reading the samples prepared using spectrophotometric methods. As a result of the study, it was determined that the levels of antioxidant vitamins C and E, which are an important defense mechanism of the immune system, decreased with the effect of tebuconazole.

Keywords: Fish, Fungicide toxicity, Vitamin C and E

1. Introduction

Being in the group of azoles, tebuconazole is used as a fungicide in paddy fields against fungal threat. Fungicides, which are generally used to prevent fungus in wheat and barley, pass to these plants and from there to the human body and spread to the heart muscle, liver, skin and red blood cells (Figure 1)

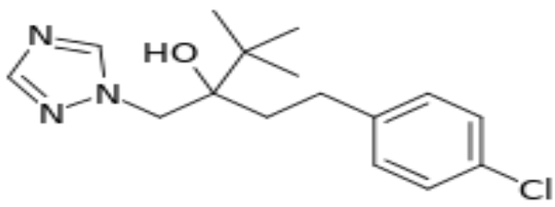

Figure1. Structural formula of Tebuconazole.

Consumption of foods with high fungicide residue amounts can cause serious health problems. Being systemically effective, tebuconazole also prevents the synthesis of ergosterol in the fungus and can cause toxic effects on organisms in the aquatic environment for a long time. Reactive oxygen species (ROT) originating from environmental pollutants can cause structural and functional changes in the cells of aquatic organisms and may also cause changes in biochemical

parameters.Studies have revealed that antioxidants neutralize free radicals and prevent cell damage. Damage by free radicals in tissues is thought to be the main cause of arteriosclerosis and heart disease (Parvez ve Raisuddin, 2005).

Fish today is considered as an important source of protein, with the study of nutritional components and knowing the effect of nutrients on our health. In terms of antioxidant compounds, besides land-based foods, seafood has an important place. Fish are also rich in the various antioxidant vitamins they contain. Antioxidant vitamins strengthen the immune system. They show these effects by preventing oxidation. Free radicals are also responsible for oxidation. Antioxidants are protective substances that neutralize free radicals. The main antioxidant vitamins are vitamins C and E. Vitamin E is an antioxidant vitamin that dissolves in fat and it accumulates in the body due to this feature. It ensures the maturation of cells and the development of male and female reproductive organs. Vitamin E deficiency leads to premature aging of cells, miscarriage and infertility. The antioxidant value of vitamin E in foods is higher than drug forms. Vitamin C is one of the most important antioxidant vitamins and has the ability to neutralize many different free radicals (oxidants). For this reason, it is effective in strengthening the immune system, protection from cancer and heart health. It is also involved in the absorption and activation of some important vitamins minerals (Floyd, 1990). The most important of these is iron and therefore it is very effective in eliminating anemia. Another feature of vitamin C that concerns us all is in the production of collagen, that is, it is important for the health of hair, nails, skin and gums. It takes part in the formation of bone-connective tissue and wound healing. The presence of vitamin C is very important for iron absorption (Lloyd,1992).

Van fish is an important fish species that is consumed by the local people in its fresh form found in Lake Van. Because of these features, it is of great economic importance. However, in studies conducted in recent years, it has been determined that Lake Van is contaminated by chemicals. This work; It was made to determine the toxic effects of tebuconazol, a pesticide group, which is widely used in the Van Lake basin, on antioxidant vitamins C and E of Van Fish.

2. Materials and Methods

2.1. The fish

80 Van fish caught from Lake Van were brought alive and put into water tanks. Fishes were divided into concentration group and their control group. Each group received tebuconazole at a concentration of 2.5 mg / L at 0, 24, 48, 72 and 96 hours. At the end of these periods, the heart tissues of the fish were taken by applying the anesthetic substance aminobenzonate methanesulfonate (MS222, 100mg / lt).

2.2. Vitamin analysis

The tissues taken are homogenized and their supernatants are taken for analysis. Spectrophotometric method of Martinek (1964) was used for Vitamin E analysis in supernatants. For Vitamin C analysis, Natelson's (1961) UV spectrophotometric method was used.

2.3. Statistics

The values obtained as a result of the analysis will be expressed as mean ± standard error. ANOVA and then Tukey's test will be performed for multiple comparisons of values emerging from different sampling areas, and the difference will be revealed. The difference between values will be made according to 0.05.

3. Results

It is observed that the vitamin E levels of the Fungicide-treated Van Fish group decreased as time progressed, both compared to the control group in their own hours and compared to the initial control group (Figure 2).

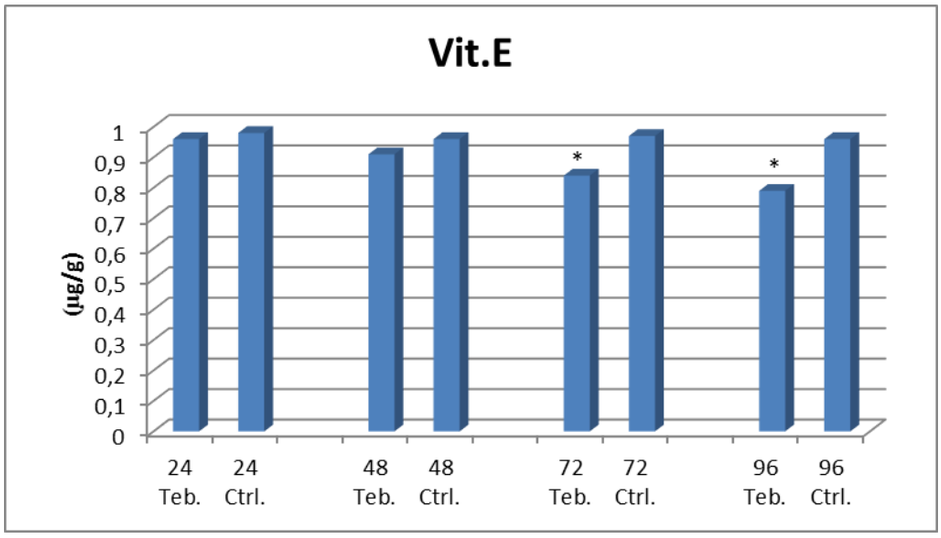

Figure 2. Change over time in vitamin E levels of Van fish treated with fungicide compared to control group.

It is observed that vitamin C levels of the Fungicide-treated Van Fish group decreased as time progressed, both compared to the control group and the initial control group (Figure 3).

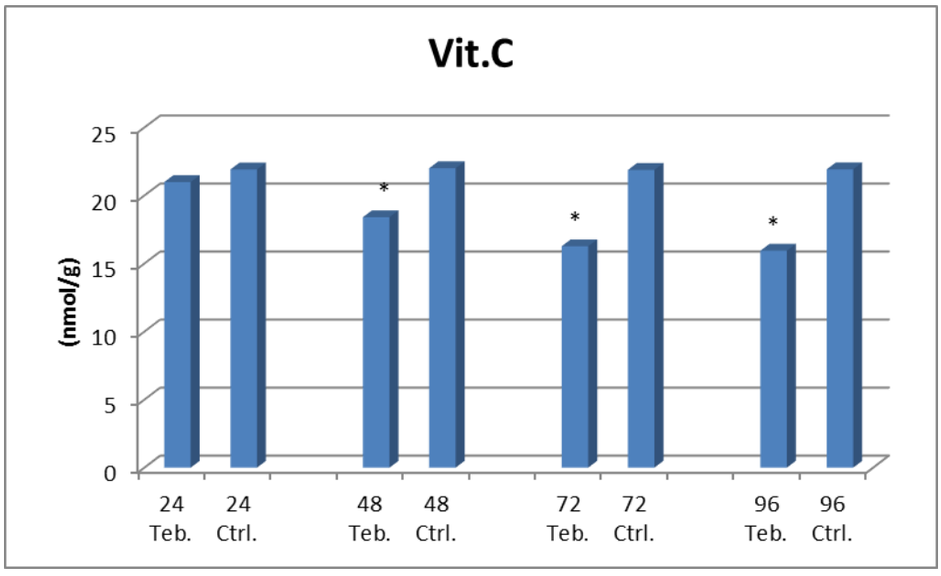

Figure 3. Change over time in vitamin C levels of Van fish treated with fungicide compared to control group.

4. Discussion

In this study, some antioxidant enzymes, vitamins and fatty acids of Van fish, which is an important food source due to its easy digestion and low fat content, which are increasingly important and necessary for adequate and balanced nutrition, were examined. In the present study, it is aimed to investigate how changes in vitamin E and C levels are affected in Van fish exposed to fungicidal toxicity. For this purpose, changes in heart tissue vitamin E and C levels were examined. A study was carried out on the effects of vitamins A and E on growth, feed utilization and some tissues of rainbow trout juveniles under oxygen stress. In this study, antioxidant vitamins A and E vitamins were added to the diets of rainbow trout juveniles for 12 weeks. The effects of vitamins A and E on 2 different dissolved oxygen levels (4.4 mg / l O_2 concentration and 7.6 mg / l O_2 concentration), some tissues (muscle, liver, kidney) and blood serum of fish fed with these rations were determined (Keleştemur, 2009.). It is seen that the results determined in this study are close to the vitamin A and E levels determined in the study. Köprücü and Özdemir (2002), in their study to determine the amounts of some vitamins (C, E) in the muscle tissue of rainbow trout, determined that the fish muscle tissue contains an average of 17.54 µg / g vitamin C, 8.73 µg / g vitamin E and 4.23% crude fat. They reported that the differences between vitamin C, E vitamins and crude fat levels in rainbow trout muscle tissue samples were insignificant ($p > 0.05$), and that Rainbow trout muscle tissues contained higher levels of vitamins C and E compared to many other commercial fishery products. Differences are observed between the levels determined in this study and the levels in the study. According to the differences obtained, it is thought that the environmental conditions in which the fish are located, the type and feeding style of the fish, the age of the fish or the working conditions in the laboratory may be caused. It has been reported that vitamin C levels in *Oreochromis niloticus* treated with Imidacloprid (IMID) toxicity decrease in the state of toxicity and with the increase of the process (Mohammed et al. 2020). These studies are in parallel with the study we present.

5. Conclusion

This study shows that tebuconazole has a toxic effect and causes oxidative stress in Van fish. In addition, as time progresses, it is observed that the effects of toxic substances increase and the levels of vitamin E and C, which are antioxidant functions, decrease. As a result, it is seen that the mixing of Tabuconazol, a wheat and barley fungicide widely used around Lake Van, into the lake with rain and irrigation water is harmful for the health of Van fish. When the study is evaluated both in terms of Van fish and ecologically, it can be said that the negative effects of pesticides on living things are very high.

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Investigation of *Anaplasma* Species with Veterinary and Public Health Significance in Sheep and Goats[#]

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Abstract:

This study was carried out to investigate *Anaplasma* species which are important for veterinary and public health in sheep and goats in Niğde province by using molecular methods. Blood samples were taken from randomly selected 690 animals (520 sheep and 170 goats), which were between 1 and 10 years old and from different study sites (Merkez, Bor, Çamardı, Ulukışla, Altunhisar ve Çiftlik) in Niğde province by using the vacutainer tubes containing EDTA. After the genomic DNA extractions from blood samples, the *Anaplasma* spp. 16S rRNA genes were amplified by PCR. Species-specific polymerase chain reaction (PCR) assays were performed on positive samples for the presence of *Anaplasma bovis*, *Anaplasma capra*, *Anaplasma ovis*, *Anaplasma platys*-like, and *Anaplasma phagocytophilum*. At the same time, the animals were checked for ixodid tick infestation and collected ticks were examined for identification under the stereo-microscope. The results of PCR analysis show that the overall *A. ovis* prevalence was 63.3% (437/690) in small ruminants. A total of 361 sheep (69.4%) and 76 goats (44.7%) were found to be infected with *A. ovis*, whereas no positivity was detected for *A. bovis*, *A. capra*, *A. platys*-like, and *A. phagocytophilum*. *Anaplasma ovis* positivity was observed at the highest percent in May (%74.6) while the lowest in June (%52.4). In total, 1361 ticks (579♀, 782♂) were collected from sheep and goats in Niğde. Ticks were identified as *Rhipicephalus bursa* (383, 28.1%), *R. turanicus* (607, 44.6%), *Hyalomma marginatum* (7, 0.5%), *Hy. excavatum* 247, 18.1%), *Hy. anatolicum* (23, 1.7%) *Haemophsalis parva* (21, 1.5%) *Hae. punctate* (7, 0.5%) *Hae. sulcate* (40, 2.9%) and *Dermacentor marginatus* (26, 1.9%). In conclusion, the present study demonstrates a high prevalence of *A. ovis* 63.3% (437/690) in sheep and goats in Niğde province.

Keywords: sheep, goat, *Anaplasma*, Niğde, tick

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1. Introduction

Anaplasmosis is caused by organisms of the genus *Anaplasma*, which are gram negative bacterium and transmitted to cattle, sheep, goats, wild ruminants, carnivores and humans by ticks (Aubry and Geale 2011; Atif 2016). *Anaplasma ovis* and *Anaplasma phagocytophilum* species have been reported to cause clinical and subclinical infections in sheep and goats (Atif 2016). *Anaplasma ovis* usually causes mild disease in ruminants. It has been observed that it causes clinical infections in sheep and goats, especially in the presence of various factors (co-infections or stressful situations). *Anaplasma ovis* infections have been reported to be endemic throughout the world, including Europe, China and the United States (Friedhoff, 1997). *Anaplasma phagocytophilum*, which causes the infection called tick-borne fever in ruminants, causes

important infections in ruminants, equines, canines, and humans. Tick-borne fever can cause direct (lamb death) and indirect loss (growth reduction) in sheep, moreover, up to 30% lamb deaths have been reported due to *A. phagocytophilum* (Stuen 2013). In 2015, a new species named *A. capra* was discovered in goats and ticks in China. In addition, this species has been reported to cause infection in humans (Li et al. 2015). *Anaplasma bovis* causing monocytic anaplasmosis in cattle has been reported in sheep and goats (Ben Said et al. 2015). Similarly, *A. platys* causing cyclic thrombocytopenia in dogs, it has been reported in sheep and goats as *A.platys*-like (Ben Said et al., 2017; Liu et al., 2012). The importance of *A. bovis* and *A. platys*-like in sheep and goats is unknown.

This study aimed to investigate *Anaplasma* species which have veterinary and public health importance in sheep and goats in Niğde.

2. Materials and Methods

Six-hundred-ninety blood samples collected from apparently healthy sheep (n=520) and goats (n=170) belonging to 28 flocks of Niğde province, in Central Anatolia of Turkey (with an altitude of 1240 m, 37° 58’ N longitude-34°41’ E latitude) between April-July 2014. Annual average of precipitation is 348.8 mm, average temperature is 11.1°C and average relative humidity is 55% in Niğde.

Genomic DNA was extracted from the thawed blood with a GF-1 Blood DNA Extraction Kit (Vivantis Technologies Sdn. Bhd. Revongen Corporation Center, Malaysia) according to manufacturer’s instructions. The DNA concentration (ng/µL) and purity (A260nm/A280nm) of each sample was determined using a nanodrop spectrophotometer.

To determine the presence and frequency of *A. bovis* (16S rRNA) (Kawahara et al. 2006), *A. capra* (16S rRNA and gltA) (Li et al. 2015; Yang et al. 2016), *A. ovis* (groEL) (Haigh et al. 2008), *A. platys*-like (groEL) (Alberti and Sparagano 2006) and *A. phagocytophilum* (msp4) (de la Fuente et al. 2005a) in small ruminants, species specific PCRs were set up using different primer sets. The PCR reactions were performed in PCR Sprint (Sensoquest, Germany) as previously described (Ozubek and Aktas 2017).

3. Results

Of the 690 samples examined by PCR, 437 (%63.3; CI 59.1-66.9) were infected with *Anaplasma* spp. As a result of the species specific PCR, only *A. ovis* positivity was detected sheep and goat, %69.4 (CI 65.3-73.3) and %44.7 (CI 37.1-52.5) respectively. No positivity was detected in type-specific PCRs of different gene regions specific to *A. phagocytophylium* (Msp4), *A. bovis* (16S rRNA), *A. capra* (16S rRNA and gltA) and *A. platys*-like (groEL).

Table 1. Distribution of the species-specific PCR results by months.

Months	Host	Number of samples	*Anaplasma* spp.	*A. ovis*	*A. phagocytophylium*	*A. bovis*	*A. capra*	*A.platys*-like
April	Sheep	130	99 (%76,1; 67.9-83.2)	99; (%76,1; 67.9-83.2)	-	-	-	-
	Goat	40	16 (%40; 24.9-56.7)	16 (%40; 24.9-56.7)	-	-	-	-
	∑	170	115 (%67,6; 60-74.6)	115 (%67,6; 60-74.6)	-	-	-	-
May	Sheep	140	118 (%84,3; 77.2-89.9)	118 (%84,3; 77.2-89.9)	-	-	-	-
	Goat	45	20 (%44,4; 29.6-60)	20 (%44,4; 29.6-60)	-	-	-	-
	∑	185	138 (74,6; 67.7-80.7)	138 (74,6; 67.7-80.7)	-	-	-	-
June	Sheep	140	79 (%56,4; 47.8-64.8)	79 (%56,4; 47.8-64.8)	-	-	-	-
	Goat	45	18 (%40; 25.7-55.7)	18 (%40; 25.7-55.7)	-	-	-	-
	∑	185	97 (%52,4; 45-59.8)	97 (%52,4; 45-59.8)	-	-	-	-
July	Sheep	110	65 (%59,1; 49.3-68.4)	65 (%59,1; 49.3-68.4)	-	-	-	-
	Goat	40	22 (%55; 38.5-70.7)	22 (%55; 38.5-70.7)	-	-	-	-
	∑	150	87 (%58; 49.7-66)	87 (%58; 49.7-66)	-	-	-	-
Total	Sheep	520	361 (%69,4; 65.3-73.3)	361 (%69,4; 65.3-73.3)	-	-	-	-
	Goat	170	76 (%44,7; 37.1-52.5)	76 (44,7; 37.1-52.5)	-	-	-	-
	∑	690	437 (%63,3; 59.1-66.9)	437 (%63,3; 59.1-66.9)	-	-	-	-

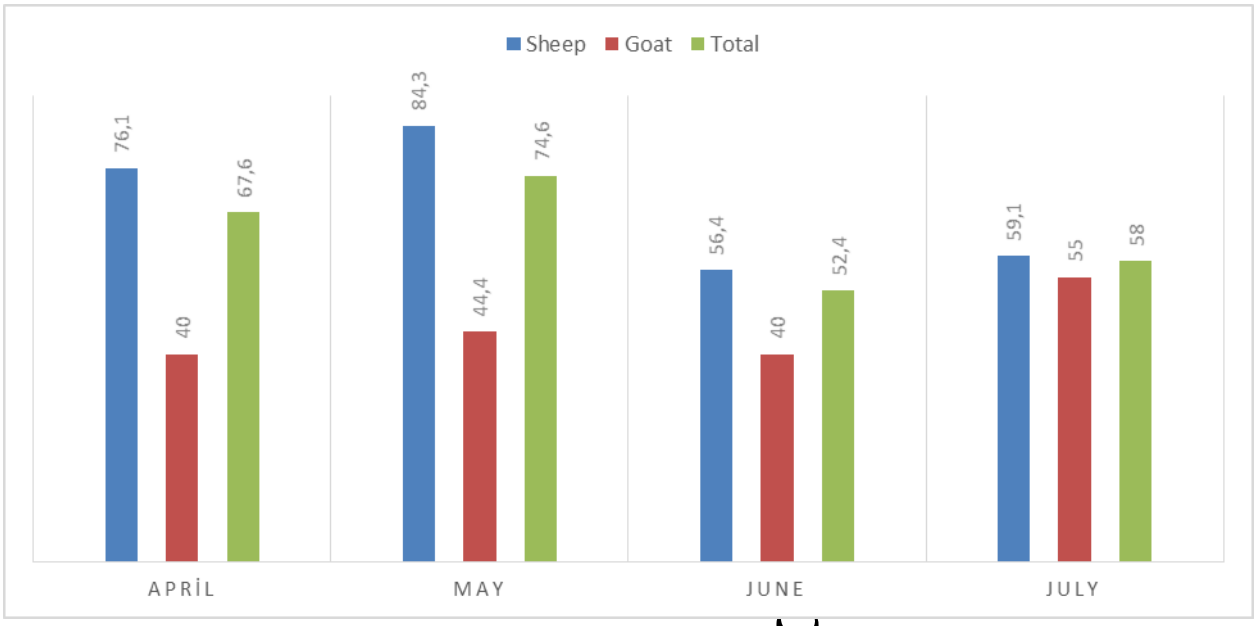

Figure 1. Distribution of *A. ovis* positivity according to the months in which the samples were collected.

According to Table 1 and Figure 1, *A. ovis* positivity was observed to be the highest in May (74.6%) and the lowest in June (52.4%). It was observed that there was the highest positivity for sheep in May (84.3%) and for goats in July (55%). The difference between the months was not statistically significant ($p > 0.05$).

Table 2. Distribution of ixodid tick species collected from sheep and goats by host and collection date.

	R. bursa		*R. turanicus*		*Hy. marginatum*		*Hy. excavatum*		*Hy. anatolicum*		*Hae. parva*		*Hae. punctata*		*Hae. sulcata*		*D. marginatus*	
	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat
April	-	3	26	7	6	-	6	-	-	-	14	-	7	-	24	-	25	-
May	48	13	383	82	-	-	176	1	21	-	-	-	-	-	16	-	1	-
June	87	43	69	29	1	-	19	-	-	-	4	-	-	-	-	-	-	-
July	118	71	9	2	-	-	45	-	2	-	-	-	-	-	-	-	-	-
Total	253	130	487	120	7	-	246	1	23	-	21	-	7	-	40	-	26	-
∑; %	383; 28,1		607; 44,6		7; 0,5		247; 18,1		23; 1,7		21; 1,5		7; 0,5		40; 2,9		26; 1,9	

According to Table 2, it was found that sheep and goats in the region were infested with 9 different tick species while blood samples were collected. 1361 ticks were collected from sheep and goats with tick infestation, 383 (28.1%) of them were *R. bursa*, 607 (44.6%) were *R. turanicus*, 7 (0.5%) were *Hy . marginatum*, 247 (18.1%) of *Hy. excavatum*, 23 (1.7%) of *Hy. anatolicum*, 21 (1.5%) *Hae. parva*, 7 (0.5%) *Hae. punctata*, 40 (2.9%) *Hae. sulcata* and 26 (1.9%) *D. marginatus* were identified.

4. Discussion

Ovine animals constitute the main source of animal husbandry with a total number of over 55 million animals in Turkey (TUIK). Anaplasmosis is a tick-borne disease that has a significant impact on animal husbandry due to the economic burden of disease-related morbidity and mortality caused by various *Anaplasma* species such as *A. marginale*, *A. bovis*, *A. ovis*, and *A. phagocytophilum* (Aubry and Geale 2011; Atif 2016).

In this project, 5 different *Anaplasma* species, including *A. bovis*, *A. capra*, *A. ovis*, *A. platys*-like and *A. phagocytophilum*, which can cause serious infections in sheep and goats, have been examined by species-specific PCR. As a result of the species-specific PCR, only *A. ovis* positivity was detected in 361 of 520 sheep, 76 of 170 goats, and 437 of a total of 690 animals. *A. bovis*, *A. capra*, *A. platys*-like and *A. phagocytophilum* were not detected. *Anaplasma ovis* (31.4-67%) and *A. phagocytophilum* (2.4-66.7%) species have been reported in sheep and goats in Turkey (Renneker et al. 2013; Altay et al. 2014; Öter et al. 2016; Bilgiç et al. 2017). *Anaplasma ovis* has been reported from Iraq, Sudan, Portugal, (Renneker et al., 2013), Tunisia (Belkahia et al., 2014), Hungary (Hornok et al., 2007), Italy (de la Fuente et al., 2005b), China (Han et al., 2017) and Slovakia (Derdáková et al., 2011), 66.6%, 41.6%, 84.2%, 70.1%, 72.7%, 87%, 14.3% and 22.6%, respectively.

Anaplasma bovis, *A. capra*, *A. platys*-like and *A. phagocytophilum* were not detected in this study. *Anaplasma bovis*, which is the agent of monocytic Anaplasmosis of cattle, it was detected in 42.7% and 23.8%, in sheep and goats respectively in a study conducted in Tunisia (Ben Said et al., 2015). *Anaplasma phagocytophilum*, also known as tick-borne fever, causes economic losses in sheep and goats by affecting them directly and indirectly. Mortality rates of up to 30% have been reported in lamb herds (Grova et al., 2011). *Ixodes ricinus* ticks are the main vector for *A. phagocytophilum.* Since *I. ricinus* generally prefers humid climates, they cannot be easily adapted to arid regions (Cerny et al. 2020). In this study conducted in Niğde region, which has an arid climate, *I. ricinus* and *A. phagocytophilum* were not found in sheep and goats. *Anaplasma phagocytophilum* has been reported between 8.5% and 66.7% in sheep and goats respectively in Turkey (Öter et al., 2016; Benedicto et al., 2020). *Anaplasma* platys-like has been reported in sheep, goats and camels in Tunisia at 11%, 22.8% and 17.7%, respectively (Belkahia et al. 2015). The pathogenicity of this species in sheep and goats is currently unknown.

5. Conclusion

We reported that *A. ovis* and couple of tick species are frequent in sheep and goats, Niğde province. Further studies are needed to determine the tick vectors (*R. bursa*, *R. turanicus*) and geno-types of *A. ovis*.

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Biogenic Iron Nanoparticles: Synthesis, Characterization and Antibacterial Activity

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Abstract:

Nanotechnology; It has emerged as one of the key disciplines with the support of advanced research and technological advances in various scientific fields such as physics, chemistry, biology, environment, materials science, medicine and pharmacy. Nanoparticles (NPs), which form the basis of nanotechnology, have remarkable properties due to their specific dimensions, shapes, compositions, larger surface area/volume ratios and purity of individual components. NPs produced from biological materials are known as biogenic NPs and the related synthesis process is called green synthesis. Plant mediated biosynthesis of metallic NPs takes place through biomolecules found in plant biomass and containing organic functional groups. Biological synthesis, also known as green synthesis; It is the preferred practical method for obtaining NPs easily and ecologically without the need for high pressure, high temperatures values and toxic chemicals. Green synthesis of NPs is performed using different biomaterials such as bacteria, fungi, yeast, viruses, microalgae and plant biomass extract. In this study, iron nanoparticle biosynthesis by *Malva vulgaris* plants was investigated. Characterization was performed by UV-VIS absorption spectroscopy, FT-IR, XRD and SEM analysis. The antibacterial effect of FeNPs obtained from *Malva vulgaris* demonstrated antibacterial effect against all tested bacteria strains. The results obtained showed that *Malva vulgaris* plant extract can be used as a good bioreducing agent for the synthesis of iron nanoparticles.

Keywords: Nanoparticle, Green Synthesis, *Malva vulgaris*, Antibacterial Activities

1. Introduction

Nanotechnology has become an area of interest to synthesize nanoparticles of various sizes, shapes and chemical compositions and their possible bio-medical applications for human benefit (Husseiny et al. 2007, Kalita et al. 2012, Ajitha et al. 2016). This technology offers the ability to produce materials with desired properties mainly by controlling their size and shape. (Nowack et al. 2007). They have a size less than 100 nm. Different metallic nano materials can be produced using titanium, zinc, magnesium, silver, iron and gold. Nanoparticles have a wide range of uses, from a variety of daily use materials such as cosmetics or clothing, to various industrial production branches such as solar and oxide fuel cells for energy storage. (Hasan et al. 2015). Green nano-biotechnology means synthesizing nano-sized materials using biological methods that include products such as microorganisms and plants with the help of various biotechnological tools. In chemical and physical methods, high radiation, high concentration

stabilizers and reducers are used, which are harmful to the environment and human health. Since less energy is used in biological synthesis, it does not harm the environment. This is why green synthesis is chosen according to chemical and physical methods because it is cost-effective, environmentally friendly and easily measurable for large scale nanoparticle synthesis. In addition, there is no need to use high temperatures, pressure, energy and toxic chemicals. (Parveen et al. 2016, Kalishwaralal et al. 2010).

Iron oxide nanoparticles show superparamagnetic properties below 20 nm. Superparamagnetic nanoparticles can be functionalized with a variety of agents to improve their performance in solution, including stability and recovery. Among their most important features, they can be easily collected using an external magnet and can be easily released into solution. (Bhateria et al. 2019).

In this study, synthesis of FeNPs from iron oxide solution was investigated using the peeling extract of Malva vulgaris as a natural reducing agent. The aim of this study is to examine the antibacterial activity of FeNPs using antibacterial peeling extract against opportunistic pathogenic microflora.

2. Materials and Methods

Preparation of plant extract

Leaves of the *Malva vulgaris* plant were used to prepare the extract. The leaves of the plant were collected and after a series of washing with tap water, finally rinsed with distilled water and dried in room temperature. The leaves in the dried form were ground to size and 10 g of the plant was mixed in 100 ml of distilled water at room temperature for 1 day. It was then made ready for synthesis after filtration with Whatman No.1 filter paper. Finally, the filtered extract was preserved at 4 °C for further studies in order to store the sample from environmental conditions.

Synthesis of copper nanoparticles

An aqueous 0.001 M iron acetate nitrate was prepared with 50 ml of distilled water at room temperature. Later, 20 ml of plant extract was added to the above solution at room temperature while stirring magnetically at 600 rpm for one day. The blue color solution changes to pale bluish green within 15 min due to the fast bio-reduction of iron ions, confirming the formation of FeNPs and there was no colour change further. The obtained FeNPs were further purified by centrifugation at 8,000 rpm for 10 min with substantial redispersion of the pellet in distilled water. The schematic synthesis procedure of bio-FeNPs using *Malva vulgaris* extract is depicted in figure 1. Finally, the synthesized FeNPs were stored in a clean amber bottle for further analysis.

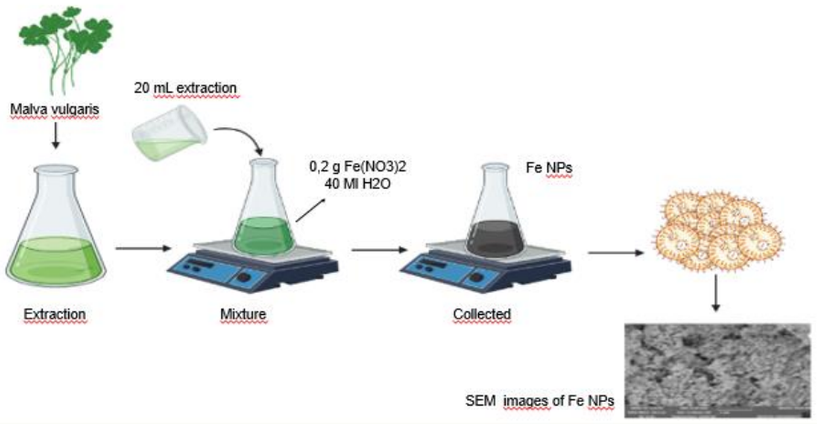


Figure 1. Schematic synthesis procedure of bio-FeNPs using *Malva vulgaris* extract.

Characterization of the synthesized FeNPs

Product samples were subjected to UV–Visible spectroscopic (THERMO Model Multiscaner spectrophotometer) of FeNPs. The interactions of plant extract and FeNPs were analyzed with FTIR spectroscopy. For FTIR measurements, sample ground was analyzed using an FTIR spectrophotometer (Shimadzu Iraffinity-1, Japan) in the range of 400–4000 cm−1. Particle morphology and size was measured by SEM (TESCAN, MAIA3 XM). The crystal structure and particle size of FeNPs was calculated using XRD analysis. XRD analysis was performed using a powder X-ray diffractometer (Rigaku, Smartlab).

Antimicrobial Activity

For investigation of antibacterial activities of the nanoparticle, Broth Micro-dilution Assay was used (Brandt et al., 2010). Briefly, Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacterial cultures were inoculated into Nutrient Broth for 24 h at 37ºC. Then, new cultures were prepared until 0.5 McFarland Unit at 37ºC. In total 200 µL of microtiter plate wells, 10 µL of bacterial cultures were inoculated with NB containing different concentrations of the nanoparticle suspended in ethanol (0-200 µg/mL). Negative controls were prepared using Nutrient Broth without bacteria. Positive controls did not contain any nanoparticle but respective amount of ethanol. Absorbances of microtiter plates were read at 600 nm using a micro-plate reader before (0^{th} h) and after (24^{th} h) the incubation at 37ºC. Bacterial viability was measured as percentage of compound-treated bacterial groups to the positive control (bacterial viability of positive control was taken as 100%). Minimum inhibitory concentrations (MIC) were calculated using the plot of nanoparticle concentration versus relative bacterial growth. One-way ANOVA with Tukey's test was used to analyze the data and $p<0.05$ was considered as statistically different.

3. Results and Discussion

The characterization of the synthesized FeNPs was carried out by UV–Vis spectroscopy. The UV–Vis spectra of FeNPs showed two distinctive peaks at 330 nm and 365 nm (Fig. 2). As per the previously reported literature, these two bands might have been primarily due to the absorption and scattering of radiation by the magnetic nanoparticles. The absorption band at 365 nm indicated the formation of nano-sized FeNPs.

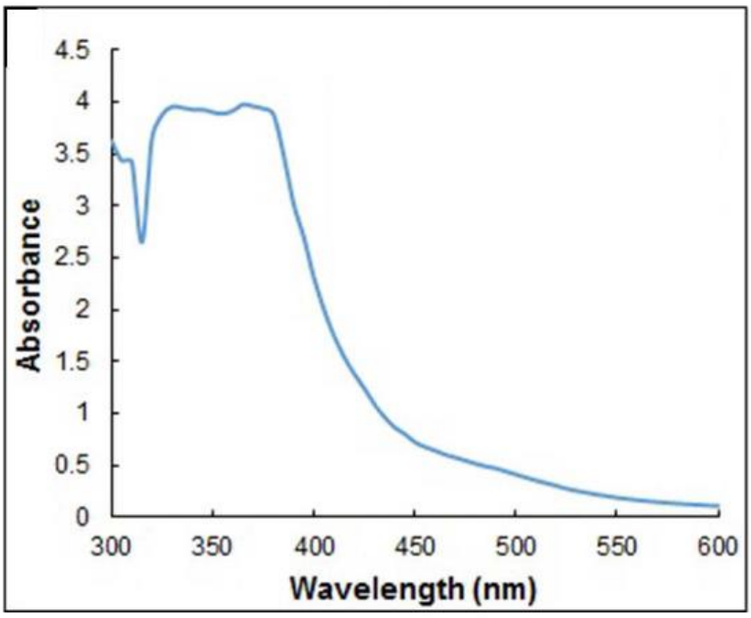


Figure 2. UV-Vis absorbance spectrum of FeNPs.

The phenolic O–H stretch appeared at 3302.38 – 3264.84 cm^{-1}.The aromatic C=C stretching frequency which appeared at 1539.81 cm^{-1}. The C=O stretching frequency appeared at 1622.64 cm^{-1}. Thus was characterized by IR spectroscopy. The band at 612.63 cm^{-1} was due to Fe as shown in Figure 3.

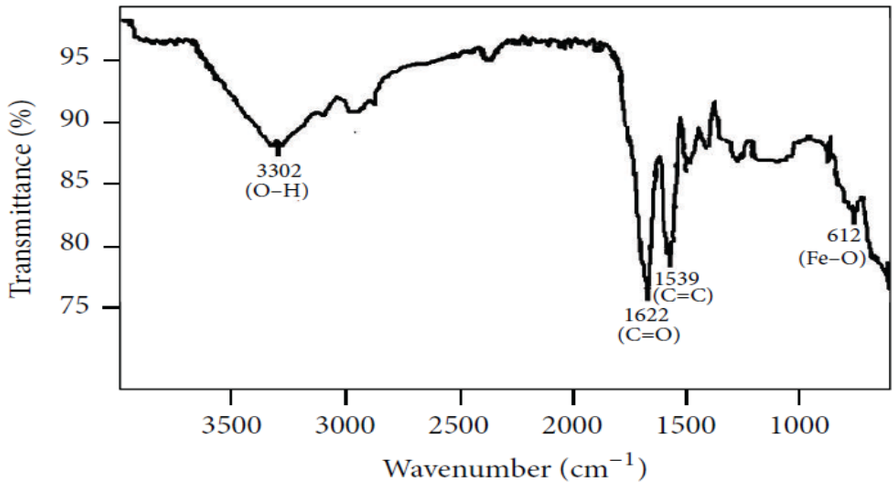


Figure 3:FTIR absorbance spectrum of FeNPs.

In order to study the morphology and size of the bio-synthesized FeNPs, SEM images were recorded at different magnifications (Fig. 4). The formation of FeNPs as well as their morphological dimensions through the SEM study demonstrated that the average size was around 15-20 nm with the shape of spherical nature. The SEM image further confirms the production of a high density of FeNPs synthesized through the *Malva vulgaris* extract.

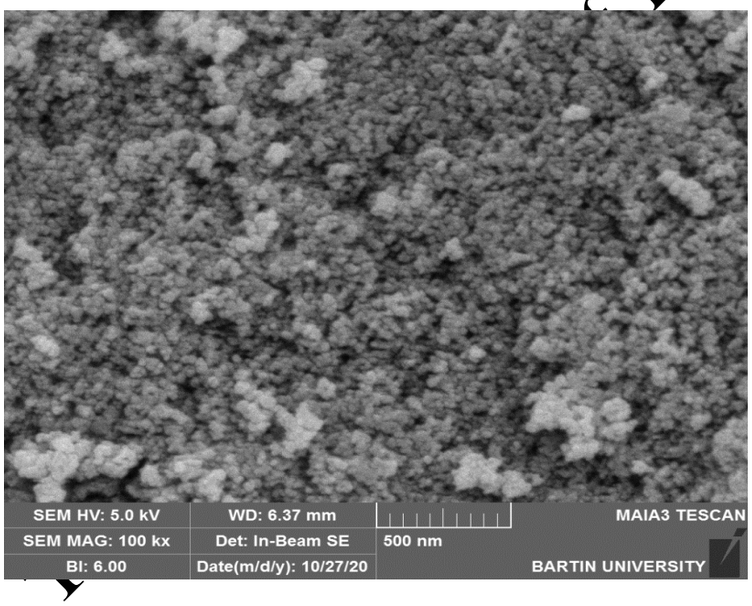
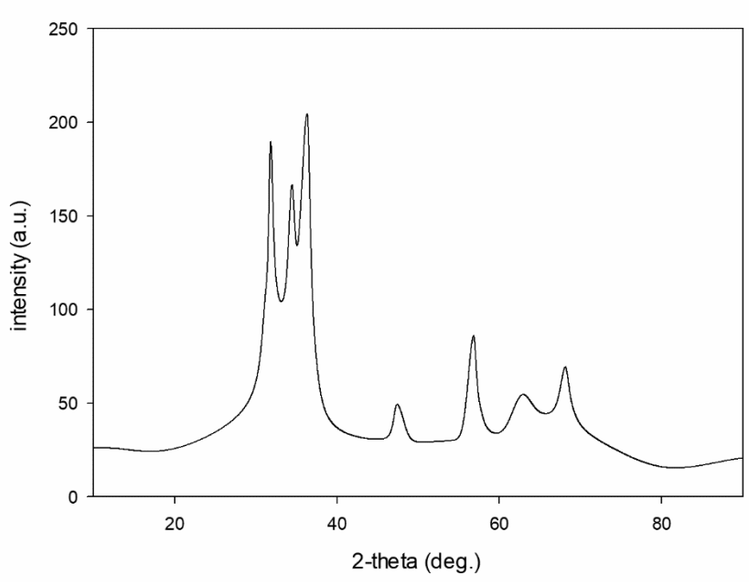


Figure 4: SEM images of bio-synthesized FeNPs at different magnifications.

The x-ray diffraction (XRD) profile of biosynthesized FeNPs is depicted in figure 5. From the XRD profile, distinct diffraction peaks at 2θ = 31.72, 34.34, 36.19, 56.57 and 68.07 are perceived, respectively which are characteristic of face centered cubic (fcc) structure of metallic Fe_3O_4 (JCPDS No. 85-1436).



Figüre 5. X-ray diffraction profile of bio-synthesized FeNPs using *Malva Vulgaris* extract.

In the present study, we examined the anti-bacterial effects of the nanoparticle. The results showed that this nanoparticle showed anti-bacterial effects against very common bacterium *E. coli* (Figure 6A). Around 30% to 50% inhibition was observed in the bacterial growth of *E. coli* at the final concentrations ($p<0.05$).

On the other hand, it also showed an inhibition against *S. aureus* (Figure 6B). Around 13% to 36% inhibition was observed in the bacterial growth of *S. aureus* at the final concentrations.

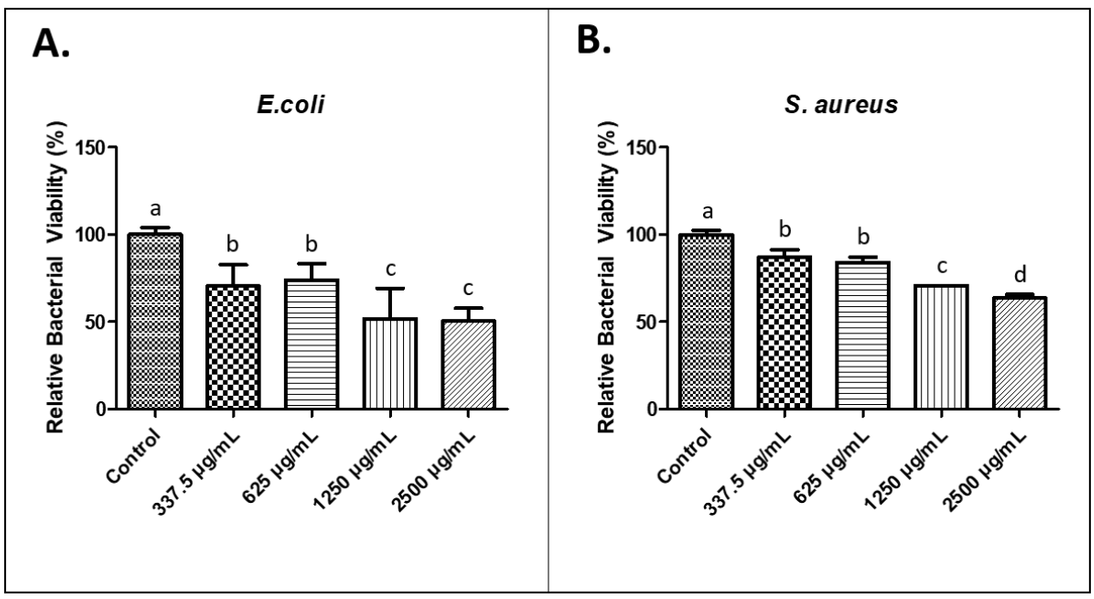


Figure 6. Antibacterial effects of FeNPs on *E. coli* (A.) and *S. aureus* (B.). Different lower cases indicate the means are statistically different ($p<0.05$) according to one-way ANOVA and Tukey's test.

4. Conclusion

The structural, morphological and optical properties of the nanoparticles synthesized in the research were characterized by using SEM, UV-Vis, FTIR and XRD. Dimension measurements and formal characterization of FeNP were examined using SEM and the results obtained showed that FeNP smaller than 50 nm with spherical morphology were formed. In this study, the formation of FeNP synthesized from *Malva vulgaris* extract was analyzed by UV-Vis spectroscopy and it was observed that the highest peak value was 330-365 nm. In this study, in vitro susceptibilities of FeNPs synthesized by green synthesis method against gram-positive and gram-negative bacteria were determined by broth dilution method. The FeNPs obtained showed approximately 50% inhibition against *E. coli* bacteria, while it showed 36% inhibition against S. aureus bacteria. These results show that FeNPs can be an alternative to existing antibiotics / antimicrobial products to overcome the antibiotic resistance crisis, which has been a serious problem in recent years.

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Preparation and Antibacterial Effect of Novel Amino Acid Methyl Ester Schiff Base

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Abstract:

Amino acid-derived Schiff bases have attracted more attention because of incorporating amino acid component to the structure, taking important parts in chemical processes in living organisms. There are so many studies about the synthesis and the biological activities of amino acid Schiff bases and metal complexes. The purpose of this work was to synthesize novel Schiff base, from amino acid methyl ester and salicylaldehyde derivatives. Structures of the obtained Schiff base compounds were elucidated by FT-IR and UV-Vis spectrometry, elemental analysis, ^{1}H-NMR and ^{13}C-NMR techniques. Besides, antibacterial activities of compound were investigated. Schiff bases were synthesized by condensation reactions of salicylaldehyde derivative and amino acid methyl ester in alkaline chloroform media. All analysis results were in accordance with suggested Schiff base structure. Antibacterial activities of these synthesized compounds were investigated by *E.coli* and *S.aureus* bacteria. As a result, these compounds synthesized have a great potential for biological activity.

Keywords: amino acid schiff bases, antibacterial effect, NMR spectra

1. Introduction

Schiff bases are important intermediates in enzymatic reactions involving the interaction of enzymes with substrates having carbonyl or amino groups. In bioinorganic chemistry, the interest in Schiff bases stems from the purpose of obtaining synthetic models of metal-containing centers in metalloproteins and contributing to the development of medical chemistry. Thus, Schiff bases and complexes have various applications in the biological, clinical and analytical fields (Ellis et al., 1997; Eglof et al., 2009). In recent years, there has been an increasing interest in the chemistry of the amino acid Schiff bases (Sari et al., 2013). Amino acids are an important class of compounds with -COOH and -NH_2 potential donor groups capable of coordinating metal ions (Frauscher et al., 1995). Since amino acids contain primary amine group, they react with aldehydes Schiff base. Since the –NH_2 group in amino acids is activated by the inductive effect of the -COOH group, they react with aldehydes more easily than other aliphatic primary amines. A large number of amino acids Schiff base are being studied for its important biological properties. These schiff bases are antibacterial, non-enzymatic valuable models, O_2^-. removal effect, as

corrosion inhibitor, as tumor radio-imaging agents, as active compounds in drug making,… etc. exhibit activities (Frauscher et al., 1995; Xiao et al., 2009; Sari et al., 2013). For this reason, a large number of amino acid Schiffs have been reported in some literature (Costa Pessoa et al., 1999; Costa Pessoa et al., 2000; Maurya et al., 2003; Ando et al., 2004; Yue et al., 2006). Within the scope of this study, firstly, new Schiff bases were obtained from the condensation reaction of amino acids and aldehydes and were characterized. In the last stage of the study, the antibacterial activity of the synthesized new schiff base was examined.

2. Materials and Methods

Synthesis of the amino acid Schiff base

The first of the schiff bases synthesized within the scope of the study, phenylalanine methyl ester hydrochloride (1.0 g, 5.51 mmol), 5-bromo-2-hydroxybenzaldehyde (0.74 g, 3.68 mmol) and triethylamine (0.37 g, 3.68 mmol) in $CHCl_3$ for 24 hours at boiling temperature. stirred under cooler. After the mixing process was terminated, the crude product obtained was extracted several times with $CHCl_3$ and water, dried with $MgSO_4$ and the solvent was removed by evaporator. The Schiff base was obtained as a yellow solid. These synthesized schiff bases have been characterized in our previous work (Taş et al., 2018).

Antibacterial Effect

Broth Micro-dilution Assay was used for investigation of antibacterial activities of the Schiff base (Brandt et al., 2010), with some modifications. Briefly, Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacterial cultures were inoculated into LB Broth from frozen stocks for 24 h at 37ºC. Then, new cultures were prepared until 0.5 McFarland Unit at 37ºC. In total 200 µL of microtiter plate wells, 10 µL of bacterial cultures were inoculated with NB containing different concentrations of the compound dissolved in ethanol (0-200 µg/mL). Negative controls were prepared using LB without bacteria. Positive controls did not contain any compounds, but respective amount of ethanol. Absorbances of microtiter plates were read at 600 nm using a micro-plate reader before (0^{th} h) and after (24^{th} h) the incubation at 37ºC. Bacterial viability was measured as percentage of compound-treated bacterial groups to the positive control (bacterial viability of positive control was taken as 100%). Minimum inhibitory concentrations (MIC) were calculated using the plot of nanoparticle concentration versus relative bacterial growth.

3. Results and Discussion

Antimicrobial Activity

In the present study, we examined the anti-bacterial effects of Schiff base. The results showed that this compound showed anti-bacterial effects against very common bacterium *E. coli* (Figure 1). Around 25% inhibition was observed in the bacterial growth of *E. coli* at the final concentration of 100 µg/mL, while almost 70% in the concentration of 200 µg/mL ($p<0.05$). Furthermore, MIC for *E. coli* was calculated as 290 µg/mL.

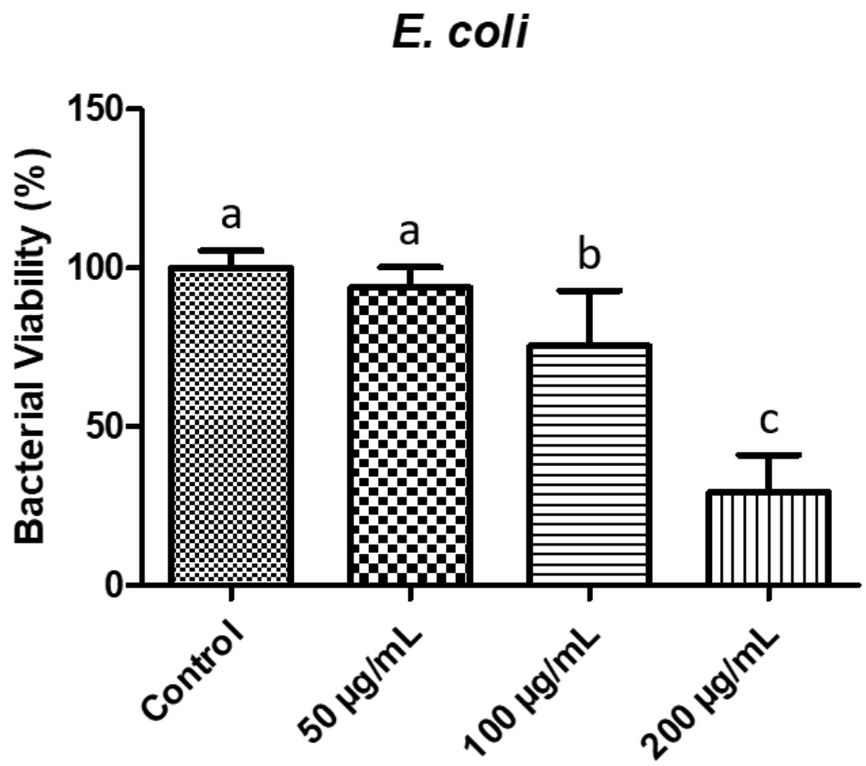

Figure 1. Antibacterial effects of the Schiff base on *E. coli*. Different lower letters indicate there is significant change according to one-way ANOVA and Tukey's test ($p<0.05$).

On the other hand, it also showed an inhibition against *S. aureus* (Figure 2). Around 60% inhibition was observed in the bacterial growth of *E. coli* at the final concentration of 200 µg/mL ($p<0.05$). MIC for this bacterium was calculated as 340 µg/mL.

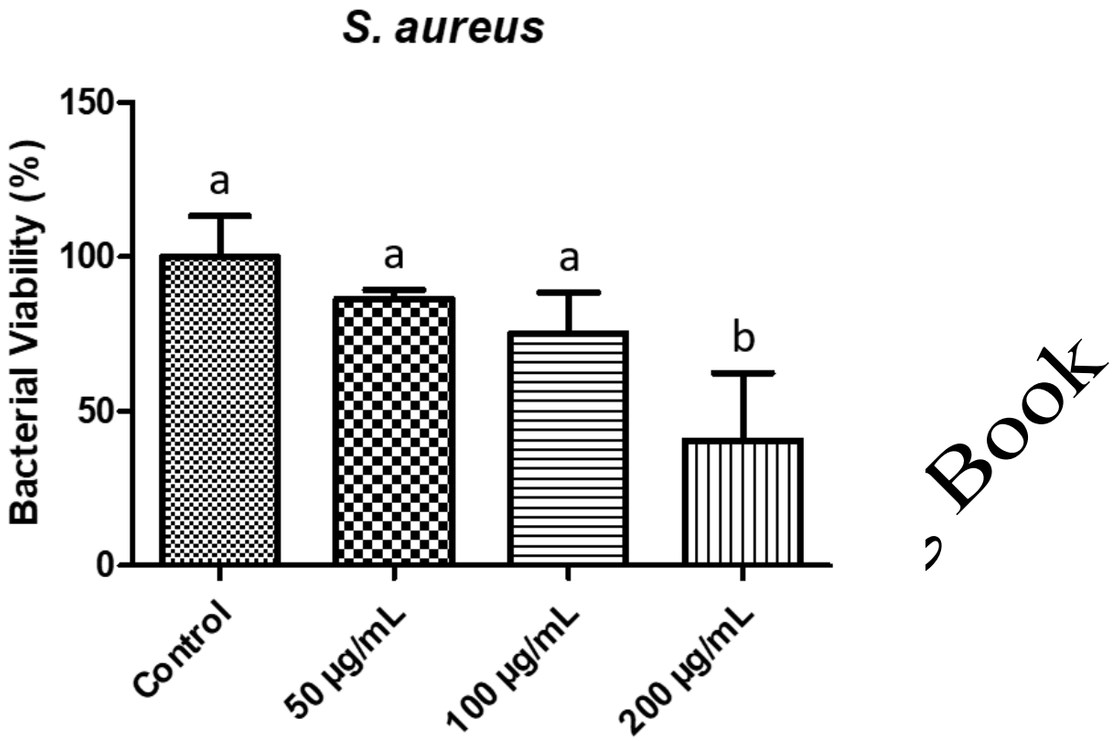

Figure 2. Antibacterial effects of the Schiff base on *S. aureus*. Different lower letters indicate there is significant change according to one-way ANOVA and Tukey's test ($p<0.05$).

4. Conclusion

The amino acid Schiff base ligand were successfully synthesized. In the present study, we examined the anti-bacterial effects of Schiff base. While the obtained Schiff showed about 70% inhibition against some *E. coli* bacteria, it showed 60% inhibition against *S. aureus* bacteria. The antibacterial effect value of the new compound is promising.

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Investigation of the cytotoxic effects of GLP-1R agonist on 3T3-L1 adipocytes

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Abstract:

The frequency of obesity, which is a multifactorial disease, poses a serious risk to public health. The world health organization has declared obesity a more serious global chronic health problem than malnutrition. Obesity is a risk factor for some of the preventable deaths in type 2 diabetes, cardiovascular disease, and many types of cancer. Therefore, there is a need for a successful obesity treatment. Pharmacological strategies developed to treat obesity are evaluated by focusing on drugs in phase 2 and 3 clinical development based on the physiological regulation of energy homeostasis. Incretin-based treatments are currently being developed to control weight. While incretins increase glucose-dependent insulin secretion in the pancreas, they suppress glucagon secretion. The aim of this study is to investigate the cytotoxic effect of Glucagon-like peptide- 1 receptor agonist on 3T3- L1 adipocytes *in vitro*. Commercially available 3T3- L1 fibroblast cells (ATCC® CL-173) were transformed into adipocyte cells. 3T3- L1 adipocyte transformation was detected by the oil red o staining method. Cytotoxic activity of glucagon-like peptide-1 receptor agonist on 3T3- L1 adipocytes was determined by 3- (4,5-dimethyldiazol-2-yl) -2,5 diphenyl Tetrazolium Bromide method. Different doses of glucagon-like peptide-1 receptor agonist were applied to 3T3- L1. adipocytes for 48 hours and IC50 value was calculated with GraphPad Prism 5.0 program. The IC50 value was 688 nM after the application of glucagon-like peptide-1 receptor agonist to 3T3- L1 adipocytes for 48 hours. Cytotoxic effect of glucagon-like peptide- 1 receptor agonist on adipocytes provides a promising approach in the treatment of obese patients.

Keywords: Obesity, 3T3-L1 adipocyte, Glucagon-like peptide-1 receptor agonists (GLP-1RA)

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1. Introduction

The continuous increase in obesity prevalence worldwide turns obesity into an international public health problem. Nearly 700 million adults diagnosed with obesity worldwide there are patients (Martinussen et al. 2017). Epidemiological studies show obesity as the key to chronic diseases. It demonstrates that ongoing obesity and its associated chronic and unresolved inflammatory state cause imbalance of adaptive homeostatic mechanisms, leading to adipose tissue dysfunction and the development of associated comorbidities (chronic metabolic diseases, including type 2 diabetes, cardiovascular diseases and various cancers). (Reilly and Saltiel 2017). Obesity is often characterized by an increase in adipocyte count or adipocyte volume. In adults, the adipocyte count is usually unchanged, so adult obesity is generally considered an increase in

adipocyte size, i.e. adipogenesis (Langhans 2018). Prevention of adipogenesis formation is important for the development and prevention of adult obesity. Obesity is associated with various risk factors such as genetic characteristics, behavior, energy consumption, psychology, environment, and socioeconomic status (Ford ES and Mannino 2005). Energy intake and expenditure is regulated by complex systems that include afferent signals and efferent effectors. Central circuits in the brain combine environmental signals that indicate nutrient intake and energy stores as well as higher cortical factors such as emotional and reward pathways (Simpson et al. 2008; Murphy and Bloom 2006). The complexity of the nerve circuits that control food intake and energy balance in the hypothalamic nuclei explains some of the limitations involved in the prevention and treatment of obesity. In addition to integrating signals from neurotransmitters and hormones, the hypothalamic systems that regulate energy homeostasis are affected by food (Sayan Özaçmak and Bayraktaroğlu). Consequently, to maintain energy homeostasis, changes in nutritional status produce coordinated responses that affect appetite and/or energy expenditure (Simpson et al 2008).

Incretin-based treatments are currently being developed to control weight. While incretins increase glucose-dependent insulin secretion in the pancreas, they suppress glucagon secretion. Glucagon-like peptide-1 receptor agonists (GLP-1RA) is an incretin-based therapy. (Göksu and Ünal 2017). There is a strong correlation between food consumption and incretin hormone release. (Elliott et al. 1993; Orskov et al. 1996). Studies have shown that GLP-1 has an effect on appetite and food intake through peripheral and central mediators (Baggio and Drucker, 2007). İmprovement in lipid profiles and cholesterol homeostasis has been shown in obese patients taking such medications (Mostafa et al. 2015), however, the mechanisms involved are not yet fully known. GLP-1 is an incretin hormone secreted by enteroendocrine L-cells in the ileum and colon in response to nutrient intake, particularly fats and carbohydrates, causing glucose-dependent insulin secretion (Campbell and Drucker 2013). GLP-1 is also synthesized as a neurotransmitter in a small community of neurons in the nucleus tract solitarius in the brainstem (Özaçmak and Bayraktaroğlu 2017). GLP-1 has a wide range of physiological effects, such as stimulation of insulin secretion and glucose-dependent suppression of glucagon secretion, inhibition of gastric emptying and food intake, and reduction of weight loss (Campbell and Drucker 2013). In different studies, GLP-1 agonists have been shown to reduce circulating levels of pro-inflammatory C-reactive protein (CRP) (Mazidi et al. 2017) and interleukin (IL) -6 (Daousi et al. 2013) in patients with T2DM. However, it is still unclear whether GLP-1 modulates inflammation indirectly by interacting with its receptor expressed on circulating immune cells or indirectly promoting weight loss and glycemic recovery (Drucker, 2016).

The aim of this study is to investigate the cytotoxic effect of GLP-1 receptor agonist on 3T3-L1 adipocytes *in vitro*.

2. Materials and Methods

Cell Culture

The 3T3L-1 fibroblast cell line to be used in the study was commercially available from the American Type Culture Collection (ATCC) (Manassas, USA). Cells were incubated in a medium containing DMEM (Dulbecco's modified Eagle's medium) medium + 10% Fetal Bovine Serum (FBS) + penicillin (100 units / ml) and streptomycin (100 µg / ml) in a 5% carbon dioxide oven at 37°C.

Transformation of 3T3-L1 Fibroblast Cells into Adipocyte Cells

Differentiation of 3T3-L1 fibroblast cells Miard et al. (2009) was made according to the protocol. Differentiation in cells was determined morphologically by the method of Lillie and Ashburn (1943) with the oil red o staining method.

Application of GLP-1RA to 3T3-L1 Adipocyte Cells

In our study, exenatide was used as GLP1-RA, which is in phase IV stage for diabetes treatment and has recently received phase II approval for obesity treatment by FDA and EMA as an anti-obesity drug due to its effect on weight loss, but has not completed phase studies for obesity (Martinussen et al. 2017). GLP-1RA 1 nM, 5 nM, 10 nM, 50 nM, 100 nM, and 250 nM were added to 3T3-L1 adipocytes and the cells were incubated for 48 hours. Only culture medium was added to the control cells.

Application of MTT Test to 3T3-L1 Adipocyte Cells

MTT analysis was done according to Yerlikaya et al method. The data was analyzed with the GraphPad Prism 5.0 program (GraphPad Software, Inc., La Jolla, CA, USA) and a graph was generated. To calculate the IC_{50} value, the data were normalized by nonlinear regression analysis using the GraphPad Prism 5.0 program.

Cell Viability Analysis

Viability rates of cells after GLP-1RA administration were calculated compared to untreated control cells. The viability of untreated cells was considered to be 100% and the viability percentages of cells were calculated as follows.

% viability: (Treated cell / untreated cell) X100

3. Results

Cells were cultured in standard medium for 24 hours before applying exenatide to 3T3-L1 adipocyte cells. Cell culture medium was applied 250, 100, 50, 10, 5, and 1 nM exenatide and incubated for 48 hours. Only culture medium was added to the control cells. As a result of GLP-1

RA application, the cells were tested for MTT. Statistical analysis of the results obtained with the MTT test was performed with the GraphPad Prism 5.0 program. According to statistical analysis after 48 hours 250, 100, 50, 10, 5, and 1 nM application to adipocyte cells, the IC_{50} value of GLP-1 RA at 48 hours was calculated as 688 nM (Figure 1). This result shows that exenatide has a cytotoxic effect on adipocytes.

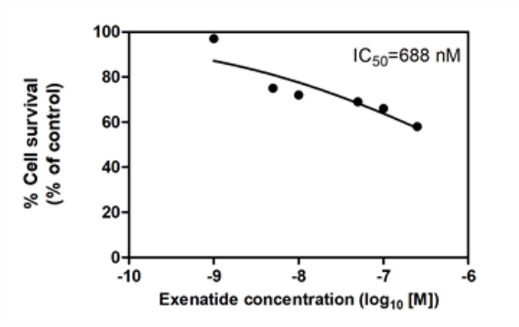

Figure 1. MTT graph after 48 hours exenatide application to adipocyte cells.

In order to analyze the survival rate of adipocyte cells as a result of application of different concentrations, 250, 100, 50, 10, 5, and 1 nM exenatide was applied to the cells grown in 96 microplates until they reach the logarithmic phase. Then, MTT cytotoxicity test was performed. Cell viability was determined as 58%, 66%, 69%, 72%, 75% and 97%, respectively, when compared to the control after 250, 100, 50, 10, 5 and 1 nM exenatide administration. It was determined that 250, 100, 50, 10 and 5 nM exenatide administration had proliferative effects in adipocyte cells. Administration of 1 nM exenatide had no proliferative effect on 3T3-L1 adipocyte cells (Figure 2).

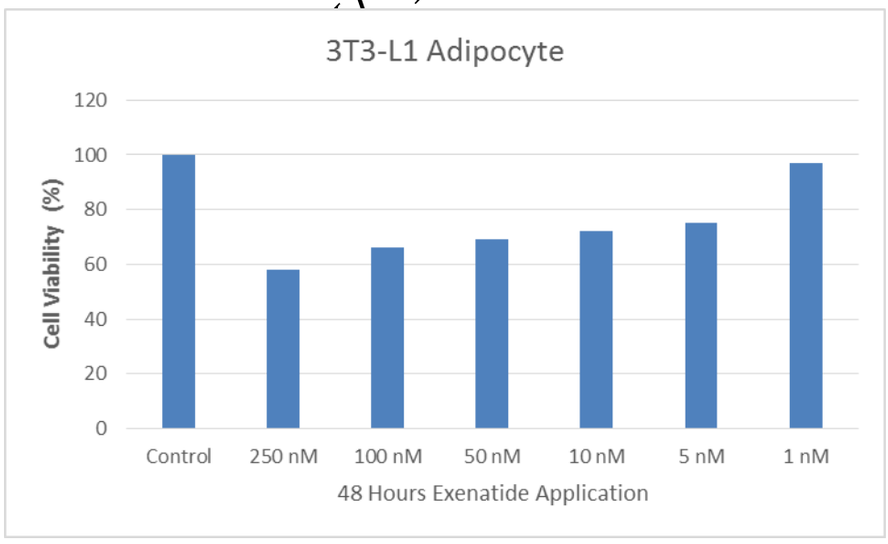

Figure 2. Cell viability (%) graph after 48 hours exenatide application to adipocyte cells.

4. Discussion

The increasing prevalence of obesity poses a major threat to public health, and available pharmacological treatment options remain limited (Martinussen et al. 2017). In addition, obesity brings a huge financial burden to healthcare services. Bariatric surgery is the most effective treatment for severe obesity, emphasizing the urgent need for new and improved drug therapies (Martinussen et al. 2017). Based on the physiological regulation of energy homeostasis, pharmacological strategies to treat obesity are evaluated focusing on drugs in phase 2 and 3 clinical development (Martinussen et al. 2017). Combination therapy that targets multiple ways of controlling energy balance may be necessary to achieve serious weight loss while minimizing side effects (Martinussen et al. 2017).

GLP-1R agonists liraglutide and exendin-4 are in phase IV for diabetes treatment (Özaçmak and Bayraktaroğlu 2017), but recently approved as an anti-obesity drug by the FDA and EMA in Phase II due to evidence of their significant effects on weight loss (Martinussen et al. 2017). In our study, it was used in phase II, which has not yet completed its phase studies, and the most prescribed exenate by the Ministry of Health (Martinussen et al. 2017). Liraglutide, a GLP-1R agonist, has been found to inhibit cell growth, differentiation and glucose uptake in human adipose cells (Cantini et al. 2015). This finding suggests that different molecular targets and signaling pathways act. Despite the studies conducted, the nature of this alternative molecular target remains unclear until now (Cantini et al.2017). Liraglutide inhibits proliferation and differentiation in preadipocytes (Cantini et al. 2015). These effects may contribute to the effects of GLP-1 receptor agonists on body weight and insulin sensitivity. The possibility of a direct effect of GLP-1 receptor agonists on adipose tissue and pre-adipocytes has not yet been fully investigated (Cantini et al. 2015). In a study conducted by Li et al to investigate the effects of Exendin-4 on the expression of a new adipokine C1q / TNF-related protein (CTRP3) in 3T3-L1 adipocytes, they found that Ex-4 increased CTRP3 mRNA and protein expression in 3T3-L1 adipocytes. (Li et al.2015). In the study that Xu et al. (2016) examined lipolitic and oxidative changes in adipose tissue by the GLP-1 receptor (GLP-1R) agonist exenatide (exendin-4) and looked at the role of SIRT1 in this process, they found that, by regulating the SIRT1 expression and activity of exendin-4 in differentiated 3T3L1 adipocytes *in vitro*, increases lipolysis and fatty acid oxidation. These data may indicate that one of the mechanisms of GLP-1R agonist on weight loss may be through SIRT1 (Xu et al. 2016).

5. Conclusion

As a conclusion, the IC_{50} value was found to be 688 nM by applying different doses of exenatide to 3T3-L1 adipocyte cells in the study. This cytotoxic effect on adipocytes may provide an alternative therapy in obesity treatment. However, in addition to preliminary results, more molecular analysis is needed for more descriptive answers.

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Determination of Foodborne Pathogens and Some Hospital Isolates Biofilm Formation Ability

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Abstract:

Biofilm-forming ability of pathogens is a key factor for the persistence in both food plants and hospital equipments and to exhibit virulence factors. Due to their biofilm structure, they develop a mechanism of resistance to antimicrobial agents, biocides, and heat. The aim of this study was to determine the biofilm formation potential of foodborne pathogens and some hospital isolates. For this purpose, 17 foodborne pathogens such as *Bacillus cereus* DSM 4312, *Escherichia coli* ATCC 35218, *Escherichia coli* O157 NCTC 12900, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* NCTC 8213, *Klebsiella pneumoniae* ATCC 70063, *Listeria monocytogenes* ATCC 7644, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 33592, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Salmonella* Typhimurium ATCC 14028, *Salmonella enteritidis*, *Listeria monocytogenes* (isolated from frozen food industry plant) and 3 hospital isolates such as *Acinetobacter baumannii* AYE, *Klebsiella pneumoniae*, *Staphylococcus aureus* MRSA. The assessment of biofilm formation of foodborne pathogens and hospital isolates was undertaken using a crystal violet assay on microtitre plates. The results show that all the tested foodborne pathogens produced biofilms. Among these foodborne pathogens, *E. coli* O157 NCTC 12900, *E. coli* ATCC 25922, both of *E. faecalis*, *L monocytogenes* ATCC 7644, *S. enteritidis* and *S.* Typhimurium ATCC 14028 were weak biofilm producers, while *K. pneumoniae* ATCC 70063 and *S. aureus* ATCC 6538 produced moderate biofilms. The rest of test pathogens were classified as strong biofilm producers. Additionally, *S. aureus* MRSA of the hospital isolate produced biofilm but *K. pneumoniae* was not capable of producing biofilms. Findings from this study indicate that the foodborne pathogens are an important biofilm producer. In conclusion by adding different strains to the strains screened in this study, a database may be created for future biofilm studies.

Keywords: biofilm, foodborne, pathogen, crystal violet assay

1. Introduction

Biofilms are a survival and protection strategy for pathogens. Microorganisms capable of producing biofilms are the main cause of surface-to-food cross contamination and can cause food spoilage. Also, if the biofilm is composed of foodborne pathogen bacteria, food poisoning can be occurred (Somrani et al. 2020). Several studies have demonstrated that foodborne pathogens and clinic isolates are able to form biofilms on surfaces that are commonly found in food processing environments and hospital equipments (Babapour et al. 2016, Doijad et al. 2015, Silva et al.

2018). Moreover, studies have reported that biofilm causes at least two-thirds of all clinical infections in various life-threatening infections in humans (Xu et al. 2020).

In a study by Zadernowska and Chajęcka-Wierzchowska (2017), some isolates of the Salmonella spp. collected from food samples (meat, raw white sausage, smoked meat and cheese) were classified as moderate biofilm producers. Dygico et al. (2020) *Listeria monocytogenes* reported that formed biofilms on different surfaces related to the mushroom production environment. Similarly, Liu et al. (2020) reported that formed biofilm of *Listeria monocytogenes* isolated from beef processing plants. Chen et al. (2020) determined *Staphylococcus aureus* strains isolated from different food samples (food poisoning incidents, restaurant food, raw meat, baked food, cooked meat product, fresh juice) as a biofilm producer. Pavlickova et al. (2017), found that biofilm produces of *Escherichia coli* strains isolated from chicken meat and wildlife. Igbinosa and Beshiru (2019) revealed that significant Enterococcus species isolated from ready-to-eat seafood produce biofilm. Kwon et al. (2017) determined that *B. cereus* strains isolated from traditional Korean soybean paste form biofilms on different surfaces (e.g., stainless steel, plastic, or glass). Surgers et al. (2019) determined that *Escherichia coli* and *Klebsiella pneumonia* strains isolated from patients enrolled in a cross-sectional study formed biofilms. Similarly, in a study by Seng et al. (2017) reported that biofilm-producing methicillin resistant coagulase negative staphylococci (MR-CoNS) strains showed a high prevalence in community and hospital environments.

After bacterial cells attach to a surface, they produce extra polymeric compounds such as proteins, DNA and polysaccharides (Bansal et al. 2019). A biofilm structure consists of 10% biofilm bacteria cells and 90% extracellular polymeric substances (EPS) (Gebreyohannes et al. 2019, Xu et al., 2020). In addition, biofilms can protect to bacteria cells due to this structure from proteases released by host defense cells and environmental stress factors such as heat, cold, disinfectants and antimicrobials (Chen et al. 2020, Montso et al. 2021, Song et al. 2019). Thus, bacteria in biofilms are difficult to mechanically remove, and exhibit significantly resistance to disinfectants (Silva et al. 2018).

The crystal violet assay is the most frequently used biofilm formation quantification technique in 96-well microtitre plates (Dygico et al. 2020, Lade et al. 2019). This method were modified for various biofilm formation assays by researchers (Djordjevic et al. 2002). Tang et al. (2020) and Liu et al. (2020) were not fixed the biofilm mass with alcohol during analysis before crystal violet staining, however Xu et al. (2020) and Guo et al. (2019) were fixed the wells. The aim of this study is to determine the biofilm formation level of standard foodborne pathogens and some clinic isolates, and to create data for future biofilm studies.

2. Materials and Methods

2.1. Microorganisms

The bacterial cultures were provided American Type Culture Collection (ATCC) (13 out of 20), DSMZ-German Collection of Microorganisms and Cell Cultures (1 out of 20), National Collection of Type Cultures (1 out of 20). The rest of cultures used throughout the study, kindly provided by hospital (2 out of 20), frozen food industry (1 out of 20) and Bursa Uludag University Veterinary Faculty (2 out of 20). Stock cultures were maintained in Mueller Hinton broth (Merck, Darmstadt, Germany) supplemented with 20% glycerol at -20 °C, until analysis.

2.2. Preparation of bacterial cultures

All bacterial strains inoculated in Tryptic Soy Broth (Merck, Darmstadt, Germany) with 0.6% yeast extract (Merck, Darmstadt, Germany) (TSBYE). The overnight bacterial suspension was adjusted to a turbidity of 0.5 McFarland units and diluted with TSBYE to ensure a concentration of 1.0 x 106 CFU mL-1 in well. For each bacterial culture, two tubes were inoculated and tested.

2.3. Evaluation of biofilm-forming ability

The evaluation of biofilm-forming ability was performed by quantification of biofilm biomass on microtiter plates using the crystal violet assay according to method of Lee et al. (2016) with slight modification. Briefly, 20 µL suspension (1.0 x 106 CFU mL-1) were added to wells which filled with 180 µL TSBYE, previously. Then, the plates were incubated at 37 °C for 48 h to allow biofilm forming. Following incubation, the medium was discarded, and the wells were gently washed three times with sterile distilled water. The plates were air-dried in the inverted position for 30 min at room temperature. After fixation with methanol, plates were air-dried in the inverted position for 10 min. Subsequently, the wells were stained with 200 µL 0.1% cristal violet for 30 min and removed. The plates were washed three times with sterile distilled water and resolubilized with 96% ethanol. The destaining solution was transferred to a new plate and the absorbance was determined at 595 nm using microplate reader (Epoch; Bio-Tek Instruments, Winooski, Vermont, USA). For negative control, 20 µL sterile distilled water was used and the absorbance (optical density, OD) of negative control was called ODnc. Also, OD indicates the absorbance measurement of each bacteria. The biofilm-forming capacity of bacteria was classified as weak (ODnc < OD ≤ 2×ODnc), moderate (2×ODnc < OD ≤ 4×ODnc) or strong (OD > 4×ODnc) biofilm producer (Stepanović et al. 2003). The crystal violet assay was done with two replicates of bacterial cultures and four repetitions under identical conditions for each bacteria (n=8).

2.4. Statistical Analysis

Results were given as the mean and standard deviation. Statistical analyses were performed using the SPSS Version 22.0 (IBM, Armonk, New York, USA). Significant differences among means

was analyzed with one-way analysis of variance ANOVA followed by Duncan post-hoc test. For all tests, the confidence level for significance was 95% ($P < 0.05$).

3. Results

Biofilm formation abilities of tested microorganisms were classified according to negative control absorbance value after performing crystal violet assay (Table 1). The microorganisms exhibited varying levels of biofilm formation from weak to strong. Among Gram negative bacteria, 5 out of 8 are weak, 1 out of 8 is moderate and 2 out of 8 are strong biofilm producers. It was observed that the number of strong biofilm producers was higher in Gram positive bacteria (Fig. 1).

Table 1. Classification of Biofilm Formation.

Evaluation	OD595nm	Classification of biofilm
Negative control	0.067±0.004	-
ODnc < OD ≤ 2×ODnc	0.067 < X ≤ 0.134	Weak
2×ODnc < OD ≤ 4×ODnc	0.134 < X ≤ 0.268	Moderate
OD > 4×ODnc	X > 0.268	Strong

OD: optical density, nc: negative control

Our results indicated that biofilm production of same genus varied greatly between different species. Similarly, differences in biofilm ability were found between species and sub-species. *E. coli* ATCC 35218 produced strong biofilm while *E. coli* O157:H7 NCTC 12900 and *E. coli* ATCC 25922 produced weak biofilm. However, two *E. faecalis* and two Salmonella spp. gave same absorbance values that were not statistically different ($P > 0.05$).

As presented in Table 2, *M. luteus* ATCC 9341, *S. aureus* ATCC 29213, *A. boumanni* AYE, *S. epidermidis* ATCC, *E. coli* ATCC35218, *S. aureus* ATCC 25923, methicillin-resistant *S. aureus* ATCC 33592, *S. aureus* ATCC 12228 and *L. monocytogenes* isolate from frozen food industry are strong biofilm producers. When the absorbance values were examined, it was determined that the highest biofilm producer was *M. luteus* ATCC 9341, significantly.

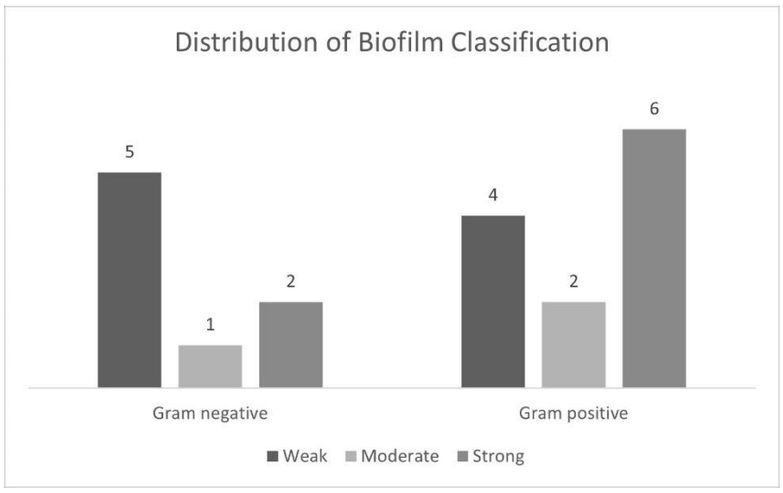

Figure 1. Biofilm producers according to Gram properties.

Table 2. Biofilm formation abilities of test microorganisms.

Microorganisms	**Gram staining properties**	**OD595nm**	**Biofilm class**
A. boumanni AYE (MDR)	Gram negative	0.782±0.113c	Strong
E. coli ATCC35218	Gram negative	0.594±0.061ef	Strong
E. coli O157:H7 NCTC 12900	Gram negative	0.079±0.004j	Weak
E. coli ATCC 25922	Gram negative	0.122±0.045hi	Weak
K. pneumoniae ATCC700603	Gram negative	0.264±0.004fgh	Moderate
K. pneumoniae hospital isolate	Gram negative	0.076±0.013j	Weak
S. enteritidis	Gram negative	0.076±0.005j	Weak
S. typhi ATCC14028	Gram negative	0.080±0.010j	Weak
B. cereus DSM 4312	Gram positive	0.096±0.018j	Weak
E. faecalis ATCC29212	Gram positive	0.074±0.004j	Weak
E. faecalis NCTC8213	Gram positive	0.091±0.008j	Weak
M. luteus ATCC 9341	Gram positive	1.328±0.226a	Strong
S. aureus ATCC 25923	Gram positive	0.454±0.081de	Strong
S. aureus ATCC 29213	Gram positive	1.086±0.344b	Strong
S. aureus ATCC 33592	Gram positive	0.592±0.180d	Strong

(MRSA)			
S. aureus ATCC 6538	Gram positive	0.177±0.052hi	Moderate
S. aureus (MRSA) isolate	Gram positive	0.199±0.032ghi	Moderate
S. epidermidis ATCC 12228	Gram positive	0.543±0.132d	Strong
*L. mono*octogenes ATCC7644	Gram positive	0.086±0.010j	Weak
L. monocytogenes isolate	Gram positive	0.326±0.085efg	Strong

a-j Different letters indicate statistical differences between values ($P < 0.05$). Data are presented as mean ± standard deviation of two independent experiments (n=8). MDR,multi drug resistant; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures; DSM, German Collection of Microorganisms and Cell Cultures; MRSA, methicillin resistant *Staphylococcus aureus*.

4. Discussion

Most of microorganisms prefer to be in the biofilm structure rather than the planktonic cell. The heterogeneous layers formed within the biofilm structure provide protection against nutrient restriction, fluid flow, drying, toxic chemical gradients, UV light, and various conditions such as pH and temperature. Therefore, biofilm formation is one of the survival strategies of microorganisms, including pathogens (Hall-Stoodley and Stoodley 2005). Although biofilm formation is not the only necessary factor for pathogenicity, it is likely that pathogens in the biofilm structure cause a higher rate of disease than planktonic cells. Biofilm structure can facilitate the virulence expression due to the pathogen microorganism's ability to regulate quorum-sensing networks, reach high population in a short time and resist some antibiotics (Høiby et al. 2010, Neopane et al. 2018). Pre-determining the biofilm production capabilities of pathogens struggled in both food industry and medicine allows us to choice more effective methods.

In this study, 20 pathogen microorganisms evaluated for their biofilm-forming ability. After crystal violet assay, OD values of M. luteus ATCC 9341 was found highest, significantly. *Micrococcus luteus* is one of the normal flora members of the oral cavity, but it has recently been considered an opportunistic pathogen, causing infection in immunocompromised individuals (Hillis et al. 2018). In a study by Maifreni et al. (2015), M. luteus isolate from an Italian Microbrewery was determined as a strong biofilm producer. Most of the *S. aureus* strains we used in the study are capable of producing strong biofilms. Similar results was found by Neopane et al. (2018) and Unlu et al. (2018). However, *S. aureus* ATCC 6538 and methicillin resistant *S. aureus* (hospital isolate) exhibited moderate biofilm formation. Unlike our results, Condò et al.

(2020) classified as a strong biofilm producer for *S. aureus* ATCC 6538. Recent studies indicated that *A. boumanni* developed remarkable antibiotic-resistance and called multi drug resistant (Bardbari et al. 2018, Tomaras et al. 2008). In our study, *A. boumanni* AYE which is a human multi drug resistant isolate was classified as strong biofilm producer. Our results revealed that the biofilm forming ability varied between same species. While *E. coli* ATCC35218 produced strong biofilm, *E. coli* ATCC 25922 and *E. coli* O157:H7 NCTC 12900 produced weak biofilm. *E. coli* ATCC 25922 was used in a study by conducted Conte et al. (2016) as a positive control for biofilm study but classification was not given. *L. monocytogenes* is one of the most common microorganisms in the food industry that cause the biofilm problem (Dygico et al. 2020). In our study, *L. monocytogenes* ATCC 7644 was classified weak biofilm producer. This result was similar to that reported by Altuntas et al. (2020) and Jaradat and Bhunia (2003). However, *L. monocytogenes* isolated from frozen food industry produced strong biofilm. There was no signifcant difference ($P > 0.05$) between biofilms formed in classified as weak biofilm producers.

Singh et al. (2017) evaluated the parameters affecting biofilm formation for *S. aureus* and reported that the medium, incubation time, fixation or not (methodology), and glucose addition were effective in biofilm formation. In addition, the biofilm proficiency of the microorganisms depends on the production of pili and secretion systems. Therefore, it should be considered that different strains may differ in response to different conditions. We can explain the differences between our study and the results in the literature with the difference in all parameters affecting biofilm formation.

5. Conclusion

In conclusion, it was determined that crystal violet assay was used in many studies determining the biofilm formation characteristic of microorganisms in the literature, but the application of the method and some results obtained differed from the literature data. Therefore, it is thought that the CV method should be optimized in order to be able to compare future studies correctly. With the similar results obtained, a database can be created for biofilm studies. In addition, the findings in this study may suggest to select of particularly strong biofilm producers to evaluate the antibiofilm properties of natural compounds. However, before biofilm formation, reducing the number of planktonic cells and preventing adhesion to the surface should be adopted as a priority biofilm control strategy.

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The Effect of Occupational Health Nursing Course on Compliance of Nursing Students with Standard Precautions

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Abstract:

The aim of this study is to evaluate the effect of the Occupational Health Nursing course on compliance of nursing students with standard precautions. A quasi-experimental research design that was with a pre-post-test design without a control group was used in this research. The sample of the study consists of 75 third grade students who chose the Occupational Health Nursing elective course and agreed to participate in the study. Occupational Health Nursing elective course that takes part in the 5th term of the nursing faculty curriculum is two hours per week during a term. As data collection tools were used the sociodemographic characteristics questionnaire and Compliance with Standard Precautions Scale. Socio-Demographic Characteristics Questionnaire consisted of questions including age, gender. Compliance with Standard Precautions Scale are 20 items on a Likert-type scale ranging from 1="never" to 4="always". As calculating the scale scores, a score of 1 was interpreted as an "always" response, while 0 was for the other responses. A total range score of 0–20 is expected, with higher scores signifying better compliance with standard precautions. IBM SPSS (Statistical Package for the Social Sciences) software (version 20) was used for data analysis. The paired sample t-test was used to compare mean scores of the Compliance with Standard Precautions Scale at the pretest and posttest. A p-value of <0.05 was considered significant. The mean age of nursing students is 21.69±0.97. 94.7% of them are women. The pre-test and post-test mean scores of the nursing students in Compliance with Standard Precautions Scale were 14.53±2.41, 15.05±2.48, respectively. There were statistically significant differences between the pre and post-test Compliance with Standard Precautions Scale scores of nursing students ($p<0.05$). The results of this study demonstrated that Occupational Health Nursing Course provided to increase compliance of nursing students with standard precautions. In order to reduce occupational accidents by increasing the compliance of nursing students with the standard precautions, it is recommended to increase the subjects and practices regarding occupational health in the nursing curriculum.

Keywords: nursing education; nursing students; occupational health, standard precautions

1. Introduction

Standard precautions (SPs) are defined as considered basic preventive measures to manage health-related infections and reduce occupational health hazards (Siegel et al., 2007). The main purpose of SPs is to reduce the risk of possible contamination by protecting the patient, other patients, healthcare professionals, and other members of the team in the presence of infection accompanying the patient's condition during health care (Siegel et al., 2007). There are five parameters on the basis of SPs: providing hand hygiene, the use of personal protective equipment, decontamination of spilled wastes, proper disposal of wastes, and prevention of cross-infection (WHO, 2007).

Although clinical practices are an integral part of nursing education, they also involve various health risks for students. In the hospital environment where infections are quite common, the risk of students encountering the agent is quite high (Cruz, 2019). Although nursing students have less clinical experience, they are exposed to the same health risks as nurses during their practical training. Nursing students who try to gain clinical practice skills and establish their professional identities in a high-risk work environment are at risk from many aspects.

Nursing students often experience occupational hazards such as needle-stick injuries, stress, violence, etc. during their practical training (Suliman et al., 2018; Togan et al., 2015; Yao et al., 2010). Boucaut & Knobben (2020) reported that sharps injuries were to be the most common occupational incidents among nursing students. Çakar et al. (2019) showed that the frequency of needle-stick injuries during the clinical practice of nursing students was found to be 27.8%. Togan et al. (2015), found that needle-stick injuries occur most frequently in healthcare students.

Although standard precautions are clear, understandable, and easy, there are problems with the implementation of standard precautions. Studies show that healthcare workers including nursing students have low knowledge, attitudes, and compliance to SPs (Kermode et al., 2005; Luo et al., 2010; Pan et al., 2008; Kim & Oh, 2015). Non-compliance of nurses to SPs is directly related to a lack of knowledge about SPs. Therefore, SPs training is to recommended to start in the early stages of nursing education and to become a part of the curriculum. The aim of this study is to evaluate the effect of the Occupational Health Nursing course on compliance of nursing students with standard precautions.

2. Materials and Methods

Study design and sampling

A quasi-experimental research design with a pre-posttest without a control group was used in this research. The sample of the study consists of 75 third grade students who chose the Occupational Health Nursing elective course and agreed to participate in the study. This study was conducted between September 2020 and January 2021 at the Faculty of Nursing in Turkey.

Data collection

As data collection tools were used the sociodemographic characteristics questionnaire and Compliance with Standard Precautions Scale. Socio-Demographic Characteristics Questionnaire consisted of questions including age, gender. Compliance with Standard Precautions Scale are 20 items on a Likert-type scale ranging from 1=“never” to 4=“always”. The original version of the scale was developed by Lam (2014) and was translated to Turkish by Samur et al. (2020). The scale’s items evaluate compliance with the use of PPE, disposal of sharps and wastes, decontamination of spills and used articles, and prevention of cross infection. As calculating the

CSPS scores, a score of 1 was interpreted as an "always" response, while 0 was for the other responses. A total range score of 0–20 is expected, with higher scores signifying better compliance with standard precautions.

Intervention

Occupational Health Nursing elective course that takes part in the 5th term of the nursing faculty curriculum is two hours per week during a term. The course takes place the subjects such as Risk Assessment in Workplace, Occupational Hazards, Occupational Diseases, Work Accidents, Employee and Employer Responsibilities, Occupational Risks for Health Care Workers, etc.

Data analyses

IBM SPSS (Statistical Package for the Social Sciences) software (version 20) was used for data analysis. The paired sample t-test was used to compare mean scores of the Compliance with Standard Precautions Scale at the pretest and posttest. A p-value of <0.05 was considered significant.

3. Results

The mean age of nursing students is 21.69±0.97, 94.7% of them are women. The pre-test and post-test mean scores of the nursing students in Compliance with Standard Precautions Scale were 14.53±2.41, 15.05±2.48, respectively. There were statistically significant differences between the pre and post-test Compliance with Standard Precautions Scale scores of nursing students ($p<0.05$).

Table 1. Compliance with Standard Precautions Scale Scores of Nursing Students

	CSPS Pre-test x±Sd	**CSPS Post-test x±Sd**	**t**	**p**
Nursing Students (N=75)	**14.53±2.41**	**15.05±2.48**	**-6.07**	**0.00***

*p<0.05.

4. Discussion

This study evaluated the effect of the Occupational Health Nursing course on compliance of nursing students with standard precautions. The standard precautions have a crucial role in decreasing the risk of infection among patients, and healthcare professionals such as doctors, nurses, students, etc. Kim and Oh (2015) reported that the use of infection prevention precautions is low among nursing students. Moon et al. (2019) stated that Korean nursing students' compliance rate with SPs was low. Alshammari et al. (2018) found that the CSPS means of nursing students was 12.02±4.50. Xiong et al. (2017) found that nursing students had low levels of knowledge and application of SPs, and mixed media education intervention can

improve nursing students' level of knowledge, attitude, and compliance with SPs. It was found that after the intervention the CSPS means of nursing students was 15.05±2.48 in this study. Occupational Health Nursing elective course affected compliance of nursing students with standard precautions. After the course, the CSPS means of nursing students increased statistically compared to before the course.

5. Conclusion

The results of this study demonstrated that Occupational Health Nursing Course increases compliance of nursing students with standard precautions. In order to reduce nursing students' occupational exposures by increasing the compliance of them with the standard precautions, it is recommended to increase the subjects and practices regarding occupational health in the nursing curriculum. It is thought that starting occupational health and safety education before practical training could be more effective in reducing occupational health risks.

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Analysis of ORM1 Levels in Tissue and Urine Samples of Patients with Bladder Cancer: Literature Review and Preliminary Experiment Results [#]

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Abstract:

Orosomucoid-1 (ORM1), also known as Alpha 1-Acid Glycoprotein (AGP) is an acute-phase protein weighing 41-43 kDa and is synthesized from the liver. Changes in ORM1 level have been reported in many diseases. Our first aim in this study was to evaluate the importance of ORM1 in urological malignancies, based on the literature. Articles in PubMed between 1955 and 2021 were reviewed with the help of MeSH terms. There were 7 studies on prostate cancer, 5 on bladder cancer, and 2 on renal cancer, and no studies on testicular or penile cancer. As a result, it was understood that ORM1 has three important effects on urological malignancies. (a) Serum and urine ORM1 levels and glycoforms can be used as a valuable biomarker. (b) ORM1 is a drug-binding/carrier protein with polymorphic variants that can alter the pharmacokinetics of anticancer drugs such as docetaxel. (c) ORM1 has significant anti-inflammatory, immunomodulatory and pro-angiogenic properties. Secondly, we analyzed the ORM1 levels in tumor tissues, normal tissues adjacent to the tumor (NAT), and urine samples of 16 patients with bladder cancer (13 male, 3 female). Information such as age (81 ± 8), height (165 ± 10 cm), weight (78 ± 13 kg), BMI (29 ± 6), tumor size (25 ± 18 mm), and pathological stages (6 pTa and 8 pT1 or other) of the patients were also recorded. For analyzes, tissue samples were homogenized and total protein extracted. Protein concentrations were measured colorimetrically by the Bradford method, and protein quality was assessed by SDS-PAGE. Tissue and urine ORM1 levels were analyzed by ELISA. As a result of this preliminary experimental study, there was no statistically significant difference between tumor tissues and NAT's ORM1 levels (P= 0.5915). Urinary ORM1 levels of patients with pTa stage were slightly decreased compared to urine samples of pT1 or other stage patients, but this decrease was not statistically significant (P= 0.4206). Urinary ORM1 levels were found to be weakly positively correlated (r= 0.4657, P= 0.1270) with the bodyweights of the patients. Performing creatinine correction in urine samples and repeating the study with a larger cohort will be informative.

Keywords: orosomucoid-1, alpha 1-acid glycoprotein, urological malignancies, bladder cancer, urine, biomarker

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1. Introduction

Orosomucoid-1 (ORM1), also known as Alpha 1-Acid Glycoprotein (AGP) is an acute-phase protein weighing 41-43 kDa and is synthesized from the liver. ORM1 is found at a certain level in serum under normal conditions, however, it has been reported that its level in physiological fluids changes in many diseases, especially cancer (Pitekova et al., 2019). The relationship between cancer and ORM1 has been the subject of many studies. In this study, we examined all studies related to urological malignancies and ORM1 in the literature, and we summarized them in this section. Besides we experimentally investigated the level of ORM1 in biological samples of bladder cancer (BC) patients.

Urological Malignancies and ORM1

Serum protein profile and anti-kidney antibodies were investigated in the study conducted by Holm *et al.* on 17 patients with renal carcinoma (RC) in 1980. As a result of Serum Protein Electrophoresis analysis, researchers stated that ORM1 level increased in patients with RC compared to the healthy control group, and also serum ORM1 levels of patients with metastatic RC (n= 6; 2.2 ± 0.5 g/L) compared to patients with non-metastatic RC (n = 11; 0.8 ± 0.4 g/L) was found to be statistically significantly ($P< 0.05$) higher (Holm et al., 1982). In the study conducted by Tanaka *et al.* on 33 RC patients, the plasma levels of pazopanib, an angiogenesis inhibitor, were analyzed for dose optimization in 2020. They analyzed plasma albumin and ORM1 levels as factors that could alter the pharmacokinetics of pazopanib, but only albumin level was found significantly associated with effective pazopanib concentrations (OR: 1.37; $P= 0.0234$) (Tanaka et al., 2020).

In the study conducted by Robey *et al.* in 1985, they investigated the ORM1/prealbumin ratio, which is called cancer serum index (CSI), in serum samples of 130 prostate cancer (PC) patients and 21 healthy controls and found that CSI was statistically significantly higher in patients with PC ($P< 0.05$) (Robey et al., 1985). In the study performed by Kuvibidila *et al.* in 2004, the levels of ORM1 and some other parameters were investigated by ELISA from serum samples of 27 PC patients and 72 healthy controls. However, it was reported that the difference between the ORM1 level of the patients with PC (0.958 ± 0.06 g/L) and the healthy control group (0.863 ± 0.03 g/L) was not found statistically significant ($P= 0.1112$) (Kuvibidila et al., 2004). In the study conducted by Kanoh *et al.* in 2011, serum levels of various inflammatory biomarkers including ORM1 were investigated in PC patients with (n= 10) and without (n= 23) a_2-macroglobulin deficiency and healthy controls (n= 10). The researchers stated that the serum ORM1 level of PC patients increased statistically significantly ($P< 0.05$) compared to healthy controls (67.5 ± 25.5 mg/mL). However, no significant difference was found between PC patients with (100.8 ± 38.90 mg/mL) and without (102 ± 39.36 mg/mL) a_2-macroglobulin deficiency (Kanoh et al., 2011).

Prostasomes are exosome-like vesicles that are important in PC progression and normal reproduction. In the study conducted by Kovak *et al.* in 2013, prostasomes were isolated from semen samples obtained from healthy male participants, and potential galectin-3 ligands were purified and identified. Researchers stated that ORM1 and some other glycoproteins could be binding ligands for galectin-3 (Kovak et al., 2013). Orosomucoid-1 is a drug-binding and drug-carrier protein, and its genetic variants are frequently discussed in pharmacogenetic studies. As one of these, in the study conducted by Younis *et al.* in 2014, the effect of rs250242 polymorphism in *ORM1* gene on docetaxel pharmacokinetics in 26 PC patients was investigated and it was shown that this variant significantly changed the systemic docetaxel clearance (P= 0.03) (Younis et al., 2014). In the study conducted by Wang *et al.* in 2015, proteomic biomarkers were investigated with MALDI-MS and MCAEF using tissue samples from 3 PC patients. Researchers identified several peptides, including ORM1 at *m/z* 5002.2 and 6704.2, and stated that the peptide changes they found when compared with the non-cancerous regions of tissue samples were statistically significant (P< 0.05) (Wang et al., 2016). In the study conducted by Matsumoto *et al.* in 2019 on a large sample, including a group of 60 castration-resistant PC patients, the potential of the serum *N*-glycan profile to be a diagnostic and prognostic biomarker for the disease was investigated. Researchers emphasized the importance of ORM1 as a tri- and tetra-antennary *N*-glycan carrier glycoprotein (Matsumoto et al., 2019). To the best of our knowledge, there is no study investigating the relationship between penile or testicular cancers and ORM1.

Bladder Cancer and ORM1

One of the first studies dealing with the relationship between BC and ORM1 was conducted by Gozzo *et al.* in 1977. Researchers investigated the presence of ORM1 using monospecific antisera against ORM1 in urine samples from 11 patients with papilloma, 18 patients with BC (I to IV stages), and 21 healthy controls. As a result of the study, ORM1 positivity was observed in 64% of urine samples of patients with papilloma, 55% of urine samples of patients with BC, and 13% of urine samples of healthy controls. Besides, it was stated that the highest reactivity with monospecific antisera was in urine samples of patients with BC (Gozzo et al., 1977). In this study, ORM1 positivity in 13% of urine samples of healthy controls was evaluated as false positive, but it was shown in the study conducted by Sweeney *et al.* in 1994 that ORM1 may be found at a certain level in the absence of BC. Researchers collected saline bladder washouts of 9 healthy male and 4 healthy female and analyzed them with 2-DE and as a result showed the presence of spots of many proteins, including ORM1, which they thought may be derived from the bladder urothelium (Sweenwy et al., 1994). In the study conducted by Irmak *et al.* using different proteomic techniques in 2005, ORM1 levels were investigated in urine samples of 45 BC patients at different stages, 10 follow-up patients, and 7 healthy controls. Researchers have

shown that urinary ORM1 levels in patients with BC are increased in a stage-dependent manner, and they suggested that ORM1 may be used as a biomarker for BC (Irmak et al., 2005). In their study in 2009, this research group showed that ORM1, whose immunomodulatory properties are known, also has pro-angiogenic properties, which suggests that ORM1 may contribute to angiogenesis in cancer (Irmak et al., 2009). In the study of Ongay *et al.* in which serum samples of 8 BC patients and 8 healthy controls were performed in 2010, researchers stated that the CZE-MS-based analysis has a great potential to use ORM1 isoforms as biomarkers in BC (Ongay et al., 2010). In the study conducted by Li *et al.* in 2016 using urine samples of 112 BC patients, 21 cases with benign bladder damage and 53 healthy controls, urinary ORM1 levels were analyzed by ELISA, and correcting for creatinine (Cr) expression was performed. Researchers found that ORM1-Cr levels were statistically significantly higher ($P< 0.0001$) in patients with BC (7172.23 ± 3049.67 ng/mL) than benign cases (2493.48 ± 830.37 ng/mL) and healthy controls (2243.16 ± 969.01 ng/mL), and it was found to be correlated with pathological stages of BC. Also, they stated that with a cut-off value of 3912.97 ng/mL, urinary ORM1 may be used with 91.96% sensitivity and 94.34% specificity for early diagnosis of BC (Li et al., 2016). In their study in 2016, Pan *et al.* introduced a new platform, Polymer-Based Reverse Phase GlycoProtein Array (polyGPA), noting the limitation in the use of the Reverse Phase Protein Array (RPPA) platform for detecting glycoproteins due to their high post-translational modifications. By using ORM1 standards and endogenous ORM1 in human plasma to evaluate the sensitivity, specificity, and quantitation capacity of the polyGPA platform, researchers recorded 99% specificity and 10-fold increased sensitivity compared to the RPPA platform. In the same study, researchers stated that A2M and C4B glycoproteins could be valuable biomarkers for BC by analyzing urine samples of 16 BC patients and 8 healthy controls with polyGPA (Pan et al., 2016).

2. Materials and Methods

To evaluate the importance of ORM1 in urological malignancies, based on the literature, articles in PubMed between 1955 and 2021 were reviewed with the help of this MeSH terms: "(urological malignancies OR urologic neoplasms OR renal cancer OR kidney cancer OR urinary bladder cancer OR bladder neoplasm OR penile cancer OR penis neoplasm OR testicular cancer OR testis neoplasm) AND (orosomucoid OR orm OR alpha 1-acid glycoprotein OR agp)".

All samples used in the experimental study were obtained from the urology service of Istanbul Medeniyet University Goztepe Training and Research Hospital between 2018 and 2019, and the study protocol was approved by the ethics committee of this hospital. Tumor tissues, normal tissues adjacent to the tumor (NAT), and urine samples of 16 patients (13 male 3 female) diagnosed with BC were collected. Information such as age (81 ± 8), height (165 ± 10 cm),

weight (78 ± 13 kg), BMI (29 ± 6), tumor size (25 ± 18 mm), and pathological stages (6 pTa and 8 pT1 or other) of the patients were also recorded. For analyzes, tissue samples were homogenized and total protein extracted by T-PER™ Tissue Protein Extraction Reagent (Pierce Biotechnology, Rockford, IL, Catalog No: 78510). Protein concentrations were measured colorimetrically by the Bradford (Bio-Rad Laboratories, Hercules, CA, USA, Catalog No: 5000002) method, and protein quality was assessed by SDS-PAGE. Orosomucoid-1 levels in proteins extracted from tissue homogenates and native urine samples were analyzed by Human AGP1 PicoKine™ ELISA Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog No: EK1486). GraphPad Prism 7.0 software (GraphPad Prism Software Inc., San Diego, CA, USA) was used for the creation of tables and statistical analysis.

3. Results

As a result of the literature review, it was seen that there were 7 studies on prostate cancer, 5 on bladder cancer, and 2 on renal cancer, and no studies on testicular or penile cancer. When all these studies, which we summarized in the introduction section, are examined, it was understood that ORM1 has three important effects on urological malignancies. (a) Serum and urine ORM1 levels and glycoforms can be used as a valuable biomarker. (b) Orosomucoid-1 is a drug-binding/carrier protein with polymorphic variants that can alter the pharmacokinetics of anticancer drugs such as docetaxel. (c) Orosomucoid-1 has significant anti-inflammatory, immunomodulatory and pro-angiogenic properties.

As a result of our preliminary experimental study, there was no statistically significant difference between tumor tissues and NAT's ORM1 levels (P= 0.5915) (Figure 1A). Urinary ORM1 levels of patients with pTa stage were slightly decreased compared to urine samples of pT1 or other stage patients, but this decrease was not statistically significant (P= 0.4206) (Figure 1B). Urinary ORM1 levels were found to be weakly positively correlated (r= 0.4657, P= 0.1270) with the bodyweights of the patients.

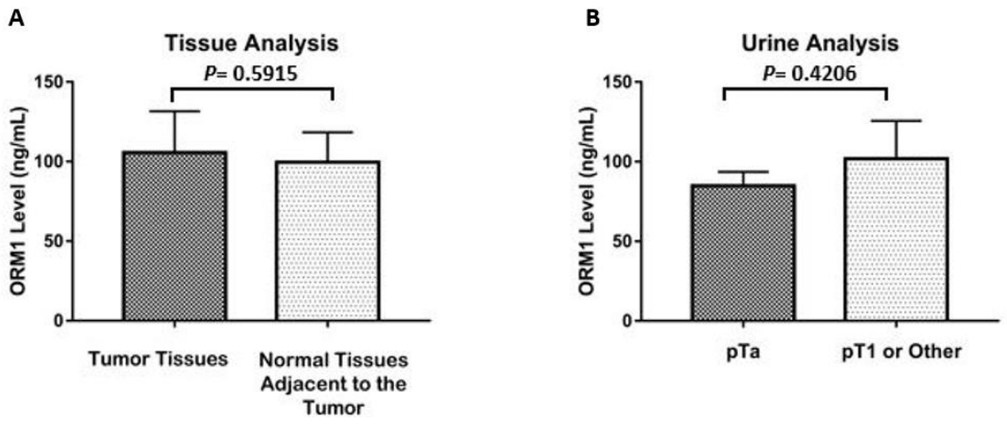

Figure 1. Orosomucoid-1 levels in proteins extracted from tumor tissues and NAT homogenates and native urine samples. (A) Orosomucoid-1 levels of bladder tumor tissues and NAT. (B) Orosomucoid-1 levels in urine samples from patients with pTa and pT1 or other stages.

4. Discussion

The importance of ORM1 in many diseases, especially cancer, is emphasized by researchers. However, very few studies appear to have addressed the relationship between urological malignancies and ORM1. With the demonstration that the urinary ORM1 level in bladder cancer is increased, the researchers thought that ORM1 and its glycoforms may be used as biomarkers for BC. For this reason, most of the studies have been done on urine samples. To the best of our knowledge, ORM1 levels in bladder tumor tissues and NAT have not been investigated in any previous study. In this respect, our study is the first, however, we found that there was no significant difference between tumor tissues and NAT's ORM1 levels. (P= 0.5915).

The importance of correcting for Cr is emphasized for more precise results in the quantitation of ORM1 from urine samples (Hou et al., 2014; Li et al., 2016). Considerable limitations of our study are that Cr levels in urine samples were not investigated, the number of samples was small and no samples were belonging to healthy controls. In our study, urinary ORM1 levels were found to be weakly positively correlated (r= 0.4657, P= 0.1270) with the bodyweights of the patients. Considering the effects of obesity, weight gain, and energy homeostasis on ORM1 (Range et al., 2013; Sun et al., 2016), it can be said that our result is compatible with the literature.

5. Conclusion

Orosomucoid-1 may have important effects on urological malignancies, both with its altering the pharmacokinetics of chemotherapeutics and its immunomodulatory and pro-angiogenic properties. Although no statistically significant difference was found in our study, studies in the

literature indicate that the urinary ORM1 level may be used as a valuable stage-dependent biomarker for BC. Repeating the study with a larger cohort will be informative.

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Investigation of Prevalence of *Blastocystis* spp. in Patients Admitted to Our Hospital with the Diagnosis of Gastroenteritis

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Abstract

Introduction: *Blastocystis* species are common protozoans in worldwide. *Blastocystis* spp. may cause gastroenteritis symptoms such as diarrhea, abdominal pain, bloating, gas, cramps, vomiting, nausea, and anorexia. The aim of this study is to investigate the prevalence of *Blastocystis* species in patients with gastrointestinal complaints.
Materials and Methods: The parasitology data between December 2018-December 2020 in Selcuk University Medical Faculty Hospital Medical Microbiology Laboratory were retrospectively analyzed. According to the results, the prevalence of *Blastocystis* spp. was determined in various age groups.
Results: *Blastocystis* spp. positivity was found in 256 (8.1%) of the patients out of 3147 patients diagnosed with gastroenteritis. Of the patients who were found to be positive for *Blastocystis* spp., 52 were in the 0-18 age range, 87 were in the 26-40 age range, and 35 were over 60. Of the *Blastocystis* spp. positive samples, 65 (25.3%) sent from the Emergency Medicine clinic, 44 (17.1%) from the Gastroenterology clinic, 32 (12.6%) from the Pediatric Emergency clinic and 30 (11.7%) from the Child Health and Diseases Unit.
Conclusion: *Blastocystis* spp.is one of the most common parasite agents in patients with gastroenteritis. Some *Blastocystis* subtypes are thought to be pathogenic, while others are non-pathogenic. Therefore, prospective studies are needed to answer the question of whether *B. hominis* infection is a cause of gastroenteritis symptoms.

Keywords: *Blastocystis* spp, gastroenteritis, direct microscopy

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1. Introduction

Blastocystis spp. was placed in the *Stramenopilis* group based on molecular data obtained from Small Subunit Ribosomal RNA (SSU rRNA) gene sequence studies. Blastocystis is an anaerobic pathogen. The parasite has four important morphological forms: vacuolar, granular, ameoboid, and cyst form. Vacuolar and granular forms are common in feces and in-vitro cultures (Yoshikawa and Iwamasa 2016). For the identification of *Blastocystis* isolates detected in humans and animals, SSUrRNA-based studies were conducted and divided into subtypes (*Blastocystis* ST1-17). ST3 is the most frequently detected subtype in human epidemiological studies (Tunalı et al. 2018).

Blastocystis is a parasite within the scope of the "Water Sanitation and Health program" of the World Health Organization. It is more common in developing countries due to low socio-economic level, improper infrastructure conditions and hygiene (Almeida et al. 2017). Human

transmission occurs with *Blastocystis* cysts. It lives in the thick intestine of man. The parasite is transmitted to humans by cysts as a result of contaminated food and drink, animal contact, and it lives in the large intestine (Tan, 2008).

Blastocystosis may be asymptomatic or manifest with nonspecific gastrointestinal symptoms. *Blastocystis* spp. may cause gastroenteritis symptoms such as diarrhea, abdominal pain, bloating, gas, cramps, vomiting, nausea, and anorexia (Kevin et al. 2010).The examination material for the diagnosis of parasitosis caused by *Blastocystis* is feces. The aim of this study was to investigate the presence of *Blastocystis* spp. in patients with gastrointestinal complaints and to determine its prevalence in various age groups.

2. Materials and Methods

A total of 3147 patients who were admitted to the Medical Microbiology Laboratory of Selcuk University Medical Faculty Hospital between December 2018 and December 2020 and asked for parasitic examination by the clinicians were evaluated retrospectively. Stool samples were examined for parasites by direct microscopic examination (native, lugol) and stool concentration methods. The diagnosis was made by seeing the typical forms of the parasite in the stool. According to the results, the prevalence of *Blastocystis* spp. was determined in various age groups. In addition, the units (clinic or service) where samples were sent were investigated.

3. Results

In 256 (8.1%) of 3147 patients diagnosed with gastroenteritis, *Blastocystis* spp. positivity was detected.The patients with positive *Blastocystis* spp, 135 (53.1%) were male and 121 (46.9%) were female. Of the patients with positive *Blastocystis* spp, 135 (53.1%) were male and 121 (46.9%) were female. Of the patients who were found to be positive for *Blastocystis* spp., 52 were in the 0-18 age range, 38 were in the 19-25 age range, 87 were in the 26-40 age range, 44 were in the 41-60 age range, and 35 were over 60. Of the positive specimens with parasites, 65 (25.3%) were from the Emergency Medicine clinic, 44 (17.1%) from the Gastroenterology clinic, 32 (12.6%) from the Pediatric Emergency clinic, 30 (11.7%)) From the Pediatric Health and Diseases clinic, 25 (9.7) from the Family Medicine clinic, 20 (7.8) from the Medical Microbiology clinic, and 14 (5.4) from the Infectious Diseases clinic.

Table 1. Distribution of *Blastocystis* spp. positivity by age.

Groups	Age range	Number of patients	
		n:256	%
Group 1	0-18	52	(20,3)
Group 2	19-25	38	(15,1)
Group 3	26-40	87	(34,1)
Group 4	41-60	44	(17,1)
Group 5	≥ 60	35	(13,4)

Table 2. Distribution of *Blastocystis* spp. positive patient groups by clinics.

Clinics	*Blastocystis* spp. positive	
	n:256	%
Emergency Medicine	65	(25,3)
Gastroenterology	44	(17,1)
Pediatric Emergency	32	(12,6)
Child Health and Diseases	30	(11,7)
Family Medicine	25	(9,7)
Medical Oncology	20	(7,8)
Infectious Diseases	14	(5,4)
Child Allergy and Immunology	10	(4,0)
Endocrinology and Metobolism Diseases	8	(3,2)
Nephrology	4	(1,6)
General Surgery	4	(1,6)

4. Discussion

The distribution of gastroenteritis factors varies according to age and geographical regions. Numerous bacteria, parasites and viruses cause gastroenteritis. It has been determined in recent studies that *Blastocystis* spp and *Entamoeba coli*, which are protozoans generally accepted as

flora members, may cause intestinal symptoms. In addition, it was concluded that it would be useful to evaluate these parasites in terms of pathogenicity (Endeshaw et al.2007, Kaya et al.2005).

Mumcuğlu et al. aimed to investigate *Blastocystis* spp. frequency and its relation with Inflammatory Bowel Syndrome (IBS). According to the results of the study, *Blastocystis* spp. in terms of incidence, there was no significant difference between the IBS patient group and the Control Group-1 (patients with gastroenteritis complaints) ($p > 0.05$), while a significant difference was found between KG-2 (healthy volunteers) ($p < 0.05$).For this reason, there may be a link between *Blastocystis* spp. and the sypmptoms.

Maçin and Musayeva, investigated the prevalence of *Blastocystis spp*. in pediatric patients with gastrointestinal complaints. *Blastocystis* spp was detected in 233 (31.5%) of the positive samples with parasites. According to the results of the study, *Blastocystis* spp. was found to be one of the most common parasite agents in children with gastroenteritis.

Pektaş et al., investigated the relationship between *Blastocystis hominis* infection and Inflammatory Bowel Syndrome (IBS). According to the results of the study, *B.hominis* was found positive in 13 of 52 patients. Gastroenteritis complaints such as abdominal pain, gas, and diarrhea were observed together in all patients with positive IBS and *B.hominis*. In addition, parasites were detected in 96 (4.4%) samples in the examination of 2160 stool specimens, and *B.hominis* was seen the most in 48 (2.2%). In conclusion, a significant relationship was found between IBS and gastrointestinal symptoms of the disease and the incidence of *B.hominis*.

Ulçay et al. conducted a research to determine which laboratory methods should be used for the detection of intestinal protozoans causing gastroenteritis in immunosuppressive people with diarrhea. Conventional methods for stool samples taken; nativ-lugol (NL), trichrome, modified acid fast (MAF), serological methods; Enzyme Linked Immunosorbent Assay (ELISA), Direct Fluorescent Antibody (DFA) and molecular method; The study was carried out using polymerized chain reaction (PCR). According to research data, *B.hominis* was detected in 3 of 36 diarrhea samples and 1 in 44 samples without diarrhea. As a result of the study, it was determined that protozoans such as *Giardia intestinalis*, *Cryptosporidium parvum*, *B.hominis* and *Entamoeba histolytica* may be responsible for prolonged diarrhea in immunocompromised patients.

5. Conclusion

According to our results, *Blastocystis* spp. is one of the most common parasite in patients with gastroenteritis. According to the results of the conducted studies, it has been determined that there may be a relationship between the incidence of IBS, Crohn's disease, ulcerative colitis, urticaria

and immunosuppressive conditions and the incidence of *Blastocystis* spp. In addition, although the pathogenic role of *Blastocystis* spp. in humans is still controversial, it has been determined that the presence of parasites may be associated with gastroenteritis symptoms according to scientific research results. For this reason, large-scale prospective case-control studies on *Blastocystis* spp.-related diseases will be of great importance in terms of revealing more concrete data on the pathogenicity of the parasite.

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Blastocystis spp. Detection in Patients with Urticaria and Determination of Subtypes

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Abstract:

Blastocystis species are enteric protozoans commonly seen in humans and animals. Today, 17 subtypes have been identified, nine of which have been isolated from humans. Some subspecies detected in humans are thought to be related to pathogenicity, and some subspecies to be apatogenous. In study, it was aimed to detect *Blastocystis* spp. in patients diagnosed with urticaria, to determine the frequency of urticaria associated with the parasite, and to compare these patients with control group patients. Direct microscopic examination was performed using the native-lugol method. *Blastocystis* subtypes were determined using PCR and sequencing methods. İncluded a patient group consisting of 100 patients with urticaria who applied to the Dermatology outpatient clinic of Selcuk University Faculty of Medicine between February 2019 and February 2020, and a control group consisting of 100 healthy volunteers who applied to the Medical Microbiology Laboratory of Selcuk University, Faculty of Medicine. A questionnaire containing information such as patient complaints, socio-economic level and hygiene was applied to the patient group. *Blastocystis* spp. was found positive in 9 (9%) people in the patient group and in 5 (5%) people in the control group. *Entamoeba* spp. and *Blastocystis* spp. were found together in 2 (2%) of the positive samples belonging to the patient group. *Entamoeba* spp. and *Blastocystis* spp. were detected together in 1 (1%) of the positive samples belonging to the control group. *Blastocystis* subtype was found distribution determined in the patient group: ST2 (n = 4, 44,4%), ST3 (n = 3, 33,3%), ST1 (n = 1, 11,1%), ST4 (n = 1, 11,1%), *Blastocystis* subtype distribution determined in the control group: ST3 (n = 2, 40%), ST1 (n = 2, 40%), ST2 (n = 1, 20%). The high rate of ST2 in the urticaria patient group as a result of determining subtypes may indicate that it plays an important role in terms of its pathogenicity. The relationship between ST2 and the symptoms of bloating and abdominal pain is striking according to the data obtained from the questionnaire applied to patients with urticaria.

Keywords: *Blastocystis* spp., urticaria, PCR, subtype.

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1. Introduction

Although *Blastocystis hominis* (*B.hominis*) is the most common parasite among human intestinal protozoans, its pathogenicity is still under discussion (Ok, 1995). In immunocompromised patients, the parasite's resistance to treatment by causing serious infections has caused the parasite to come to the fore more frequently. (Ok, 2007). *B.hominis*, an anaerobic protozoan, lives in the human colon epithelium and lumen. This parasite shows significant morphological variability and karyotype diversity (Noël et al 2005).

Studies show that the prevalence of *Blastocystis* in humans appears to be lower in developed countries (1.5% to 10%) than in developing countries (30% to 50%), and even in a particular country, reported prevalence rates vary greatly (Stenzel and Boreham 1996). The distribution of *Blastocystis*, which differs from country to country, also differs in various regions of the same country. The incidence and prevalence of the parasite is increasing due to reasons such as poor socio-economic level, unsuitability of infrastructure conditions, contaminated use of food and beverages, and hygiene. *Blastocystis* spp. cosmopolitan epidemiology Turkey also shows a wide spread. This parasite has been found both in the human intestine and in vertebrate animals such as mice, rats, chickens, cattle and pigs (Saygı 2016).

The subtypes of the parasite differ according to their reservoirs, geographical distribution and transmission routes. Today, 9 subtypes (ST1-ST9) of Blastocystis spp have been identified in humans. ST3 is the most common subtype found in humans. Seventeen different subtypes of the parasite that may cause harmful or beneficial effects on the host through various mechanisms have been identified (Alfellani et al 2013).

Studies have shown that different subtypes are dominant in different regions (Popruk et al 2013, Wawrzyniak et al 2013). In similar studies, higher rates of ST1-ST4 subtypes have been reported in humans compared to other hosts. Apart from these four subtypes of people; It has been detected in birds, dogs, rats, non-human primates, pigs, and cattle (Villanueva-Garcia et al 2017). ST6 and ST7 are common in poultry and ST5 in pigs. Only ST8 has been isolated from non-human primates. In none of the studies conducted, ST9 could not be isolated from any organism other than human (Yoshikawa et al 2016). *Blastocystis* isolates that infect insects, amphibians and reptiles are still not classified according to subtypes (Betts et al 2018).

Also, different *Blastocystis* subspecies have different pathogenic potentials (Clark 1997). ST1, ST2 and ST4 are held responsible for gastrointestinal symptoms (Kaneda et al 2001). It is associated with Irritable Bowel Syndrome (IBS) and ST7 (Poirier et al 2012). It has been reported that ST1 is associated with increasing pathogenicity (El Safadi et al 2013). It has been suggested that ST3 may cause the pathogenic potential only when the amoeboid form is present (Yan et al 2006).

To investigate the suspected pathogenicity of various *Blastocystis* subtypes; Approaches such as Polymerase Chain Reaction (PCR) based methods (Forsell et al 2017), genomic studies (Gentekaki et al 2017), protein activity studies or metagenomic analysis of fecal microbiota (Forsell et al 2017, Siegwald et al 2017) methods such as can be used.

As in other parasitic diseases, important factors for protection from *Blastocystis* infection are personal hygiene, education and environmental cleanliness (Saygı 2016).

In our study, we aimed to evaluate the *Blastocystis* spp. in children and adults diagnosed with urticaria, to determine the frequency of urticaria associated with the parasite, and to compare these patients with healthy control groups. For this, stool samples taken from the people included in the study were examined by direct examination (native-lugol method) and stool concentration method. Patient samples with *Blastoctysis* spp. detected in stool after examination were studied by PCR method. Paired base sequencing analysis was performed to identify the *Blastocystis* subtypes. In addition, a questionnaire study was conducted with patients diagnosed with urticaria. Participation in the research is entirely voluntary.

2. Materials and Methods

Stool samples obtained from 100 patients with urticaria and 100 volunteers sent to the Medical Microbiology Laboratory were examined. The subjects were informed to give stool samples on three different days within 10 days. Stool samples were examined directly in microscopy using native-lugol method. *Blastocystis* spp. positive cases were added to formalin tube and ependorpha and stored in two ways. *Blastocystis* spp. positive cases were stored at -20ºC until they were included in the study. In addition, a questionnaire containing information such as patient complaints, socio-economic level and hygiene was applied to the patient group in the study, and the relationship between the presence of *Blastocystis* spp. and these variables was investigated.

The samples to be taken into the study were taken out of the -20 ° C cooler and expected to become normal at room temperature. Genomic DNA isolation was performed using (Norgen Biotek, Canada) commercial kit. *Blastocystis* isolates were put into operation on a PCR, Sensoquest (Labcycler, Germany) thermal cycler. PCR products were analyzed by 1.5% agarose gel electrophoresis, followed by gel staining with ethidium bromide and photographed (Gel Logic 200 Imaging System, Kodak).

In the purification stage, the PCR product was purified by using the "HighPrep ™ PCR Clean-up System" (AG-60005) purification enzyme for the single band samples obtained, following the kitin procedures. For Sanger Sequencing samples, the ABI 3730XL Sanger sequencer (Applied Biosystems, Foster City, CA) and BigDye Terminator v3.1 Cycle Sequencing Kit were used in the Macrogen laboratory (Applied Biosystems, Foster City, CA).

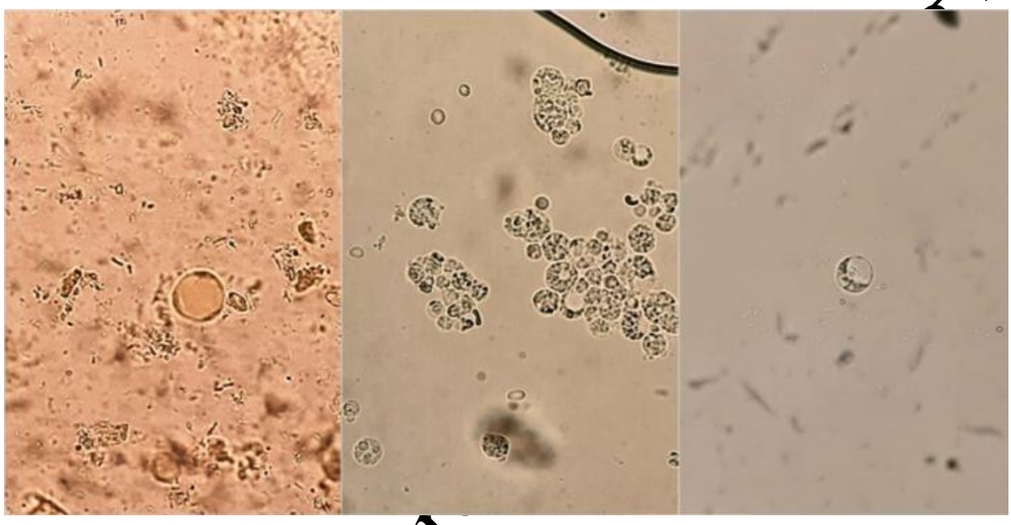
3. Results

A total of 200 stool specimens were taken into the study and *Blastocystis* spp. was accepted as positive if at least one microorganism was seen in a microscope field at x400 magnification. The distribution of parasites in the patient and control groups is given in Table 1.

Table 1. Parasite distribution according to patient and control groups.

Parasite distribution	**Patient group n=100**		**Control group n=100**		**Total n=200**	
	n	(%)	n	(%)	n	(%)
***Blastocystis* spp.**	7 (7%)		4 (4%)		11 (5,5%)	
***Blastocystis* spp. + *Entamoeba* spp.**	2 (2%)		1 (1%)		3 (1,5%)	
***Entamoeba* spp.**	-		2 (2%)		2 (1%)	

Different forms of *Blastocystis* in different sizes were seen in our study. Vacuolar forms are given in figure 1.



Fiqure 1. Vacuolar forms of *Blastocystis* spp., seen in different sizes in direct microscopic examination.

After direct microscopic examination, genomic DNA isolation was performed from 14 stool specimens with *Blastocystis* detected and *Blastocystis* SSU rRNA gene-specific PCR was studied and amplification was observed at the expected size (~ 119bp) (**Figure 2**).

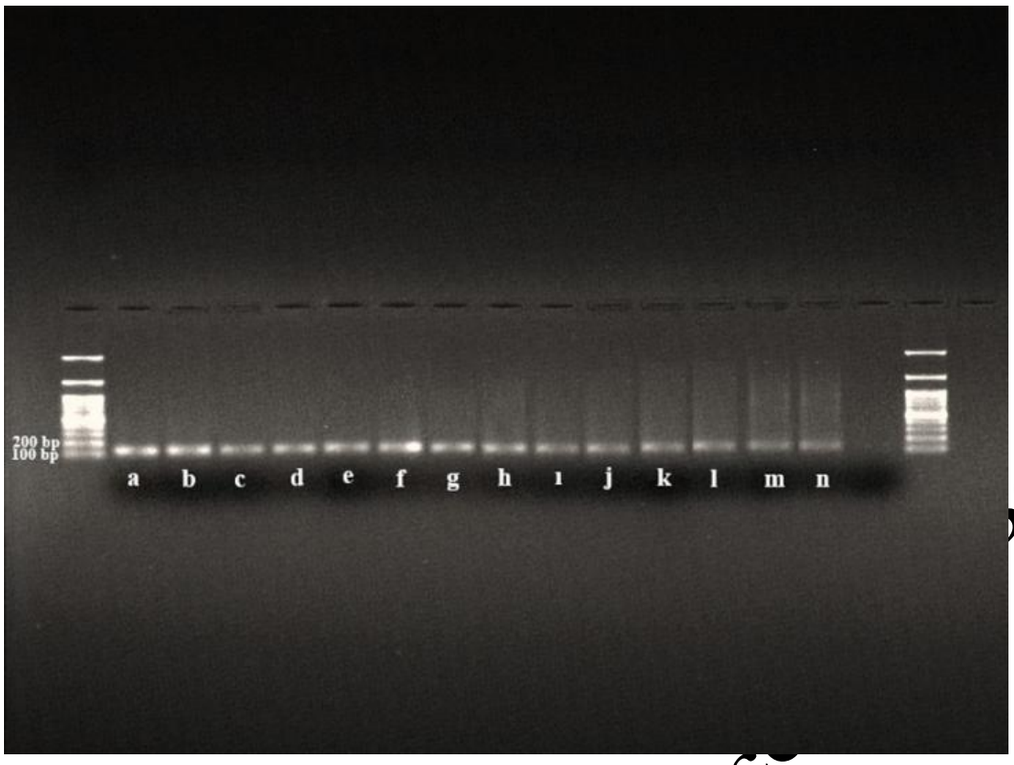


Figure 2. Gel image of *Blastocystis* spp. detected positive by PCR. *Blastocystis* spp. genes in the patient group (a, b, c, d, e, f, g, h, ı) and control group (j, k, l, m, n) are seen at the size of 119 bp.

DNA sequencing was read bidirectionally. DNA sequences obtained from stool samples using DNA isolation and PCR methods were sequenced with reference sequences and possible false nucleotide readings were corrected by examining the chromatogram images (Figure 3).

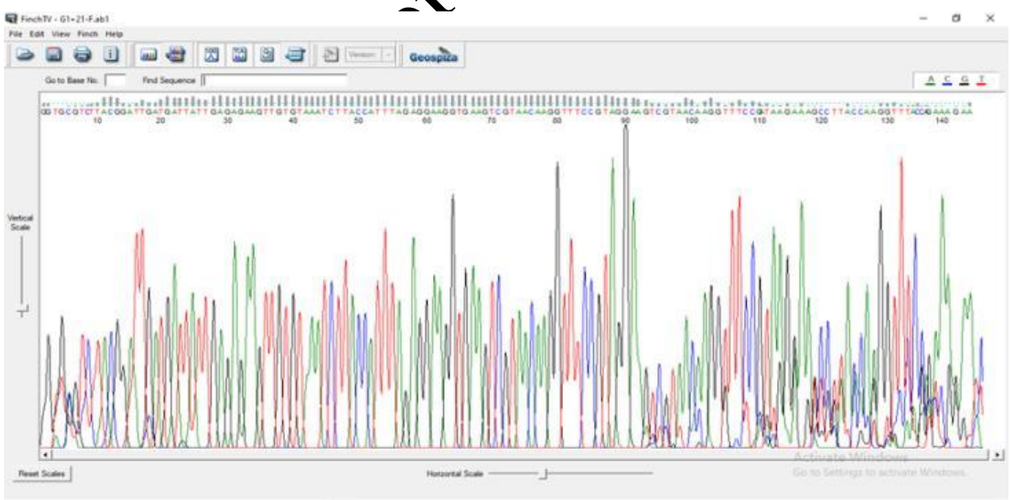


Figure 3. Chromatogram file of one of *Blastocystis* DNA sequences read bidirectionally.

Table 2. Distribution of *Blastocystis* subtypes according to patient and control groups.

Subtype	**Patient**		**Control**	
	n=100	**(%)**	**n=100**	**(%)**
ST1	1 (%1)		2 (%2)	
ST2	4 (%4)		1 (%1)	
ST3	3 (%3)		2 (%2)	
ST4	1 (%1)		-	
Total	9 (%9)		5 (%)	

According to the results of the survey, 87 (87%) of the urticaria group patients stated that they lived in the city center and 13 (13%) lived in the district. 11 (11%) of the patients stated that they used ready-made water, 28 (28%) mains water, 61 (61%) mains water and ready-made water. While giving stool samples, 11 (11%) of the patients stated that they used antibiotics and 4 (4%) reported that they fed pets.Symptoms of *Blastocystis* spp. positive urticaria group patients are shown in **Table 3**.

Table 3. Distribution of patient complaints according to acute and chronic urticaria patient groups.

Symptoms	**Acut urticaria (n=36)**	**Cronic urticaria (n=64)**	**Total (n=100)**
	n (%)	n (%)	n (%)
İtching	34 (94,4%)	59 (92,1%)	93 (93%)
Redness	31 (86,1%)	58 (90,6%)	89 (89%)
Blister	11 (30,5%)	35 (54,6%)	46 (46%)
Fever	8 (22,2%)	36 (56,2%)	44 (44%)
Swelling	9 (11,1%)	31 (48,4%)	40 (40%)
Skin rash	3 (8,3%)	19 (29,6%)	22 (22%)
Abdominal pain	1 (2,7%)	8 (12,5%)	9 (9%)
Diarrhea	3 (8,3%)	1 (1,5%)	4 (4%)
Nausea	1 (2,7%)	3 (4,6%)	4 (4%)
Constipation	-	3 (4,6%)	3 (3%)
İndigestion	-	3 (4,6%)	3 (3%)
Womiting	1 (2,7%)	1 (1,5%)	2 (2%)
Weight loss	-	-	-

Table 4. Patient group relationship between symptoms and subtypes of *Blastocystis* spp. positive patients.

Symptoms	**ST1 (n=1)**	**ST2 (n=4)**	**ST3 (n=3)**	**ST4 (n=1)**	**Total (n=9)**
Redness	1	3	3	1	8
İtching	1	3	2	1	7
Fever	1	2	2	1	5
Blister	1	1	1	-	3
Swelling	-	2	-	-	2
Abdominal pain	-	1	-	-	1

4. Discussion

Urticaria is a heterogeneous disease group with a highly variable clinical picture and may be caused by various factors (Gupta and Parsi 2006). One of the causes of urticaria is known to be protozoa and helminths. The cause of itchy lesions is histamine release in the skin as a result of the immune mechanism. It has been reported that parasites stimulate the secretion of certain immunological mediators secreted from TH2 in the lumen, such as IL-3, IL-4, IL-5 and IL-13, to induce histamine release as a result of the IgE response (Karaman 2009).

Turkey's Malatya province made a total of 100 urticaria patients were studied in 21 patients in a study, and was *Blastocystis* viewed. 11 (52.4%) of the *Blastocystis* positive patients were diagnosed with chronic urticaria and 10 (47.6%) patients with acute urticaria diagnosis. In the statistical evaluation, no significant conclusion could be reached about the relationship between allergic urticaria and parasitosis (p = 0.81) (Aycan et al 2011). In our study, *Blastocystis* spp. was detected in a total of 9 cases in the urticaria group and 6 of them were chronic urticaria and 3 were patients with acute urticaria.

In our study, 100 people were included in the patient group with urticaria. *Blastocystis* spp. was not detected in the pediatric patient group, but in 9 patients in the adult patient group. In a study, 17 patients (22.3%) in the pediatric patient group, 16 children (14.6%) in the pediatric control group, 7 patients (18.4%) in the adult patient group, and 3 patients (8.8%) in the adult control group parasites were detected and these differences were not statistically significant (p = 0.181 and p = 0.376, respectively). In the pediatric patient group (18.4%), the number of *Blastocystis* spp. positive samples was higher than the pediatric control group (13.7%). This difference was

not statistically significant (p = 0.155). In cases with parasites, improvement in CSU symptoms by approximately 50% after appropriate antiparasitic treatment was considered as an important finding (Vezir et al 2019).

There are some studies showing that the ameboid form of *Blastocystis* spp. may be associated with pathogenicity (Katsarou-Katsari et al 2008). In a study conducted in Egypt, the amoeboid form was found in 60.6% of patients with *Blastocystis* positive urticaria, but not in any healthy controls. A total of 54 patients with urticaria and 50 people from the control group were included in the study. Stool samples were examined by microscopy and evaluated by PCR. Significantly higher number of parasites (P <0.001) were detected in the patient group compared to the control group. There was no significant difference between patients with acute and chronic urticaria (P = 0.2). While 33 (61.1%) of 54 patients were positive for *Blastocystis*, only 4 (8%) of 50 controls were found to be positive (Zuel - Fakkar et al 2011). Vacuolar and cyst forms were generally seen in our study.

In our study, when the five most commonly reported symptoms (itching, rash, swelling, fever and swelling) in the patient group are evaluated, it is thought that the itching and rash symptoms may be related to the *Blastocystis* subtype distribution. In addition, the relationship between ST2 and bloating and abdominal pain symptoms is remarkable. Many studies have been conducted to determine the relationship between *Blastocystis* spp. and clinical findings. In one of these studies, a total of 554 stool specimens were included to investigate the prevalence and clinical significance of *Blastocystis* between symptomatic and asymptomatic groups. Asymptomatic and symptomatic patients were found to be 64/398 (16.08%) and 29/156 (18.58%) *Blastocystis* positive respectively. There was no significant relationship between patient symptoms and *Blastocystis* (P = 0.528), but a statistically significant relationship was observed between urticaria and *Blastocystis* positivity (P <0.05). The relationship between the presence of *Blastocystis* and symptoms was diarrhea 19/116 (16.37%), constipation 10/59 (16.94%), bloating 19/122 (15.57%), nausea 15/105 (14.28%), respectively. and urticaria was determined as 5/7 (71.42%) (Riabi et al 2017).

Host specificity and pathogenic potential of different isolates were found to be correlated with sequence variations in the SSU rRNA gene (Skotarczak 2018). The striking genetic heterogeneity among *Blastocystis* isolates indicates a relationship between different subtypes and pathogenicity. It is unclear whether *Blastocystis* is directly associated with the allergic manifestation or a common component of the gut microbiota. A study was conducted to evaluate the molecular diversity of *Blastocystis* spp. in patients with urticaria in Brazil in 2019. Stool samples of 58 urticaria patients were examined by parasitological methods and then analyzed by PCR using primers specific to *Blastocystis*. Subtypes (STs) and alleles (a) were determined using BLASTn and MLST tools. ST1, ST2, ST3, ST4, ST6 and mixed infection (ST1 + ST3) were detected in

patients with urticaria, ST1 (a4), ST3 (a34 and a36) and ST4 (a42) were the most common (Baptista et al 2019).

Today, it is accepted by many researchers that the majority of individuals infected with *Blastocystis* are asymptomatic. In addition, recent studies have claimed that *Blastocystis* may even be a marker of a healthy intestinal flora (Audebert et al 2016, Stensvold and Clark 2016). As a result of studies, it is accepted that clinical findings develop depending on many factors such as *Blastocystis* genotype, host immune response, *Blastocystis* density, microbiota, and other accompanying infections (Tan et al 2010, Andersen and Stensvold 2016).

In a study similar to our study, 16 urticaria examinations (16/133, 12%) were found to be *Blastocystis* positive in stool samples, and seven (7/133, 5.3%) had acute and nine (9/133, 6.8%) patients with chronic urticaria. ST1 was found in three patients with acute urticaria, ST2 in three, and ST3 in three, ST1 in one and ST3 in eight patients with chronic urticaria. *Blastocystis* positivity was detected in two samples (2/123, 1.6%) in the control group and two samples of them were determined as ST3. P = 0.67). *Blastocystis* positivity ($P < 0.01$) was found to be a significant difference between urticaria patients and controls on the other page. Subtype (P = 0.67) or *Blastocystis* alternative and gastrointestinal-like no significant difference was found. The symptoms of the patients with *Blastocystis* positivity were as follows: itching (16/16), gas (11/16), bloating (9/16), nausea (6/16), abdominal pain (5/16), constipation (5 / 16), diarrhea (3/16), vomiting (1/16) and weight loss (1/16). This important result is the disappearance of urticaria lesions after *Blastocystis* positive metronidazole treatment, and this reveals the possibility of a relationship independent of gastrointestinal symptomatology and subtype between urticaria and *Blastocystis* (Aydın et al 2019). In our study, the most frequently reported five symptoms in the patient group were as follows: redness 8/9, itching 7/9, fever 5/9, blister 3/9, swelling 2/9, abdominal pain 1/9.

5. Conclusion

Although the occurrence of *Blastocystis* in both symptomatic and asymptomatic patients creates doubts about whether it is pathogenic or not, the parasite is now accepted as a pathogen. The most important problem in studies on *Blastocystis* is that a suitable experimental model has not been found (Saygı 2016).

Many studies have reported that there is a close correlation between *Blastocystis* positivity and urticaria, and some studies have indicated that this parasite can cause urticaria by activating certain immune mediators (Balint et al 2014, Lepczynska et al 2016).

Blastocystis isolates, whose relation with pathogenicity were investigated in our study, were detected at a rate of 9/100 (9%) in the patient group and 5/100 (5%) in the control group, and 9 subtypes known to cause infection in humans were studied. In our study, the low rate of *Blastocystis* isolates compared to some studies is thought to be due to the good socio-economic level and attention to hygiene conditions.

Among the 9 subtypes identified in the patient group with urticaria, ST2 was found in 4 samples as the most dominant subtype. After ST2, ST3 (n = 3), ST1 (n = 1), ST4 (n = 1) followed, respectively. ST3 (n = 2), ST1 (n = 2), ST2 (n = 1) were detected in a total of 5 subtypes determined in the healthy control group. In our study, it was determined that ST2 was high in the patient group. Among all the subtypes identified in the patient and control groups, ST3 and ST2 were determined as the dominant subtypes.

When we look at the relationship between symptoms and subtypes in the urticaria patient group, 8 (8/9) of 9 *Blastocystis* spp. positive patients had rash, 7 (7/9) had itching, 6 had fever (6/9), and 3 had swelling (3/9), 2 had swelling (2/9) and 1 (1/9) had abdominal pain symptoms. Symptoms of bloating and abdominal pain were found only in patients with ST2. As a result of the questionnaire analysis, it was concluded that there may be a relationship between ST2 and symptoms.

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Evaluation of Rheumatoid Factor and Antinuclear Antibody in Rheumatoid Arthritis Patients

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Abstract

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory and destructive joint disease that affects 0.5-1% of the population in the industrialized world and commonly leads to significant disability and consequently a reduction in quality of life. In this study we aim to investigate rheumatoid factor and antinuclear antibody positivity of rheumatoid arthritis patients.
Materials and Methods: In the study, antinuclear antibodies and rheumatoid factor result of patients were retrospectively analyzed between 01.01.2020-31.12.2020 dates. Rheumatoid factor was studied with nephelometric method (Beckman Coulter Immage 800, USA). Antinuclear antibody test was carried out in serum by Indirect Fluorescent Antibody (IFA) method and was examined under fluorescence microscopy.
Results: In this study, totally 3885 blood samples were analysed and 176 of patients were prediagnosed with rheumatoid arthritis. 89 (71.7%) of the patients were female and 35 (28.3%) of patients were male. Of the 176 rheumatoid arthritis patients, 124 were rheumatoid factor positive and 24 were ANA positive.
Conclusion: The ANA test alone is not a diagnostic test for a rheumatic disease, but it supports the diagnosis of rheumatic diseases. 24 (20%) of the RA patients were also positive for ANA and RF. Rheumatoid factor is a sensitive, but nonspecific test for rheumatoid arthritis. We detected 70.4% of the RA patients were also positive for RF. We believe that If all tests were done the number of ANA positivity would increase. Therefore, we suggest that rheumatoid factor and ANA tests positivity should be controlled for rheumatoid arthritis patients.

Keywords: Antinuclear Antibody, Rheumatoid Arthritis, Rheumatoid Factor

Financial Disclosure: The authors declared that this study has received no financial support.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory and destructive joint disease that affects 0.5-1% of the population in the industrialized world and commonly leads to significant disability and consequently a reduction in quality of life. Rheumatoid arthritis (RA) is a chronic, inflammatory disease whose etiology is unknown, mostly involving synovial joints and with progressive destruction around the joint (Singh et al. 2016). Rheumatoid factors are antibodies directed against the Fc region of immunoglobulin G (IgG). It was positive in 70-80% of patients with rheumatoid arthritis. Rheumatoid factor is a sensitive but non-specific measure for RA. Since RA is a systemic disease, other parts and organs of the body are also affected in advanced stages. RF

positivity is not specific for RA. It can also be seen in infectious and autoimmune diseases with B cell activity, hyperglobulinemia and B cell lymphoproliferative diseases (Koopman and Schrohenloher 1988, Francesca et al. 2013). The antibodies that are created by the body against its own cell nuclei are called as anti-nuclear antibodies (ANA). The ANA test is a frequently used laboratory test for screening autoimmune diseases, especially in patients with musculoskeletal complaints or skin findings (Fritzler et al. 2003). In this study, we aim to investigate rheumatoid factor and antinuclear antibody positivity of rheumatod arthritis patient's.

2. Materials and Methods

In this study, results of antinuclear antibodies and rheumatoid factor were retrospectively analyzed between 01.01.2020-31.12.2020 dates. The RF test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction. Rheumatoid factor test was carried out with nephelometric method (Beckman Coulter Immage 800, USA). Due to its high sensitivity and specificity, the indirect immunofluorescence test (IIFT) using human epithelial cells (HEp-2) and primate livet is the gold standart for the detection of anti-nuclear autoantibodies (ANA). Antinuclear antibody test was carried out in serum by IIFT method and results evaluated with fluorescence microscopy. All tests were carried out with manufacturer's recommendations (Euroimmun, Lübeck, Almanya) .

3. Results

In this study, totally 3885 blood samples were analysed and 176 of patients were prediagnosed with rheumatoid arthritis. Of the 176 rheumatoid arthritis patients, 124 were rheumatoid factor positive and 24 (19.4%) were ANA positive (Figure 1).

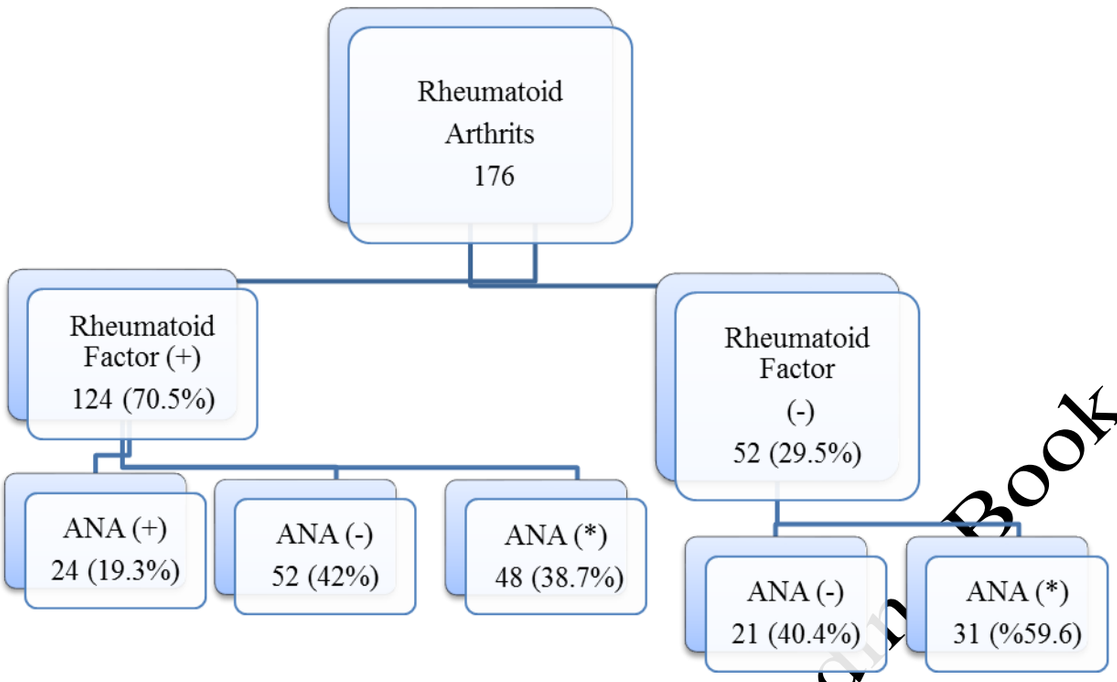

Figure 1. Evaluation of RF and ANA test results.

RF positive 89 (71.7%) patients were female and 35 (28.3%) were male. 21 (87.5%) of ANA positive patients were female and 3 (12.5%) were male. Highest positivity of both ANA and RF tests were detected over the 40 years (45.9%, 83%) patients (Table 1).

Table 1. Distribution of RF and ANA test positivity by age.

Age range	RF Test		ANA test	
	Positive	**%**	**Positive**	**%**
0-20	4	3.3	5	20.8
21-40	17	13.7	8	33.3
40≤	103	83	11	45.9
Total	**124**	**100**	**24**	**100**

Although different ANA patterns were detected, the most granular and homogeneous patterns were identified (Table 2).

Table 2. ANA patterns of positive patients.

Number	Patterns
5	1/100 Granules
2	1/100 Homogeneous
2	1/320 Homogeneous
1	1/1000 Granules
1	1/100 Nucleolar
1	1/640 Centromeres
1	1/320 Granules+ granular chromosomal
1	1/320 Granules+ granular chromosomal+cytoplasma granular
1	1/100 Homogeneous, 1/100 a few dots
1	1/100 Cytoplasma granular+1/320 Dots
1	1/1000 Nucleolar, 1/100 Granules
1	1/100 Cytoplasma granular+1/320 Homogeneous
1	1/320 Homogeneous+1/320 Granules
1	1/100 Homogeneous+1/100 Granules
1	1/320 Granules
1	1/320 Cytoplasma granular
1	Vimentin

4. Discussion

Rheumatoid Arthritis, one of the most common autoimmune diseases, is a chronic polyarthritis table that leads to disability as a result of being late in diagnosis and treatment. Early diagnosis of RA is very important in terms of preventing damage to joint tissue with treatment. In patients with typical symptoms, diagnosis can often be easily made in the first year of the disease. But often in the first period of the disease, the clinical symptoms are not obvious. In many patients with atypical progressive symptoms, it can take a long time to diagnose RA (Smolen et al. 2016, Gavrila et al. 2016).

Although rheumatoid factor (RF) is a test that is evaluated for the diagnosis of RA, it is used in rheumatic and autoimmune diseases other than RA. Rheumatoid factor is a sensitive, but nonspecific test for rheumatoid arthritis (Francesca et al. 2013). Sensitivity to sunlight may be requested in the presence of clinical signs such as rash, arthritis, Raynoud phenomenon, or in the presence of laboratory tests such as esh and CRP, which are routine and inexpensive. In previous studies, laboratory abnormalities and clinical manifestations such as arthritis, Raynaud's

phenomenon have also been reported to be rare in individuals with main positivity but without rheumatic disease (Suresh 2004). The ANA test alone is not a diagnostic test for a rheumatic disease, but it supports the diagnosis of rheumatic diseases. A positive ANA test is not always a sign of a Rheumatological disease; because low positivity can be detected in the population. Therefore, ANA test results should be evaluated with the patient's clinic (Fritzler et al. 2003, Xavier et al. 2020). In a study carried on 36 rheumatoid arthritis patients, RF and ANA positivity were found respectively 26 (72.2%) and 17 (47.2%) (Sertpoyraz 2013). In our study 124 (70.4%) of the RA patients were positive for RF, 24 (20%) of the RA patients were positive both ANA and RF. ANA test of 48 RF positive patients was not studied (ANA test was not requested).

5. Conclusion

We believe that if all RF positive patients were studied ANA, the number of ANA test positivity would increase. Therefore, we suggest that rheumatoid factor and ANA tests positivity should be controlled for rheumatoid arthritis patients.

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Evaluation of HCV-RNA, Serum Transaminase and AST/ALT Levels in ANTI-HCV Positive Patients

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Abstract

Objective: Hepatitis C Virus (HCV) is an enveloped, single-stranded RNA virus from the Flaviviridae family. HCV infection may cause 80-85% chronic liver disease, cirrhosis and hepatocellular carcinoma. In this study, it was aimed to evaluate the HCV-RNA and serum transaminase levels in patients with positive anti-HCV.
Method: Serum samples of 900 patients with anti-HCV positivity sent to Selcuk University Medical Faculty Medical Microbiology Laboratory between January 1, 2015 and December 31, 2019 were included in the study. HCV RNA, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values, which were studied simultaneously with the anti-HCV levels of the patients, were evaluated retrospectively. HCV RNA levels of the patients were studied by real time PCR, anti-HCV levels by chemiluminescence microparticle immunassay, and serum transaminase levels by spectrophotometric enzymatic method. Three patient groups with anti-HCV values between 0.9-4.99 (Group 1), 5-10 (Group 2) and > 10 (Group 3) were formed.
Results: HCV-RNA was found positive in 1.1% of group 1, 14.3% of group 2 and 64.2% of group 3. Mean AST and ALT levels were 26.9-22.6 IU / L in group 1, 38.4-43.8 IU / L in group 2 and 45.6-42.7 IU / L in group 3, respectively.
Conclusion: In our study, while there were 164 (35.8%) HCV-RNA negative samples from 458 samples with high anti-HCV values, there were 294 (64.2%) HCV-RNA positive samples. HCV infection should be evaluated with anti HCV, transaminase and viral load levels.

Keywords: Hepatitis C virus, HCV RNA, anti-HCV, transaminase, ALT/AST rat

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1. Introduction

Hepatitis C virus (HCV), which is an enveloped, single-stranded RNA virus in the Flaviviridae family, is one of the most important pathogens in terms of causing acute and chronic hepatitis, having a high level of chronicity, causing cirrhosis, hepatocellular carcinoma and death. It is one of the leading causes of liver transplantation in developed countries (Thomas 2013, Barut and Günal 2009). Approximately 700,000 people die each year due to HCV-related causes (WHO2019). According to current country data, approximately 3% of the world population is infected with HCV. According to the data of the World Health Organization (WHO), it is estimated that 71 million people have HCV infection (Shepard et al 2005).

For all these reasons, the diagnosis of HCV is extremely important. Anti-HCV antibody is examined serologically in the diagnosis of infection and HCV-RNA levels in the detection of viremia as a molecular method. In addition to all these, HCV-RNA viral load and liver transaminase levels are also monitored during the treatment and the follow-up. It is important to determine HCV genotypes in planning the treatment period and dose before treatment (Türkoğlu 2007). While the severity of inflammation and fibrosis in the liver is determined by liver biopsy, there is not any non-invasive method that can give an idea about the severity and prognosis of the disease. Therefore, although the monitoring of liver transaminase levels is not specific to the disease, it can be used in the diagnosis and follow-up of the infection (Uygun and Polat 2005). In addition, progressive liver damage can be seen in the majority of those with acute HCV infection, and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels may remain constantly high or increase and decrease intermittently in these patients (Kaşifoğlu et al 2007). ALT and AST increase is parallel in most liver diseases, and the AST / ALT ratio is ≤1. ALT activity generally exceeds AST activity in toxic and viral hepatitis, chronic hepatitis and cholestatic hepatitis. Conditions in which AST is higher than ALT are alcoholic, neoplastic or infiltrative liver disease and non-biliary cirrhosis (Günşar 2006).

In this study, it was aimed to evaluate HCV-RNA, serum transaminase and AST / ALT levels in anti-HCV positive patients.

2. Materials and Methods

Serum samples of 900 patients with anti-HCV positivity between January 1, 2015 and December 31, 2019 in the Medical Microbiology Laboratory of Selcuk University Faculty of Medicine were included in the study. HCV RNA, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values, which were studied simultaneously with the anti-HCV levels of the patients, were evaluated retrospectively. HCV RNA levels of the patients were studied by real time PCR, anti-HCV by chemiluminescence microparticle immunassay, and serum transaminase levels by spectrophotometric enzymatic method.

* Patients divided into three groups;
    * Group 1: Anti-HCV values between 0.9 and 4.99 signal-to-cut ratio (s/co) value
    * Group 2: 5-10 s/co
    * Group 3: > 10 s/co

3. Results

41.4% of the samples examined within the scope of the study are in Group 1, 7.8% are in Group 2, and 50.8% are in Group 3 (Table 1). HCV-RNA positivity was detected in 1.1% of patients in Group 1, 14.3% of patients in Group 2, and 64.2% of patients in group 3 (Table 2). Mean AST and ALT levels were found respectively as 26.9-22.6 IU/L in group 1, 38.4-43.8 IU/L in group 2 and 45.6-42.7 IU/L in group 3. When the AST/ALT ratio was examined, it was determined as 1.2 IU/L in group 1, 0.8 IU/L in group 2 and 1.1 IU/L in group 3 (Table 3).

Table 1. Distribution of anti-HCV positive patient groups.

Groups	Anti-HCV Value Range	Number of patients (n= 900)
Group 1	**0,9 - 4,99**	**372 (41,4%)**
Group 2	**5,0 - 10,0**	**70 (7,8%)**
Group 3	**10<**	**458 (50,8%)**

Table 2. Distribution of HCV-RNA results belonging to the groups.

	Group 1 (n:372)	Group 2 (n:70)	Group 3 (n:458)
HCV-RNA Negative	**368 (98,9%)**	**60 (85,7%)**	**164 (35,8%)**
HCV-RNA Positive	**4 (1,1%)**	**10 (14,3%)**	**294 (64,2%)**

*Chi-square test (X^2=376.676, ($p<0,001$)

Table 3. AST, ALT values and AST/ALT ratios by groups.

	Group 1 (n:372)	Group 2 (n:70)	Group 3 (n:458)
AST	**26,9**	**38,4**	**45,6**
ALT	**22,6**	**43,8**	**42,7**
AST/ALT	**1,2**	**0,8**	**1,1**

* AST Chi-square test : 64.389, p<0.001
ALT Chi-square test : 116.617, p<0.001

4. Discussion

HCV infection is one of the important causes of mortality and morbidity in our country and in the world. The screening and diagnosis process for HCV infection starts with the detection of antibodies against HCV. Negative antibody excludes infection in the absence of severe immunosuppression and recent exposure. If the anti-HCV antibody is found to be positive, the current medical approach is to confirm the active infection by examining HCR RNA or HCV

core antigen, and failure to do so may cause the patient not to be diagnosed and treated, and chronicity and transmission (Manns et al 2017). Serological and molecular methods are used in the routine laboratory diagnosis of HCV infections (Türkoğlu 2007).

Since anti-HCV antibodies can be detected in patients who fully recover and become chronic in HCV infections, laboratory findings are not sufficient to distinguish between a previous infection and an active infection. At the same time, antibody response occurs late or not at all in some cases (Us et al 2001).

A total of 900 patients were included in this study, which was conducted to compare the HCV-RNA levels and serum transaminase levels of anti-HCV positive patients and to evaluate their relationship with serum anti-HCV. Three separate patient groups were formed as those with anti-HCV values between 0.9 and 4.99 (Group 1), those between 5-10 (Group 2), and those with an anti-HCV value of> 10 (Group 3). As a result of the study, it was observed that there was an increase in HCV-RNA positivity due to the increase in Anti-HCV value. Similarly, average AST and ALT values were determined as 26.9 IU / L and 22.6 IU / L in Group 1, 38.4 and 43.8 IU / L in Group 2, 45.6 and 42.7 IU / L in Group 3, respectively. The AST/ALT ratios were found respectively as 1.2 IU/L in group 1, 0.8 IU/L in group 2 and 1.1 IU/L in group 3. As a result of the study (Kayman et al 2013) on 1000 serum samples in order to compare HCV-RNA levels with anti-HCV and transaminase levels of patients with HCV pre-diagnosis / diagnosis and to evaluate serum AST / ALT ratios, 52.7% of the samples had both HCV RNA and It was determined that the anti-HCV was positive in Group 3, ALT and AST levels were higher in Group 3 compared to Groups 1 and 2, and AST / ALT ratio was higher in the samples in Group 2 than in Groups 1 and 3. In the study conducted by Kaşifoğlu (Kaşifoğlu et al 2007) on 690 serum samples, anti-HCV positivity was detected in 65.9% of the samples. HCV-RNA was found positive in 51.6% of the anti-HCV positive samples. Gökahmetoğlu et al 2002 found HCV-RNA positivity as 36.5%, Sönmez et al (Sönmez et al 1996) 66%, and Çolak et al (Çolak et al 1998) 72.4%.

AST and ALT, which are in the aminotransferase enzyme group, are normally found in serum at low concentrations (30-40 IU/L). This condition is thought to be related to normal cell cycle and regeneration. As a result of the increased permeability due to the increase in hepatocyte destruction, these enzymes go out of the cell membrane and their serum levels increase for this reason. While ALT is an enzyme relatively specific to the liver, AST is found in hepatocytes as well as in heart and skeletal muscle, kidney, brain, pancreas and erythrocytes. For this reason, ALT elevation reflects higher hepatocellular destruction more specifically than AST. However, it is more appropriate to request both tests in order to exclude extrahepatic causes leading to AST elevation alone. The increase in ALT supports that the increased AST is liver-based (Uygun and Polat 2009, Nafees et al 2010).

In this study, it was observed that AST and ALT values of the patients in Group 1 were lower than the others, and the patients in Group 3 were higher. (Bacon 2002) reported that ALT levels are normal in approximately 30% of patients with chronic HCV infection. Similarly, Puoti et al. reported that 30% of those with chronic HCV infection consistently had normal ALT levels (Puoti et al 2010). Külah et al. (Külah et al 2007) found that ALT values were high in 22% of HCV-RNA positive samples and in 40% of anti-HCV positive samples. Fındık et al. (Fındık et al 2011) reported high transaminases in 53% of anti-HCV and HCV-RNA positive samples. These results show that transaminase values alone cannot be sufficient alone in the course of the disease.

In the study, it was observed that the AST/ LT ratio was 1.2 IU/L in Group 1, 0.8 IU/L in Group 2 and 1.1 IU/L in Group 3. The high rate of AST/ALT indicates that the chronic disease process reaches more advanced levels in the patients in Group 1. (Kayman et al 2012) found that the mean AST / ALT ratio in Group 2 was higher than the other groups in their study.

5. Conclusion

Considering the results obtained from the study and the literature, it can be said that it would be appropriate to evaluate HCV infection together with anti-HCV, HCV RNA and transaminase in the laboratory process in clinical management. As the S / co values increase, liver function appears to be impaired and high s/co values should be evaluated more carefully.

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Investigation of Seroprevalence of Hepatitis B, Hepatitis C and HIV in Hemodialysis Patients

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Abstract

Aim: Impairments in the immune system of patients undergoing HD cause patients to be more sensitive to infection agents. The purpose of this study; to determine the seroprevalance of HBV, HCV and HIV in patients undergoing hemodialysis treatment.
Materials and Methods: HBsAg, Anti-HBs, Anti-HBc IgM, Anti-HBc IgG, HBV-DNA, Anti-HCV, HCV-RNA and Anti-HIV parameters studied from serum samples of Chronic Kidney Patients (CKD) undergoing hemodialysis treatment at Selçuk University Faculty of Medicine It has been scanned retrospectively for ten years.
Results: HBsAg was determined in 17 (0.40%) of 4155 hemodialysis patients and Anti-HBs was positive in 454 (11.2%). Anti-HCV was detected as positive in 14 (0.33%) serum. HBV-DNA was found to be positive in 13 (76.5%) of HBsAg positive HD patients and in 12 (85.7%) of Anti-HCV positive patients. Anti-HIV antibodies were not detected in any of the serum samples.
Conclusions: The results of our study have shown that proper infection control measures in hemodialysis patients can reduce the contamination of infectious agents.

Keywords: Hemodialysis, Chronic Kidney Patients, Hepatitis B, Hepatitis C, HIV

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1. Introduction

Chronic kidney disease (CKD) is a common health problem all over the world. CKD is a disease with progressive and irreversible loss of function in the kidney's metabolic and endocrine functions as a result of a decrease in fluid electrolyte balance and a decrease in glomerular filtration. Due to the increasing frequency of CKD in the world and in Turkey, high morbidity and mortality rates are observed (Nissenson and Fine, 2009). It is defined as a serious public health problem due to the serious effect of the disease on the quality of life, the high cost of renal replacement therapies required for its treatment and the poor prognostic course (Mohanty et al., 2020). An effective hemodialysis has been found to reduce morbidity and mortality. However, providing an effective HD depends on more than one factor. Some of these factors are; dialysis dose, nutritional status of patients, presence of comorbidity, degree of anemia, socio-economic status, compliance, adequate blood flow and membrane type used for hemodialysis (Kalender et al., 2002). The purpose of this study; to determine the seroprevalance of HBV, HCV and HIV in

patients undergoing hemodialysis treatment. In addition, it was aimed to re-evaluate patients who are sensitive to HBV and to identify patients who are anti-HBs positive.

2. Materials and Methods

This study includes hemodialysis patients serum samples which were studied in Selçuk University Medical Faculty, Medical Microbiology Laboratory. HBsAg (Hepatitis B surface antigen), Anti-HBs (Hepatitis B surface antibody), Anti-HBc IgM (Hepatitis B core IgM antibody), Anti-HBc IgG (Hepatitis B core IgG antibody), HBV-DNA, Anti-HCV, (Hepatitis C antibody), HCV-RNA and Anti-HIV (Human Immunodeficiency Virus Antibody) parameters were retrospectively screened between 1 July 2010 and 1 July 2020.

"HIV-1 / 2Ag / Ab Combo" test was performed by the Architect i1000 sr (Abbott Diagnostics, Germany) device. Patients with confirmed positive serum samples were followed up in our laboratory by HIV -RNA testing (HI Virus-1 RT-PCR, Rotor Gene Q, QIAGEN, Germany). For the diagnosis of hepatitis B and hepatitis C, the Architect i1000 sr (Abbott Diagnostics, Germany) device working with the chemiluminescence method was used. HBV-DNA and HCV-RNA parameters were studied by Real-Timed PCR method using Cobas x 480 (Roche, Sweden) device.

3. Results

Serum samples from 4155 hemodialysis patients were examined. 2405 (58%) of the patients were male and 1750 (42%) were female. HBsAg was positive in 17 (0.40%) patients. Anti-HBs positivity was found in 454 (11.2%) patients. Anti-HBs antibody level was found above 100 mIU / ml in 112 patients (24.7%). Anti-HBc IgG was positive in 10 (0.24%) patients and Anti-HBc IgM in 7 (0.16%) patients. HBV-DNA positivity was found in 13 (76.5%) of the HD patients who were HBsAg positive. Anti-HCV was positive in 14 (0.33%) of the patients (Table 1**)**. In 12 (85.7%) of the patients who were Anti-HCV positive, HCV RNA was positive. Anti-HIV was negative in all samples.

Table 1. Hepatitis Markers and Seroprevalence (%).

Hepatitis Markers	Seroprevalence (%)
HBsAg	**0.40**
Anti-HBS	**11.2**
Anti-HCV	**0.33**
Anti-HBc IgG	**0.24**
Anti-HBc IgM	**0.16**

HBs Ag: Hepatitis B surface antigen; Anti-HBs: Hepatitis B surface Antibody; Anti-HCV: Hepatitis C antibody; Anti-HBc IgG: Hepatitis B core IgG antibody; Anti-HBc IgM: Hepatitis B core IgM antibody.

4. Discussion

Despite the precautions taken, viral hepatitis remains an important risk factor for both patients and employees in hemodialysis units. HBV seroprevalence in Turkey varies from region to region. However, our country is located in the middle of an endemicity zone in terms of HBV infection. According to the data of TND, the seroprevalence of HBsAg in chronic hemodialysis patients has been determined as 3.8% (Guvenir et al., 2019). HBsAg positivity in studies in hemodialysis patients in Turkey; In Çanakkale, Arabacı et al. by 4.8% in Elazig, 8.1% by Kaygusuz, and Evirgen et al in Hatay. 3.6% in Gaziantep, Sırmatel et al. by 8.7%. In a multicenter study, seroprevalence was determined as 5.5% (Çopur et al., 2013).

In 2016, a cross-sectional study was conducted with 360 HD patients in 5 hemodialysis centers in Tehran. According to the study results, HBsAg was found to be positive in 1.39% of patients (Yadegarynia et al., 2017). In the study performed among HD patients in Cameroon, HBsAg seroconversion rates were found to be 1.1% (Halle et al., 2016). In another study conducted with 113 HD patients in Vietnam, 7% HBsAg positivity was reported. The high HBsAg level in the hemodialysis unit is an indication that it will pose more risks for decentralized hemodialysis services in Vietnam, where universal measures are not taken (Duong et al., 2015).

In our study, HBsAg was found to be positive in 17 (0.40%) of hemodialysis patients. In chronic hemodialysis patients, the HBsAg seropervalance was 4.3% according to the data of TND in 2011, while it was reported as 3.8% in 2016. The reason for low HBsAg seropositivity in hemodialysis patients in our study; timely implementation of prophylaxis programs are associated with erythropoietin therapy, better blood control, separation of dialysis machines for HBsAg patients and good infection control measures (Ozturk, 2010).

In this study, Anti-HBs positivity was found in 454 (11.2%) patients. seropositive of Anti-HBs in HD patients in the studies made in Turkey, has been reported to be between 33.5-64% (Çopur et al., 2013). Evirgen et al. In the study conducted by, Anti-HBs positivity was reported to be higher

in HD patients as 72.2%. In this study, Anti-HBs antibody level was found above 100 mIU / ml in 112 patients (24.7%). In Turkey, compared the prevalence of HBV, HCV, although lower, because it is causing more severe consequences both hospital staff is very important for both normal population (Ozer et al., 2007). According to current data, it is estimated that more than 170 million people worldwide are infected with HCV. In addition, 1 million people die each year from cirrhosis or liver cancer due to HCV infection (Eleftheriadis et al., 2011). Liver Studies Association of Turkey, Turkey Anti-HCV positivity in society according to the results of his work in general was reported as 0.95%. In studies conducted worldwide, Anti-HCV seropositivity among HD patients has decreased in some countries over time, but has increased in some countries (Rabanal et al., 2010; Çopur et al., 2013; Piskinpasa et al., 2013).

In studies conducted in Kosovo, the general seroprevalence of HCV infection in hemodialysis patients ranged from 22.3% to 53% in different centers (Jakupi et al., 2018). According to the results obtained, the rate of seroconversion in hemodialysis was determined as high for HCV, low for HBV and zero for HIV (Halle et al., 2016). In a study conducted in 360 patients with CKD in Iran, anti-HCV antibodies were found positive in 3.06% of the participants. In another similar study in hemodialysis patients, anti-HCV seropositivity was found in 30 (15.30%) of 196 patients. HCV infection has a special importance in dialysis patients due to the high risk of transmission from hospital. Because HCV prevalence is higher in hemodialysis patients compared to normal society (Kansay et al., 2019). In a meta-analysis, it has been reported that HCV carriage increases the risk of death in hemodialysis patients. In studies conducted in different countries, anti-HCV positivity rate was determined to vary between 4-59% between geographical regions (Rabanal et al., 2010). The seropositivity for anti-HCV antibodies in chronic hemodialysis patients in Turkey, according to data of TND was reported as 5.2% (Guvenir et al., 2019).

According to the results of this study, anti-HCV was found positive in 14 of 4155 hemodialysis patients (0.33%).

In Cameroon, Luma et al. Research was carried out to determine HIV seroprevalence in 104 CKD patients. According to the results obtained; HIV-related nephropathy (HIVAN) 11.5% (12/104).

The results of our study shown that HIV-positive was not detected in the hemodialysis unit because the necessary disinfection rules were followed and the screening was done on time. In addition, the results we obtained in our study can be explained by low HIV seroprevalence in Konya Region. Because studies have reported that HIV seroprevalence differs according to the location of the HD unit (Eleftheriadis et al., 2011).

Studies have shown that it is sufficient to apply general disinfection rules in patients with HIV infection in dialysis units.

5. Conclusion

In view of the relatively average HCV seroprevalence at the hemodialysis center at Konya university hospital center, it is evident that the most important risk factors are the length of time of hemodialysis. The data from this study encourage us to better apply the rules of asepsis, and to use recombinant human erythropoietin.

Ethical considerations

The study protocol followed ethical guidelines of the Declaration of Helsinki. Ethical approval has been provided by the Ethics Committee from the Faculty of Medicine, Selçuk University (02/09/2020 – 2020/348).

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Author Contributions: All of the authors declare that they have all participated in the design, execution and that they have approved the final version.

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Solubility Improvement of Cefdinir Using Polyvinylpyrrolidone (PVP): Preparation of Solid Dispersions and Their Characteristic Properties

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Abstract

Cefdinir (CEF) is an antimicrobial drug with a wide spectrum of activity. It is mainly used in infections yet is not the first choice considering its solubility and bioavailability problems. The main aim of this study is to improve therapy with CEF by enhancing its solubility and improving dissolution properties. Solid dispersions have been preferred for this purpose and polyvinylpyrrolidone (PVP) has been used to develop two different formulations, PVP-1 and PVP-2. Physical mixture of PVP and CEF was also prepared for comparison. Formulations were prepared by lyophilization following probe sonication with different conditions. To ensure that our formulations have reached their purposes, further experiments were conducted. A specific and selective UV-visible spectrophotometric method was used to determine CEF concentration and the method was validated according to ICH guidelines for linearity, accuracy, and precision. Solubility studies were conducted with pure CEF, physical mixture (PM-PVP) and our solid dispersions, PVP-1, and PVP-2. Solubility studies confirmed CEF's low solubility, less than 1 mg/mL, and showed a significant difference between pure CEF and solid dispersions. While there was also a significant difference between PM-PVP and both PVP-1 and PVP-2, there were no significant differences between PVP-1 and PVP-2. Therefore, PVP-2 was chosen as the optimum formulation and dissolution studies were conducted with PVP-2 and pure CEF in three different mediums, water, pH 1.2 and simulated intestinal fluid (SIF, pH 6.8), respectively. Solubility and dissolution profiles of pure CEF and the formulations were compared and evaluated according to literature. Our formulations were found to be successful in the purpose of improving therapy with CEF by demonstrating a better solubility with better dissolution profiles in all mediums.

Keywords: Cefdinir; solid dispersions; solubility enhancement; probe sonication

1. Introduction

Cefdinir (CEF) is a semisynthetic third generation cephalosporine with a ß-lactam structure within. Its indications include respiratory infections, soft tissue infections and acute otitis media. It has activity on both gram-positive and gram-negative bacteria species (Garrepally et al. 2013). It is used via oral route and has many dosage forms available. CEF has a very low solubility in water and low bioavailability of nearly 25%, which makes it classify as a Class IV drug according to Biopharmaceutical Classification System (BCS) (Thota et al. 2014). In addition to

solubility and permeability problems, CEF's biological half life is limited to 1 – 2 hours. Its solubility is highly dependent on pH and is variable (Garrepally et al. 2013).

Solid dispersions are one of the many ways to improve a drug's solubility and even dissolution profiles by improving its aqueous solubility (Lakshman et al. 2020). Many polymers such as carboxymethyl cellulose, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP) and co-polymers and different combinations can be used to prepare solid dispersions of pharmaceutical active ingredients. The main challenge is to find the compatible and inert polymer that can be used with chosen active pharmaceutical ingredient (Schittny et al. 2020). While there are many available options to prepare solid dispersions, the method applied must be compatible with all the ingredients and one of the suitable methods is probe sonication.

Polyvinylpyrrolidone is a non-toxic, biologically compatible, and inert polymer with excellent properties. It is soluble in water and various solvents, also a great stabilizer in pharmaceutical systems. PVP is commonly used in preparation of nanosized drug delivery systems, such as nanoparticles (Koczkur et al. 2015).

In this study, preparation of solid dispersions containing CEF as active pharmaceutical ingredient and PVP as the polymer is aimed. The main goal is to improve therapy with CEF by improving its solubility and dissolution properties in different media.

2. Materials and Methods

2.1. Materials

Cefdinir is a kind gift from Sanovel İlaç (Istanbul, Turkey). All other chemicals used were of analytical grade.

2.2. Preparation of solid dispersions

0.5 g PVP was weighed and dissolved in 50 ml water. 0.5 g CEF suspension in 50 ml methanol was also prepared simultaneously and the suspension was added to the polymer solution, stirring continuously using magnetic stirrer. The obtained mixture was sonicated in a specific amplitude and duration (Sonics®, USA). All formulations were refrigerated at -80oC overnight to be lyophilized (Scanvac CoolSafePro Labogene, Denmark) to obtain the final powder form.

The physical mixture of PVP and CEF was prepared by weighing 0.5 g PVP and 0.5 g CEF in a falcon, shaken using a horizontal shaker at 250 rpm in room temperature for 24 hours. All conditions and formulation compositions are summarized in Table 1.

Table 1. Preparation conditions and compositions of the formulations.

Formulation Code	Amount (g)		Volume (mL)		Sonication conditions	
	CEF	PVP	Water	Methanol	Amplitude	Duration (min)
PM-PVP	0.5	0.5	-	-	-	-
PVP-1	0.5	0.5	50	50	75%	4
PVP-2	0.5	0.5	50	50	75%	8

2.3. Characterization

Prior to all experiments, a validated spectrophotometric method was used to determine CEF. The method was performed using a UV/vis Recording Spectrophotometer UV-1601 (Shimadzu, Japan). In all experiments, quartz cells were used, and the analysis wavelength was 287 nm. Partial analytical method validation for linearity, precision, recovery, and accuracy was performed according to ICH guidelines (ICH 2005).

2.3.1. Solubility experiments

50 mg per formulation (PM-PVP, PVP-1 and PVP-2) and pure CEF were weighed and added to falcon tubes with 10 mL distilled water and shaken vigorously using a horizontal shaker at 250 rpm in room temperature for 24 hours. The mixtures were centrifuged, obtained supernatants were collected and filtered using syringe filters with 0.45 µm pore diameter. The filtrates were diluted using distilled water and analyzed using UV spectrophotometer. All experiments were performed in triplicates in room temperature.

2.3.2. Dissolution

In vitro dissolution studies were performed on pure CEF and optimum formulation (PVP-2). The experiment took place using dialysis membrane in three different mediums, water, pH 1.2 buffer and simulated intestinal fluid (SIF, pH 6.8) for over 4 hours.

3. Results

3.1. Characterization

In validation studies of UV-Visible spectrophotometric method for CEF analysis, several parameters have been investigated. Linearity study was conducted at a concentration range of 1 – 17 $\mu g.mL^{-1}$ and the linear equation is y=0.0528x+0.004 with an R^2 value of 0,9997. For the determination of precision, relative standard deviation (RSD) values were calculated and found to be smaller from 2%. Accuracy was investigated by calculating recovery values and found to be less than 2% for all concentrations.

3.1.1. Solubility experiments

The results clearly show that while pure CEF has a solubility of less than 1 mg/mL in water, both our formulations improved solubility significantly. It is also clearly seen that physical mixture of PVP and CEF improved solubility yet there are no significant differences between PM-PVP and pure CEF and the solubility still did not exceed 1 mg/mL. Our formulations, PVP-1, and PVP-2 show improvement and reached to solubility close to 3 mg/mL. Solubility results are presented in Figure 1.

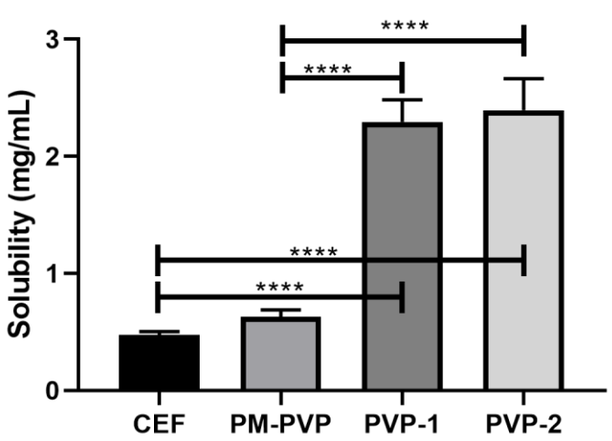

Figure 1. Results of solubility study and statistical analysis.

3.1.2. Dissolution

Since the solubility results demonstrated that there are no significant differences between PVP-1 and PVP-2 formulations, PVP-2 was chosen as the optimal formulation and dissolution studies were conducted using PVP-2 and pure CEF. In all three mediums PVP-2 showed better cumulative release in all time periods. While the difference is not very clear in water, in both pH 1.2 and pH 6.8 PVP-2 showed higher cumulative release. It is also seen that in pure CEF drug release has stopped after a while in water and pH 1.2, while PVP-2 kept releasing the drug. The *in vitro* dissolution results are shown in Figure 2.

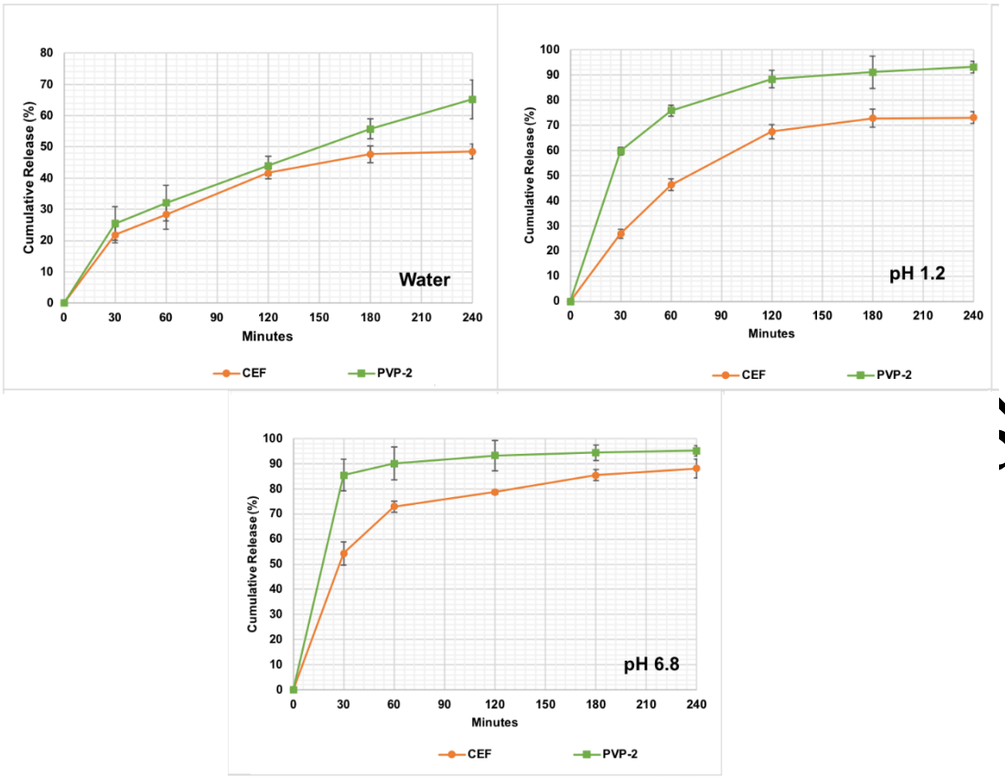

Figure 2. Results of *in vitro* dissolution study in different mediums (water, pH 1.2, pH 6.8).

4. Discussion

4.1. Characterization

Linearity, RSD, and recovery values were found to be within limits according to ICH. The results show that the current method is linear, precise, accurate and repeatable. Results of partial analytical method validation study proves that this method is not only easy and cost-friendly, and that it can be used for CEF determination routinely (Ozturk et al. 2019).

4.1.2. Solubility experiments

One of the main problems regarding CEF is its low water solubility, which is confirmed with our solubility experiments. In Figure 1, it is seen that CEF concentration in water didn't exceed 1 mg/mL. While the formulations improved CEF's solubility in water significantly compared to both pure CEF and PM-PVP, the difference between physical mixture and pure CEF did not reach to significant level. Although PVP can enhance solubility in a very low rate, it is not significant when compared and with previous studies showing that probe sonication has a positive effect on solubility enhancement (Pereira et al. 2016), it is clear that in this study the main difference is seen by probe sonication.

4.1.3. Dissolution study

Dissolution studies also confirm solubility results, showing CEF release reached higher cumulative release values in water. In water, the dissolution graphics were very similar until 2

hours. After that pure CEF dissolution has decreased while drug release from PVP-2 kept going at the same rate without any decreases. At 3rd hour it is seen that drug release in pure CEF stopped while PVP-2 kept releasing.

It is known that CEF has different solubility profiles and rates in different mediums with different pH values, showing the highest rate at pH 6.8 (Cho et al. 2017). Even though the release graphics are in very similar shapes for both pure CEF and PVP-2 in both mediums, pH 1.2 and pH 6.8, it is clearly seen that released amount of CEF is higher for PVP-2.

To take a closer look, in pH 1.2 the drug release stopped at 3rd hour for pure CEF while kept going for PVP-2, reaching above 90% cumulative release. Pure CEF reached only above 70% percent at the end of 4th hour, also showing that solubility has increased in pH 1.2 as well.

Since CEF solubility reaches a much higher value in pH 6.8 (Cho et al. 2017), drug release is much higher than in both water and pH 1.2. While pure CEF reaches above 50% at the end of 30 minutes, PVP-2 reaches above 80%, proving that solubility is also improved in an environment that CEF already has a higher solubility rate. Since cumulative amount reaches a very high level very early regarding PVP-2, a decrease is seen after 60 and 120 minutes, while pure CEF keeps releasing the drug until it reaches just below 90%, while the final cumulative release of PVP-2 hits above 90%.

5. Conclusion

CEF has many clinical uses in today's medicine, yet the problems regarding its properties limit its applications. As this study was conducted to improve solubility and improve CEF dissolution, the results show preparation of solid dispersions has been successful. This study is a small step in improving antimicrobial therapy with cefdinir and only a small part of a bigger project.

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Preparation and Characterization of Vitamin A Palmitate Loaded PLGA Based Nanodermacosmetics

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Abstract

The way nanotechnology is used in the dermacosmetic / cosmetics industry under today's conditions is to transfer very small sized products such as nanoparticles produced in nanometric dimensions to the cosmetic content. Vitamin A palmitate is a very important component of many important and various biological functions such as reproduction, embryological development, cellular differentiation, growth, immunity and vision. Vitamin A palmitate also has antioxidant and sunscreen effects. This study describes the development and evaluation of poly (lactic-*co*-glycolic acid) (PLGA) nanoparticles for dermal delivery of Vitamin A palmitate. Structures of PLGA nanoparticles were elucidated by particle size, polydispersity index (PDI) and zeta potential. The particle size of the blank formulation was 181.77 nm whereas particle size of Vitamin A palmitate containing nanoparticles was 196.33 nm. The average PDI of all nanoparticle formulations were below 0.12 and all prepared nanoparticles had a negative zeta potential value. Particle size, PDI and zeta potential values of formulations prepared confirmed the successful preparation of Vitamin A palmitate into PLGA nanoparticles. Nanoparticle formulations seem to be promising for dermal delivery of Vitamin A palmitate for dermacosmetic use.

Keywords: PLGA, Vitamin A Palmitate, Nanoparticle, Dermacosmetic, Nanocosmetic.

1. Introduction

Nano structuration modifies chemical, physical, and biological properties of materials leading to a range of new applications in health, including novel delivery systems for pharmaceutics and cosmetics, advanced materials for implants and biosensors, tissue engineering, and new tools for diagnosis (Guterres et al. 2019). The use of nanotechnology-based carriers to encapsulate drug or cosmetic active agents offers advantages such as increased absorption bioavailability, improved intracellular penetration, and controlled drug / cosmetic delivery (Öztürk et al. 2020a). It has been hundreds of years ago in the development of nanomaterials for cosmetic purposes. Gold and silver nanoparticles have been used by women as nail colors. Also, liquid formulations containing gold nanoparticles were used as anti-aging in the Middle Ages. However, in recent years, nanoscale materials have been used more widely in the development of cosmetics (Shokri, 2017). Polymeric nanoparticles have been shown to be versatile for cosmetic applications. They find special place in the cosmetic arena because these nanoparticles can improve the stability of labile

ingredients, offer efficient protection for the skin from harmful UV radiation, target the active ingredient to the desired layer of the skin or to the hair follicles, enhance photostability, antioxidant and antimicrobial effects, and control the release or the volatilization for prolonged time (Guterres et al. 2019). Poly (lactic-co-glycolic acid) (PLGA) is a United States Food and Drug Administration (FDA) approved, biocompatible and biodegradable copolymer that is widely used as a matrix for nanoparticles (Öztürk et al. 2020b). Topical application of antioxidants that act as photoprotective, which can preserve or restore a healthy skin barrier, is very common. Antioxidants are often used in anti-aging products. Vitamin A palmitate acts on keratinization, which is thought to be abnormal, as well as epithelization in dry and rough skin. It also absorbs the sun's harmful UV radiation (Gaspar et al., 2013). Therefore, the purpose of this study is to prepare and characterize Vitamin A loaded nanoparticles with better wound healing and better antiaging properties.

2. Materials and Methods

2.1. Materials

Vitamin A Palmitate was received from Dermoskin® (Matilek Foreign Trade Limited Company), Istanbul, Turkey as a kind gift. Resomer® RG 502 H [Poly (D, L-lactide-*co*-glycolide), acid-terminated, lactide: glycolide 50:50, Mw: 7.000-17.000] and Span® 60 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pluronic® F-68 (Poloxamer-188) was purchased from Alfa Aesar (Kandel, Germany). All other chemicals used were of analytical grade

2.2. Methods

PLGA-based nanoparticles were prepared by following the nanoprecipitation technique with some modifications (Öztürk et al., 2018). Briefly, a weighed amount of PLGA (60 mg) was dissolved in 3 mL acetone together with Span® 60 (20 mg). Next, 3 mL of this solution was added dropwise at a rate of 5 $mL.h^{-1}$ into 10 mL of Pluronic® F-68 aqueous solution (0.5%, w/v) under magnetic stirring. Acetone was then allowed to evaporate at room temperature under magnetic stirring for 4 h. The resulting aqueous dispersion was centrifuged to collect the nanoparticles (11.000rpm, 45min, 4ºC) (Rotina 420R, Hettich Zentrifugen, Tuttlingen, Germany). After the nanoparticles were collected, 5 mL of distilled water was added in order to wash particles. The nanoparticles dispersed in water were again subjected to the above mentioned centrifugation process. This process was repeated twice to wash the nanoparticles.

For Vitamin A Palmitate-loaded PLGA-based nanoparticle preparation, briefly, the procedure started by adding 3 mg Vitamin A Palmitate to the organic phase. Then, 3 mL of such solution with drug were added dropwise at rate 5$mL.h^{-1}$ into 10 mL of Pluronic® F-68 aqueous solution (0.5%, w/v) under magnetic stirring. Acetone was then allowed to evaporate at room temperature

under magnetic stirring for 4h. The resulting aqueous dispersion was centrifuged to collect the nanoparticles (11.000 rpm, 45 min, 4ºC) (Rotina 420 R, Hettich Zentrifugen, Tuttlingen, Germany). This process was repeated twice to wash the nanoparticles. Formulation ingredients are given in Table 1.

Table 1. Formulation ingredients.

Code	PLGA	Vitamin A Palmitate	Span 60	Acetone	Pluronic F-68
NP-1 Blank	60 mg	-	20 mg	3 mL	10 mL
NP-1	60 mg	6 mg	20 mg	3 mL	10 mL

***PLGA**: Resomer® RG 502 H, **Pluronic F-68:** 0.5%, w/v solution.

The particle size and polydispersity index of nanoparticle formulations were measured using a dynamic light scattering technique (DLS) on the Zetasizer Nano (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). Particle size and polydispersity index of nanoparticle prepared were measured by dispersing the formulation in distilled water. Zeta potential values were determined using the same instrument in a disposable folded capillary zeta cell, at 25ºC room temperature and diluted with distilled water. All samples were measured in triplicate and the average values and standard deviation of the measurements were calculated.

3. Results

Particle size, polydispersity index and zeta potential values of the formulations are presented in Table 2. Preparation of the formulations is visually presented in Figure 1.

Table 2. Particle size, Polydispersity index and Zeta Potential values

Code	Particles Size (nm)	PDI	Zeta Potential (mV)
NP-1 Blank	181.77 ± 2.38	0.12 ± 0.06	-15.47 ± 0.23
NP-1	196.33 ± 0.65	0.11 ± 0.02	-15.60 ± 0.44

*Results given as Mean± Standard Deviation (**Mean ± SD**), **PDI**: polydispersity index, **nm**: nanometer, **mV**, milivolt

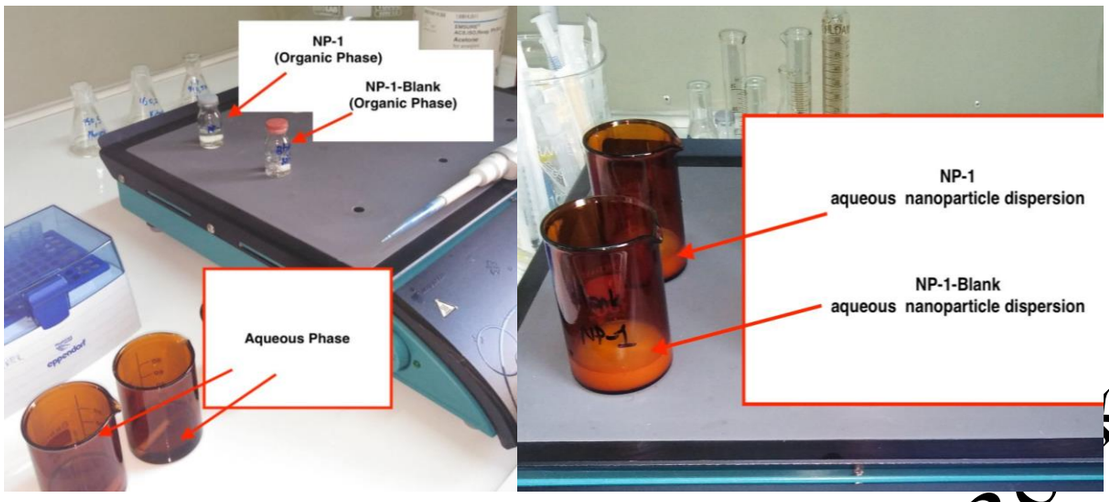

Figure 1. Preparation of Formulations.

4. Discussion

As seen in Figure 1, the formulations were developed without any aggregation or any problems. Characterizations of nanoparticles are primarily evaluated with particle size, particle size distribution (polydispersity index: PDI), and zeta potential. The relevant information is presented in Table 2. Particle size obtained in the blank formulation was 181.77 nm while particle size in the Vitamin A palmitate-loaded nanoparticles was 196.33 nm. In particle size analysis, it is important to note that particle size increases with active substance loading. Particle size growth has been reported in the literature when the nanoparticles are loaded with drug active substance (Şenel and Öztürk, 2019; Ilkan and Özdemir, 2017).

PDI value which defines particle size distribution is in the range of 0.01 and 0.7 for monophasic systems; value higher than 0.7 is indicative of a very wide particle size distribution and the value close to zero means narrow particle size distribution (Öztürk and Aygül, 2020). When the PDI results are discussed in this literature information, it can be said that blank and Vitamin A palmitate loaded PLGA nanoparticle formulations are monodisperse.

When Table 2 is examined, negative zeta potential values are seen in PLGA nanoparticles. The zeta potential was observed as -15.47 and -15.60 mV in blank PLGA nanoparticle and Vitamin A palmitate loaded nanoparticles, respectively. PLGA in neutral environment has negative surface potential attributed to terminal carboxyl groups, and this can be confirmed by the zeta potential of the negative obtained in PLGA nanoparticles (de Lima et al., 2018).

5. Conclusion

PLGA nanoparticles were prepared for dermal delivery of Vitamin A Palmitate nanoprecipitation technique. Particle size, PDI and zeta potential values of formulations prepared confirmed the successful preparation of Vitamin A Palmitate into PLGA nanoparticles. Nanoparticle formulations seem to be promising for dermal delivery of Vitamin A Palmitate for dermacosmetic use. The solid structure of the nanoparticles will be elucidated in the later stages of the study. After the completion of in vitro dissolution studies of nanoparticles, their wound healing potential and antiaging benefit will be examined at the cellular level.

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Antibiotic susceptibility of multidrug-resistant *Acinetobacter baumanii* and *Pseudomonas aeruginosa* in bloodstream Infections

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Abstract

Introduction: Bacterial bloodstream infections cause high morbidity and mortality worldwide in humans due to the emergence of drug-resistant pathogens, resulting in infections which are difficult to treat or even untreatable with conventional antimicrobials.
Objectives: The aim of this study is to describe the epidemiological aspects of bloodstream infections caused by multi drug resistant gram-negative bacilli (MDR-GNB).
Methods: A prospective study was conducted at the University Hospital of Constantine, Algeria from September 2018 to February 2019. The disk diffusion method was used for antimicrobial susceptibility testing according to Clinical & Laboratory Standards Institute methodologies (CLSI) from different antibiotics (Ampicillin, Ticarcillin, Piperacilin, Ticarcillin/Clavulanate, Piperacillin / Tazobactam, Ceftazidim, Cefepim, Aztreonam, Imipenem, Fosfomycin, Gentamycin, Pefloxacin, Sulfamethoxazol / Trimetoprim, Colistin), in order to target multi-resistant strains. The search for ESBL-producing strains was according to the recommendations of CLSI.
Results: A total of 54 isolates were collected from blood streams. Of the patients, 68% were male and 60% come from burn intensive care unit.
Regardless of the specimen, there was two nosocomial organisms, *Acinetobacter baumanii (A. baumanii)* comprised 69% and *Pseudomonas aeruginosa (P.aeruginosa)* 31%.
The isolates of *A. baumannii* and *P.aeruginosa* were resistant to commonly used antibiotics, antibiotic resistance rates of *A. baumanii* isolates were higher: Carbenicillin (100%, 64.7%), Piperacillin (100%, 64.7%), Imipenem (83%, 35.3%), Ticarcillin (91.9%, 70.6%), Ticarcillin/Clavulanate (94.6%, 47.1%), Cefepim (86.5 %, 41.2%), Aztreonam (86.5 %, 17.6%), Gentamicine (89.2%, 47.1%), Pefloxacine (86.5%, 64.7%), Tazobactam (83.8%, 41.2%), Ceftazidime (91.9%, 41.2%), Fosfomycin (86.5%, 47.1%), Sulfamethoxazol / Trimetoprim (83.8%, 94%).
Among the *A. baumannii* strains 85% were MDR while *P.aeruginosa* isolates (42%).
Colistin was the most effective against *A. baumanii* strains and *P.aeruginosa* with 100% sensitivity.
Conclusion: Bacteremia with resistant BGNNF is considered to be serious conditions, responsible for significant morbidity and mortality worldwide. Continuous monitoring of susceptibility profiles of pathogens to important antibiotics is warranted to provide appropriate antimicrobial regimens against bloodstream infections.

Keywords: blood stream infection, resistance, antibiotics, non-fermenting gram-negative bacilli.

1. Introduction

Non-fermenting Gram-negative bacteria (NFGNB), which are isolated from a variety of environments such as soil, water, plants and animals, as part of the natural bacterial microbiota but also is known as an opportunistic pathogen. This dual life-style is likely attributable to its large genome (Sommer et al. 2020). More than 120 pathogenic species species of NFGNB were identify as etiological agents involved in most hospital-acquired infections including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia* and since they are opportunistic pathogens, them pathologies are mostly acquired in hospital environments (Oliveira et al. 2017) such as bronchial tree especially in immunosuppressed and cystic fibrosis patients, which already showed to be a sever because these bacteria acquire high resistance to a wide variety of drugs, including penicillins, cephalosporins, aminoglycosides, tetracyclines, fluoroquinolones, trimetropim-sulfamethoxazole, carbapenems and polymixins(Oliveira et al. 2017)

Many studies reported that among non-fermenting gram-negative bacilli pathogens, resistant *P. aeruginosa* (Ponce de Leon et al. 2020) and *Acinetobacter baumanii* have become one of the most difficult nosocomial pathogens to control and treat with nosocomial and healthcare associated infections (HAIs) and an associated mortality of approximately 30% and significant costs (Royer et al. 2015) . The treatment of resistant these opportunistic pathogens with an ability to rapidly develop resistance to multiple classes of antibiotics, is especially challenging (Chand et al. 2021).

In Algeria, and many countries, non-fermenting Gram-negative bacilli (BGNNF) are increasingly implicated in nosocomial infections, formerly sensitive to almost all antibiotics are now responsible for infections occurring through epidemics and involving increasingly multi- drug resistant strains (MDR). Bacteremia is one of these infections with serious conditions, responsible for significant morbidity and mortality worldwide

For a better understanding of the epidemiology of infections antimicrobial resistance caused by such microorganisms, the analysis of the degree of sensitivity and resistance of NFGNB is essential, besides contributing with the proposition of new therapeutic schemes to battle them more efficiently (Oliveira et al. 2017).

Therefore, this study aimed to describe the epidemiological and resistance profile of these sever bacteria by determining the resistance profile of NFGNB, isolating and having phenotypically identification from blood cultures of hospitalized patients in a university hospital in Constantine, in the east of Algeria.

2. Materials and Methods

We conducted a prospective study at Constantine Hospital, between September 2018 and February 2019. External and internal patients are included in the study.

120 sample of positive blood culture were collected in this period. All isolates were identified by conventional techniques using a standard media. Susceptibility testing for these clinical specimens was performed using the disk diffusion method. The following antimicrobial agents were used for the susceptibility evaluation of both *A. baumannii* and *P. aeruginosa* isolates: Carbenicillin, Piperaciline, Ticarcillin/clavulanate (TCC)… etc. All the tests were done in accordance with the Clinical and Laboratory Standards Institute recommended practices CLSI 2016. All isolates of *A. baumannii* and *P. aeruginosa* were screened for ESBL producing phenotype by the synergy test (ST) CLSI 2016 and confirmation test (double disk). The lecture and the interpretation of the susceptibility were according to the CLSI instructions.

3. Results

Among 120 positive samples, 54 of NFGNB represent with *P. aeruginosa* and *A. baumannii* were isolates from clinical-samples and identified from blood specimen in the period of study. 37 (69%) and 17 (31%) were identified respectively as *A. baumannii* and *P. aeruginosa*, using biochemical tests (Figure1).

The male sex showed a highest rate (Figure 2) and was the most exposed to sepsis with (68%).

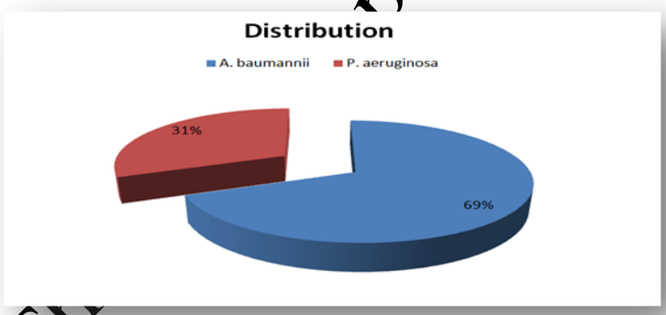

Figure 1. NFGNB genera isolated from blood cultures analysis.

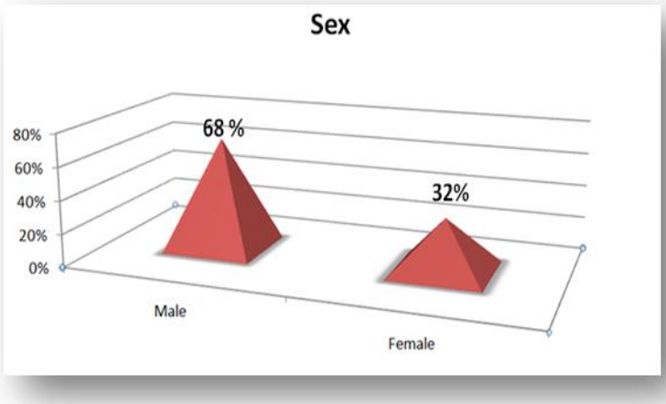

Figure 2. BGNNF distribution according to the sex.

The isolated blood cultures come from the different departments (Table 1) where the center of the burns was the largest feeder (60%).

Table 1. BGNNF distribution according to the service.

Services	Number	Rate %
Neonatal	**1**	**1.8 %**
Surgery B	**2**	**3.6 %**
Center of the burns	**32**	**60%**
Intensive care of burns	**3**	**5.45 %**
Surgery A	**1**	**1.8 %**
Intensive care of the internal medicine	**3**	**5.5%**
Nursery	**6**	**11%**
Dermatology	**3**	**5.45%**
Pediatrics	**3**	**5.4 %**

The antibiotic resistance patterns (Figure 3) highlighted that, Out of 83% *Acinetobacter baumannii* isolates was carbapenem resistant. Antibacterial susceptibility pattern of b-lactamine resistant of *Acinetobacter baumannii* isolates were highly significant to all of carbenicillin and Piperacillin with a rate of 94.6%, ticarcillin/clavulanate and ticarcillin with a rate of 91.9%, ceftazidime and Aztreonam with 86.5 %, Cefepime with 64.86% .

The susceptibility pattern of the rest of antibiotic familly on *A. baumannii* was also significant: Gentamicin (89.2%), Pefloxacin (86.5%), Fosfomycin, Piperacillin / Tazobactam, and sulfamethoxazole / Trimetoprime (83.8%).

6 (35.3%) of the *P. aeruginosa* isolates were screened as imipenem resistant and susceptible strains. The mentioned rates of the isolated *P. aeruginosa* against β-lactamines were (70.6%)

with ticarcillin, (64.7%) with Carbenicillin and Piperacillin, (47.1%) with Ticarcillin/Clavulanate, (41.2%) with Ceftazidime, (17.6%) Aztreonam, (11.76%) Cefepime.

The susceptibility pattern of the rest of antibiotic familly on *P. aeruginosa* were (94%) to Sulfamethoxazole / Trimetoprime, (64.7%) to Pefloxacin, (47.1%) to Gentamicin, Fosfomycin, piperacillin / tazobactam successively.

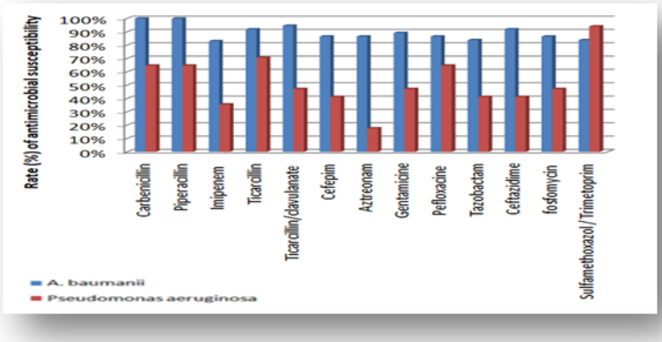

Figure 3. Comparison between antibiotic resistance rates of 13 antibiotics tested on *A. baumanii* and *P.aeruginosa* isolates.

The confirmation test showed that among the *A. baumannii* strains 28(75.7%) were MDR while for *P.aeruginosa* isolated 6(35.3%) strains were MDR.

Colistin was the most effective against *P.aeruginosa* and *A. baumanii* strains with 100% sensitivity.

4. Discussion

According to several studies including Arsalane et al. 2010, Royer et al. 2015, Alcántar-Curiel et al. 2019, the evolution of multi-resistance to antibiotics over the last years was marked by the emergence of *Acinetobacter*.

Acinetobacter sp. has been considered the second NFGNB most frequently found in the laboratory studies of this genus, behind only the Pseusdomonas sp., however the present study showed a prevalence of 69.5% for Acinetobacter sp. and only 31% for Pseudomonas

One of the explanations of antimicrobial resistance phenomenon of NFGNB is that bacterial cells somehow selectively activate genes, when necessary from the survival point of view, while silencing other ones (Bogiel et al. 2020).

During bloodstream infection the antimicrobial resistance phenomenon has been previously observed (Kruczek et al. 2016, Elmassry et al. 2019), in Ramsay et al. 2019 study P. aeruginosa's genome was confirmed for it plasticity, improving that the environmental isolates are less resistant to antimicrobials than nosocomial strains and this is probably due to an adaptation to a

hospital environment and antimicrobial therapy pressure. Also these strains are too infectious for human (Bogiel et al. 2020).

The antimicrobial resistance in *A. baumannii* and *P. aeruginosa* are associated with several mechanisms and therapeutic options for these organisms are limited, but the most important is the production of β-lactamases and has been attributed to the production of carbapenemases enzymes of for *A. baumannii*, and class B Meta β-lactamases (MBLs) associated with loss of porins and efflux system over expression for *P. aeruginosa* (Royer et al. 2015).

5. Conclusion

Beta-lactam antibiotics are the most widely used therapeutic choice for treatment of bacterial infections, accounting for 60 % of total antibiotics used. Their high efficivity, low cost, comparatively chemical diversity with minimum side effects make this class of antibiotics popular in treatment of microbial infections. Cephalosporins such as cephazolin are commonly used β-lactams in the treatment of several infections.

Bacteremia with resistant BGNNF is considered to be serious conditions, responsible for significant morbidity and mortality worldwide. Continuous monitoring of susceptibility profiles of pathogens to important antibiotics is warranted to provide appropriate antimicrobial regimens against bloodstream infections.

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Impact of Quercetin on Lymphocyte DNA Status of Hens

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Abstract

This study was conducted to examine the influence of four levels of flavonoid quercetin in deoxyribonucleic acid (DNA) status of hen's lymphocyte with using comet assay. For this purpose, one-hundred and twenty hens(forty weeks aged) were reared for 20 weeks and divided into four experimental treatments as fallow: T1(control), T2, T3 and T4: basal diet supplemented with 0, 400, 800, 1200 mg quercetin / kg diet, respectively. Formulated diet and water were presented as free at all rearing period. Blood samples were obtained with EDTA tubes at the experiment end, lymphocytes were washed and isolated for comet assay which indicate to severity of DNA breaks. Comet assay parameters findings revealed a high significant difference with respect of comet length, comet height, comet intensity and comet area for addition treatments especially T3 and T4 that supplemented with 800 and1200 mg quercetin / kg diet as compared with T1 and T2 that supplemented with 0 and 400 mg quercetin/kg diet respectively, furthermore the Medium and High fragmentation percentages of DNA had been decreased significantly in addition groups comparing with control group, too. From current results, we conclude that our study provide an evidence that quercetin may be beneficial as protective effect and maintaining the cellular genetic material against possible oxidative stresses at certain levels without adverse effects.

Keywords: DNA, quercetin, comet, hen.

1. Introduction

Quercetin is a known vegetarian flavonoid found in various foods, such as apples, onions, and green leafy vegetables. Scientific studies suggested that quercetin have wide range of biological activities that are healthy significant, including anti-oxidative, anti-cancer, and anti-viral (Formica and Regelson 1995).

Free radicals are normally formed in cellular pool due to peroxidation of fatty acids, but, excess free radicals lead to damage of cellular components, such as lipid, proteins and nucleic acids(RNA, DNA) (Agrawal et al. 2008), so quercetin has ability of hunting these radicals and restore ordinary status of the cells(Muntasser et al. 2016), also the quercetin and its glycosidic metabolites may modify biological activities like cell signaling pathways and prevention of oxidative DNA damage (Wilms et al. 2005).

Even body cells possess a perfect antioxidant system, an impairment in this system can occur and may lead to oxidative stress, that damage could causes single strand breaks in DNA, which are measured by unwinding of DNA by using of comet assay (Wilms et al. 2008).

One study, noticed that flavonoids quercetin, rutin and naringin significantly suppress intracellular reactive oxygen species that formed by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A549 cells, even quercetin was more effective than other flavonoids and vitamins C and E (Yeh et al. 2006). Chan et al. (2014) observed the quercetin at 20 and 100mg/kg/3 times/week that has been given orally and intra-peritoneal, decreased lymphocyte's DNA damage in mice .

Park et al. (2003); Wätjen et al. (2005) have reported that quercetin has protective effect of hepatoma cells, DNA strand breaks and apoptosis against H_2O_2 cytotoxicity at quercetin concentrations of 10–25 μM.

Another study suggested that it may has a stabilizing effect on fundamental structure of DNA, but prolonged therapy may disrupts double helix of DNA(Alvi et al. 1986).

Objective of the current study is to examine the impacts of quercetin on the DNA status of hen's lymphocyte.

2. Materials and Methods

Experimental animals and design:

A total of 120 ISA-Brown laying hens at age of forty weeks, all hens were reared for twenty weeks on letter floor with clean sawdust in wire pins at well ventilated and temperature-controlled house room according to the instructions of manufacturer's manual, ration and clean water were ad libitum. Hens distributed randomly into 4 treatments with three replicates/treatment (10hens/pin) and placed into 12cages with standard rearing density. The treatments were T1(control): basal diet without any addition, T2, T3 and T4: basal diet supplemented with 400, 800, 1200mg quercetin /kg diet.

Hens fed on the same basal diet which contained 17% crude protein with 2750 Kcal/kg diet metabolizable energy during experimental period. Photoperiod that used include 16h light followed by 8h dark during all experimental weeks, Basal diet was formulated to match nutritional requirements of hens(Table 1).

Table 1. Ingredients and Chemical analyses of experimental Diet.

Ingredients	**%**
Yellow corn	**37**
Wheat	**20**
Barley	**5**
Wheat bran	**5**
Soya bean meal	**18**
Concentrate protein(40%) *	**5**
Vegetable oil	**1**
Limonstone	**7**
Di-Calcium phosphate	**1**
Salt	**0.3**
Premix**	**0.2**
Total	100
Chemical analysis of basal diet	
Crude protein	**%17**
M.E (Kcal/kg)	**2750** kcal/kg
Ca	**4.6** %
Available phosphorus	**3.5** %
Methionine	**0.32**%
Methionine+cysteine	**0.68**%

*Al-wafi(Jordan): each kg contain: metabolisable energy 2100kcal/kg, crude protein 40%, crude fat 5%, fibers 2%, Ca 8%, P 2%, Methionine 2.85%, Methionine+cycteine 3.20%, Lycine 2.85%, Na 2.20%.

**Provimi: each kg contain metabolisable energy 660kcal/kg, crude protein 8%, Fat 1%, Ash 85%, Ca 15-18%, Ava P 12%, Na 5.2-5.6%, Cl 6%, Meth 8.5, Lys 2.3%, vit A 40000 iu,vit D3 80000, B1 140 mg, B2 24 mg, B6 1000mg, B12 72 mg, K3 800 mg, Niacin 280 mg, Biotin 20 mg, Pantothenic acid 200 mg, Folic acid 800 mg, Choline 2000 mg, Mg 4000 mg, Mn 4000 mg, Zinc 2000mg, Mn 4000 mg, Se 200 mg, I 1760 mg, Antioxidant 2000 mg.

Comet assay:

At end of experiment (60weeks of age) by collecting blood samples from wing vein in EDTA-tubes for six hens for each treatment(2hens/replicate) to measure DNA strand status of the lymphocyte cells, Comet assay was performed on the whole blood samples (Singh et al. 1988), by using of commercial reagent kits(Trevigen ,USA) with using the fluorescent microscopy.

At first, lymphocytes were isolated from the anticoagulant whole blood using the method described by Boyum,(1968), these cells were suspended in low-melting-point agarose in phosphate buffer saline(PBS) at 37ºC and then placed on a frosted glass microscope slide pre-coated with a layer of 1% normal-melting-point agarose. After application of a third layer of 1%

normal-melting-point agarose, the slides were immersed in cold-lysing solution (10mM Tris, 2.5M NaCl, 100mM Na2EDTA, 1% sodium Nlaurylsarcosine, 1% Triton X-100, and 10% dimethylsulphoxide) for 1 hour at 4ºC. The slides then placed in an electrophoresis tank, the DNA was allowed to unwind for 15 min in the alkaline solution. Electrophoresis was performed by using the method of Collins et al. (1995). Staining the cells with Ethedium Bromide and using of a fluorescent microscope at 40x objective has been used to cell observation, about 100 randomly selected cells were analyzed per sample. The captured images were analyzed by computer with the Interactive Image Analysis Comet Assay III (Perceptive Instrument, UK)(Figure 1), DNA strand breaks were expressed as comet length, height, intensity, and comet area by using of comet score software (Figure 2).

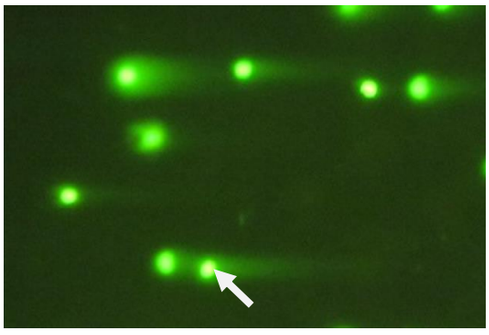

Figure1. Shape of lymphocyte DNA comet like

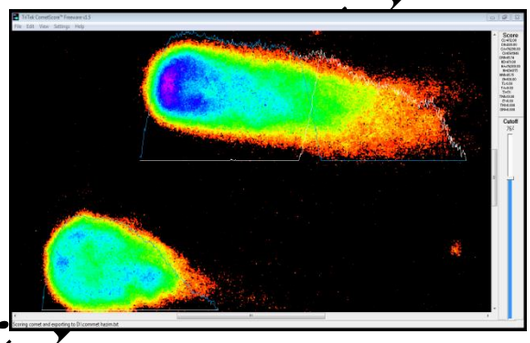

Figure2. Analysis of DNAcomet by comet score program

Data were analyzed by using SPSS (version 24.0). All numerical results have been expressed as the mean±standard error. For comparisons, the statistical significance has assessed by ANOVA. The significance level has set at P<0.05 and P<0.01.

3. Results

Table 2 which represent the influence of supplementing quercetin in fragmentation proportion of DNA of heart lymphocytes at experimental end. These data depended on number of examined cells of each treatment for DNA fragmentation. There were a significant differences among treatments regarding medium and high percent of DNA fragmentation, however, there were no significant differences among treatments considering the percent of No and low fragmentation of DNA.

Data of Medium fragmentation of DNA, indicated that there were a high significant differences ($p \leq 0.01$) among treatments, herein there was no difference between T2 and T3, which differed than T1 and T4. The lower and better value was T4(9.38) as compared with control(10.38).

With regard of High fragmentation of DNA, revealed that a significant difference among treatments, no difference among T2, T3 and T4, in addition, no differences among T1, T3 and T4, so the T2 recorded the lowest and the best percent as compared with control.

Table 2. Effect of quercetin in fragmentation (Frag) percent of lymphocyte DNA (%) of laying hen at experimental end (mean ± standard error).

Fragmentation % of lymphocyte DNA				
Treatments	**No Frag (%)**	**Low Frag(%)**	**Medium Frag (%)**	**High Frag (%)**
T1	43.67 ± 1.09	34.74 ± 1.16	10.38 ± 0.33 b	11.21±0.65 a
T2	45.22 ± 0.32	34.65 ± 0.18	11.30 ± 0.22 a	8.83±0.24 b
T3	44.48 ± 0.76	33.49 ± 0.62	12.00 ± 0.27 a	10.02±0.45 ab
T4	45.69 ± 0.34	35.02 ± 0.32	9.38 ± 0.19 c	9.90±0.44 ab
Significance	**N.S**	**N.S**	****	*****

T1=control, T2, T3 and T4 supplemented with 400, 800 and 1200 mg quercetin/kg diet respectively, Frag=Fragmentation of DNA, N.S= No significant differences between treatments, ** significant differences between treatments at (p≤ 0.01) in the same column. Different letters within the same column mean there is a significant difference among treatments.

Findings of table 3 that represents the effect of added quercetin in certain DNA criteria that consider as an indicators of severity of DNA damage which measured as pixel unite. There were a high significant differences (p≤0.01) among treatments in all parameters that related with DNA comet. Concerning the comet length, there was a high significance, so T2 and T3 didn't differed between each other, also T3 and T4 were the same thing. T4 recorded the best length (32.58%) contrast the control treatment (62.58%).

In accordance of comet height, there was also a high significance (p≤0.01) among treatments. The T2 and T3 in addition to, T2 and T4 didn't significantly differed. Herein, T3 scored low and better value as compared with control.

In regard of comet intensity and area, there were also a high significant differences(p≤0.01) among experimental treatments, there were a significant differences between quercetin-

supplemented treatments and control, here in the supplementation treatments (T2, T3 and T4) significantly differed and surpassed than control.

Table 3. Effect of supplementing quercetin on lymphocyte DNA criteria (px) of laying hen at experimental end (mean ± standard error).

Comet criteria(px)				
Treatments	**Comet length (px)**	**Comet height (px)**	**Comet intensity (px)**	**Comet area (px)**
T1	62.58 ± 1.85 a	35.08 ± 0.94 a	1574.25 ± 90.79 a	143346.17 ± 8236.25 a
T2	37.58 ± 0.97 b	23.50 ± 0.91 bc	628.00 ± 54.02 b	58871.17 ± 5289.55 b
T3	34.00 ± 1.14 bc	22.33 ± 0.68 c	547.75 ± 33.47 b	54522.42 ± 3267.76 b
T4	32.58 ± 1.20 c	25.58 ± 0.66 b	634.00 ± 36.04 b	51704.83 ± 5592.42 b
Significance	**	**	**	**

T1=control, T2, T3 and T4 supplemented with 400, 800 and 1200 mg quercetin/kg diet respectively, ** high significant differences between treatments at (p≤ 0.01) in the same column, px=pixel. Different letters within the same column mean there is a significant difference among treatments.

4. Discussion

The current study resulted in an improvement in the comet indicators of DNA which includes comet length, height, intensity and area in addition, improved Medium and High fragmentation percents of DNA. Our findings have been proved that the protective activity of quercetin for nucleic acid against damage effectors such as stress, oxidation, high climate temperature, radiation and pollution. The manner of findings was the same in all DNA criteria which, indicated there was a superiority of quercetin-supplemented treatments comparing with control.

Quercetin has potency of protecting DNA against instability caused by carcinogenic DNA, it was observed that the potent reduction by quercetin of DNA damage caused by DNA hydroxide in HepG2 cells line (Ramos et al. 2008). An Indian research group reported that the preventive potential of quercetin against formation of comet defects through by changing oxidative status of cultured lymphocytes (Muthukumaran et al. 2008).

Gupta et al. (2010) observed via in vivo study that quercetin has the protective effect through a significant reduction of the comet formation of liver cells upon induced poisoning with diethylnitrosamine.

Quercetin´s protective activity of DNA may be attributed to its anti-oxidative potential by either directly scavenging free radicals or indirectly by suppressing the single oxygen, perhaps donating hydrogen and chelating metal ions thus, minimizing peroxidation of DNA (Heo and Lee 2004).

By the way, we didn't find a study deals with quercetin and comet assay in poultry, so our findings are in agreement with Awasthy (2013) who noticed that reduction of apoptotic DNA content and DNA fragmentation in rats testicular cells after treatment these cells with different concentrations of quercetin which previously exposed to in vitro induced peroxidation. The work done by Prakash (2009) was supported by present study who showed ameliorative effects of quercetin through significant reduction in DNA anomalies and apoptotic cells of thymocytes that poisoned by the arsenic.

Wilms et al. (2008) noticed that quercetin is able of protecting leucocytes against oxidative damage that caused by hydrogen peroxide in a dose-dependent way, so the quercetin at concentrations of 1, 10 and 50 μM minimized the levels of superoxide-induced oxidative DNA defect, indicating that quercetin is a more potent inhibitor of hydroxyl radical formation and protecting DNA.

5. Conclusion

Our conclusion from the statistical analysis of DNA comet is the dominance of addition treatments as compared with control concerning of comet criteria of DNA, thus, the quercetin may has the protective potential for genetic material. The effect of quercetin on lymphocytes DNA status was much obvious with high significant differences.

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Isolation and Identification of Bacteria among Renal Failure Iraqi Patients

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Abstract

Study aim to identification of pathogenic bacteria associated with renal failure patients and to demonstrate its sensitivity to antibiotic. **Methods:** A total of 121 urine samples of renal failure patient were obtained from both Marjan and Al-Imam Al-Sadiq Hospital in Hillah city, belonging to both sexes at various age groups ranging from 15 to 85 years of age, using sterile urine cup, all specimens were immediately transported to laboratory and cultivation within 2 h. **Findings:** morphological and biochemical characterization indicated that 68(58%) of sample had positive and 53 (42%). **Results** of urine culture and biochemical tests for isolated bacteria from renal failure patient were revealed that total bacterial isolate 49 of the most popular bacteria, *Escherichia coli* is 14 (30.4%) afterwards accompanied by *Staphylococcus aureus* 9 (19.5%), *Enterococcus faecalis* 9 (19.5%), *Staphylococcus saprophyticus* 2 (4.3%), *Klebsiella pneumoniae* 5 (10.8%), *Pseudomonas aeruginosa* 2 (6.5 %), Proteus (2.1%) and *Serratia fonticola* (2.1%). **Conclusion:** According to the current study renal failure is more common in male than female in Babylon province. Urinary tract infection in female more than male in renal failure patient Escherichia coli the most common bacteria isolate.

Keywords: bacteria, kidney, *E. coli*, antibiotic

1. Introduction

A kidney impairment caused by bacterial contamination. The incidence of renal dysfunction in biliary or gastrointestinal tract infections and random bacterial peritonitis (SBP) and other forms of infections was greater than in others. Furthermore, only biliary or gastrointestinal tract infections, SBP, and urinary tract infection Clinicians are worried with infection-induced kidney diseases as early diagnosis and treatment of infections will prevent or restrict the severity of damage caused by micro-organisms causing the infections. (UTI) precipitated the progressive type of renal failure (Silvano *et al.*, 2006). Infections can cause kidney injury by either direct invasion, or by immune-mediated mechanisms that manifest as post-infectious glomerulonephritis, or glomerulone associated with infection. Depending on the microorganisms, the endemic / epidemic type and the cause of infection, clinical symptoms may be acute or chronic Both infectious viruses, microbes, mycobacteria, fungi, and protozoa have been implicated in kidney failure inducing either direct kidney damage or immune-mediated harm. Practices of infection management in significant areas of the world are limited by deprivation, social behavior, high population growth, pollution, limited exposure to clean drinking water, and insufficient health care services While, antimicrobials and vaccines have successfully eradicated and healed many infectious diseases; but injudicious antimicrobial usage and proliferation of resistant species complicated the disease seriousness including secondary renal amyloidosis with

chronic recurrent infection. A recent pattern in the developed world has been the re-emergence of various infections leading to uncertain diagnostic challenges and association with kidney disease Standard theory of evolution of virulence assumes that virulence factors are retained because These observations are disagreed with by most opportunistic pathogens (OPs),they said the reproduction of parasites, increase growth and/or spread among hosts..(Brown *et al* 2012) The spectrum of kidney diseases induced by infection is diverse Severe kidney damage (AKI), severe and persistent glomerulonephritis syndrome, nephrotic syndrome, acute nephritis-nephrotic syndrome, acute or chronic tubulointerstitial nephritis, and quickly progressing glomerulonephritis, etc. One of the most common exposures is Aki may Occurrence de novo or on pre-existing chronic kidney disease (CKD) background. Approximately 40 per cent of patients recovering from AKI have persistent renal dysfunction and rnany are developing CKD. In those with pre-existing CKD, infections frequently speed up the process of development and may contribute to renal failure in the end stage (ESRD).(Prasad & Patel, 2018).

2. Materials and methods

Sample Collection A total of 121 urine samples of renal failure patient were obtained from both Marjan and Al Imam ALSadiq Hospital in Hila city, during a period ranging from August 2019 to February 2020, belonging to both sex at various age groups ranging from 11 to 75 years of age, using sterile urine cap, all specimens were immediately transported to laboratory to laboratory and cultivation within 2 h.

Isolation of bacteria spp.

To study the morphological features of microbial isolates several methods were used , a group of microorganisms have been identified including bacteria, fungi (yeast) . The colony form was adopted as an initial diagnosis , staining smear with Gram stain bacterial was made to study microscopic characteristics and lactophenol cotton blue to fungi and simple stain , observed under oil immersion lens (100X). The bacterial isolate was appearing as Gram positive and Gram negative so the biochemical test and Vitek 2 compact system was confirmed the final diagnosis). The fungi (yeast), colonies morphology and stain.

3. Results and Discussion

Urine culture and biochemical tests for isolated bacteria from renal failer patient were revealed that total bacterial isolate 49 Of the most popular bacteria, Escherichia coli is 14 (30.4%) afterwards accompanied by *Staphylococcus aureus* 9 (19.5%) , *Enterococcus faecalis* 9 (19.5%) *Staphylococcus saprophyticus* 2 (4.3%), *Klebsiella pneumoniae* 5 (10.8%), *Pseudomonas*

aeruginosa 2 (6.5 %), Proteus (2.1%) and Serratia (2.1%) as figure (1) In the present study out of cases , had pure culture in which bacterium and fungi 46 (38%) , 12 (9.9 %) cases) and 8(an respectively , 55 (43.8%) cases did not show any growth, 2 (1.6%) cases more than one bacterium , 6 (4.9 %) cases had mixed culture show in table (1)The results were shown the pure culture in which bacterium higher than others causative agents agree with (Bhan *et al.* , 2016) .

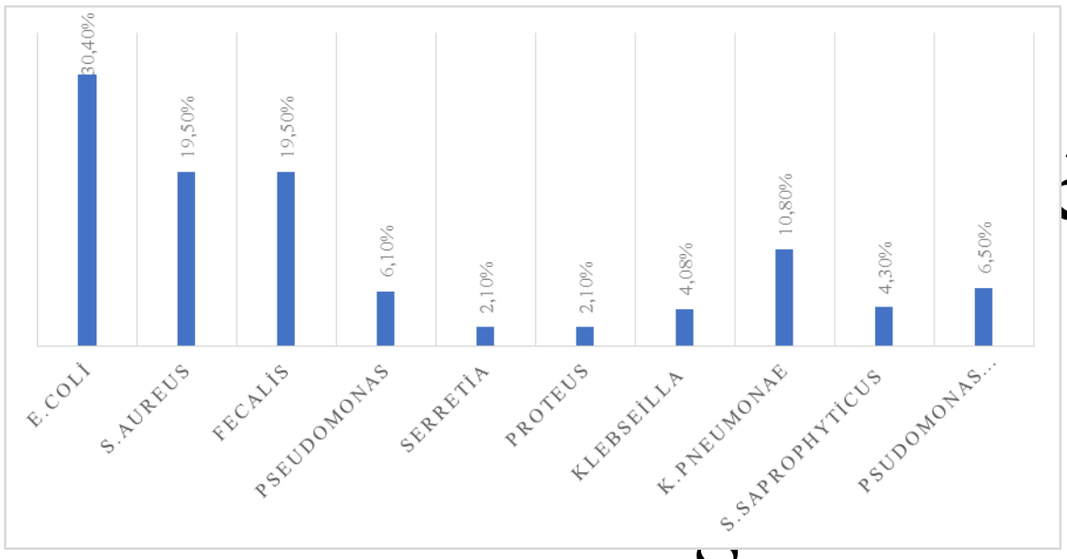

Figure 1. Types and percentage of bacterial isolates.

The bacterial infection higher than others which was (38%) while mixed (fungi with bacterial) cause infection was (9.9%%) , fungi and unknown infection was (1.65 %) in patients . differences in types of isolated infection depend on different types of environmental factors , geographical regions and health of people . This result might be shown that mixed causative agents are belong to two types bacterium to bacterium as primary infection bacterium and caused secondary causative agent with other fungi causes, also due to secondary infection as complication of primary bacterium infection . this result agree with (Jreemich, 2017) he point that groups of patients are critical to diagnosis which one of agents are first causative from other.fungi detected in other study(Hadi and Alsultany,2020).

Table 1. Main Causative Groups of Microorganisms.

No. of patient / Causative agent	Total	Age groups			%
		11-34	35-45	45-75	
Bacterial	46	8	9	29	67.6%
Mixed	8	2	0	6	11.7%
Fungi	12	2	1	9	17.6%
Unknown	2	1	0	1	2.9%
Total	68	13	10	45	100%

This result might be refer to that, the bacterial causes are more current than other and concluded that mixed group have fungi associated with infection as well as the unknown group is to related to other causative agents not included in present study (Jeong., 2019) The main bacterial types isolated in this study were shown in Table (2) .

E coli this result agree with (Jeong., 2019) *E coli* have virulence factor that responsi ble for adherin in urinary tract epithelium (Sarowska et al., 2019) the second isolate *Staphylococcus aureus* and *Enterococcus faecalis* this result agree with (Khalil, El-Balat, Zeid, Al-Mohamma, & Enan, 2020) Klebsiella pneumoniae, Serratia and Proteus result may be because bad hygene and nosocomial infection (Najar, Saldanha, & Banday, 2009) Pseudomonas aeruginosa result positive the most common nosocomial infection in renal failure patient urine this result agree with (Novoa et al., 2017) Staphylococcus aureus was important causative asgent in this study Because it is represent (13.8) of all isolate so the result agree with(L.H. *et al*., 2015) the current study agree with another study (Al-Jebouri & Al-Alwani, 2015) Escherichia coli remains the most common pathogen for both outpatients and hospitalized patients.

Table 2. Distribution of microbial isolate from renal failure patient infection.

Type case	Microbial	Total isolate	Percentage%	No.
G+VE	*Staphylococcus aureus*	10	13.8	23 31.9%
	Staphylococcus saprophyticus	2	2.7	
	Enterococcus faecalis	11	15.3	
G-VE	*Escherichia coli*	20	27.7	31 43 %
	Klebsiella	6	8.3	
	Pseudomonas aeruginosa	3	4.1	
	Proteus mirabilis	1	1.3	
	Serratia fonticola	1	1.3	
YEST	*Candida albicans*	5	6.9	18 25%
	Candida krusei	6	8.3	
	Candida glabrata	5	6.9	
	Candida parapsilosis	2	2.7	
	Total	**72**		**100**

Table 3. Biochemical test for bacteria that isolated from renal failer patient.

Bacteria	indol	Methyl red	Voges proskauer	Simmon citrate	Catalase	oxidase	Urease	motility
P. aeruginosa	–	+	–	+	+	–	-	-
E. coli	+	+	–	–	+	–	-	+
K. pneumoniae	–	+	–	+	+	–	+	-
S. fonticola	–	–	+	+	+	–	+	+
S. aureus	–	+	+	+	+	–	+	-
E. faecalis	–	+	+	+	+	–	-	-
Proteus	–	+	–	+	+	–	+	+

The current study agree with (Al-Jebouri & Al-Alwani, 2015)The prevalence of UTI was higher among female than male patients with ratio of 1.5: 1 and this was almost similar to that of (Al-jebouri, 2015)This was due to the shortness of the urethra, which is close to the vagina and anus and the lack of prostatic fluid which has antibacterial activity.

Effect of some antibiotics on microbes isolate

Some antibiotics were used to show the effect on different types of microbes isolate from renal failer patient urine . disk diffusion method was used in study the sensitivity of bacterial to antibiotic . it has been found that there is clear variation in resistance ,and most isolates shown resistance to one or more antibiotics . It has been found that most of these isolates were high resistant to beta lactam group(amoxicillin+clavulanic acid and ciftazidim) This study revealed high resistance of Gram-negative bacterial isolates to β-lactams (100 % for amoxicillin/clavulanic

acid) this study identical to (Breijyeh, Jubeh, & Karaman, 2020) they have pointed Neutralizing the antibiotics, circulating them outside the cell or changing their exterior composition resulting in disruption of the bacterial connection of the medications. Antibiotic resistance pathways are divided into four groups: inherent resistance in which bacteria may alter their structures or materials, resistance in another form in which bacteria may gain new resistance , Where bacteria can acquire new genes of resistance and DNA from other bacteria of resistance. In fact, genetic variations in DNA that may modify protein development contributing to specific components and receptors that are not recognized by the antibiotic. Report of another research extremely immune to betactame antibiotics (Shang *et al*., 2019) who found. Lipoprotein-like genes (lpl, sa2275–sa2273) that are upregulated in the main clinically prevalent MRSA clones in reaction to β-lactam induction at subinhibitory concentrations. In enzyme mediated resistance, There are three main pathways of resistance in Staphylococcus aureus to beta-lactam antibiotics: enzyme induced (penicillinase or beta-lactamase) by which the antibiotic is inactivated; intrinsic, which is not attributable to inactivation of the medication, which accounts for methicillin-resistance; which immunity, by which the inhibitory and killing activities of beta-lactam antibiotics are dissociated. (Sabath, 1982) ciftazidim is the thired generation of cephalsporin alse used in this study the result showed that all isolate of Pseudomonas aeruginosa and Staphylococcus aureus where resistance (100%,100%) respectively on the other hand (S. *et al*., 2016) had point The primary pathways are the overexpression of efflux pumps and the production of beta-lactamases, i.e. broad range beta-lactamases and metallo-beta-lactamases.. Beta-lactamases are the enzymes encoded by various bacterial chromosomal and plasmid chromosomes. Beta lactamases are produced as a form of metabolic by-products capable of hydrolyzing and destroying the beta lactam Antimicrobials. The global challenge to antimicrobial therapy is resistance induced by extended spectrum beta-lactamases (ESBLs), metallo-beta-lactamases (MBLs), and ampc beta-lactamase (AmpC) enzymes to wide-spectrum beta-lactam antibiotics. Hence the increasing pattern of antibiotic resistance attributable to beta-lactamase enzymes of widely used antibacterial agents for infect care Because of P. aeruginosa and its current impact on antibacterial therapy failure, we were encouraged to find out the resistance rate mediated by beta-lactamases and current antibiotic options for effective treatment of that organism. To the best of our knowledge, this is one of the few studies that focus exclusively on investigating the pattern of antimicrobial susceptibility in P-producing ESBL, MBL, andAmp *C. aeruginosa*. The effect of amoxicillin on the result protious (100 per cent) shows isolate where amoxicillin + These results of this antibiotic resistance are almost compatible with those of clavulanic acid resistance(Girlich, Bonnin, & Dortet, 2020) Proteus isolates the loss of porins, reduces the expression of penicillin-binding proteins (PBPs) PBP1a, PBP2 or acquires several genes of antibiotic resistance, including carbapenemase genes. In addition, resistance to non-β-lactams is also in agreement with the results of this study (Lomholt & Kilian, 2003) that found Pseudomonas aeruginosa was susceptible to ciprofloxacin

all Klebsiella pneumoniae strain isolates from renal failure patient were suciptable to ciprofloxacin , and have suggest that fourth of fluroquinolones seem to be more effective than previous generation against Klebsiella pneumoniae strain and other type of bacteria isolated from renal failer patient. In Gram-negative bacteria and Gram-positive bacteria, respectively, the process by which quinolones influence bacterial DNA synthesis is by inhibiting DNA gyrase and topoisomerase type IV. Both DNA gyrase and topoisomerase type IV are important to the relaxation of DNA supercoils during DNA replication the result agree with result point out (Aditi Priyadarshini, Mahalakshmi, & Naveen Kumar, 2019) regarding to ciprofloxacin , all bacterial isolate were susceptable to ciprofloxacin Staphylococcus spp. Pseudomonas aeruginosa ,Klebsiella pneumoniae ,Proteus, and *E. coli* were completely susceptible ciporofloxacin these result agree with those obtain (Chaudhary, 2016) except s . aureus resistance to ciprofloxacin (Kot *et al*., 2017) Antimicrobial Resistance Patterns in Methicillin-Resistant *Staphylococcus aureus* from Patients Hospitalized

Table 4. Resistant of gram positive Bacteria isolate from renal failure patients to antibiotic.

Bacteria isolate	**Total**	**Antimicrobial drug resistance (%)**										
		Antibiotic type										
		AMC	**CAZ**	**PRL**	**AK**	**MEM**	**TE**	**AZM**	**CIP**	**TOB**	**ATM**	**CN**
Staphylococcus aureus	**9**	55.55 %	100 %	100 %	100 %	33%	22.2 %	22.2%	33 %	11.11%	66.66 %	10 0%
Staphylococcus saprophyticus	**2**	50%	100 %	100 %	50 %	0	0	0	0	0	0	10 0%
Enterococcus faecalis	**9**	9%	33%	100 %	0	0	100 %	100%	0	66.66%	0	44. 44 %

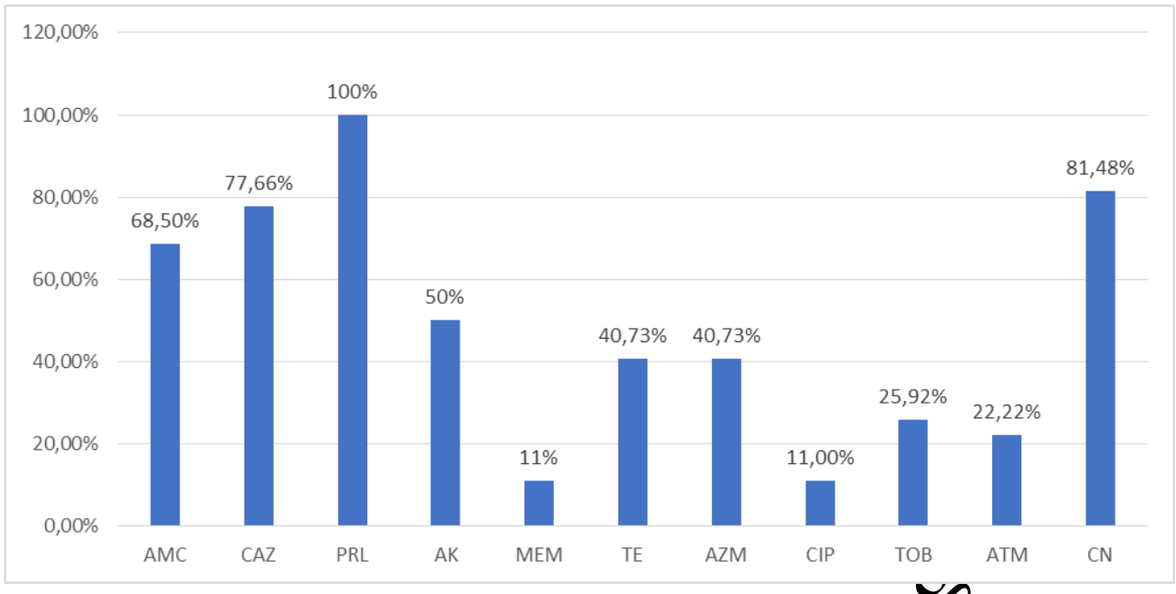

Figure 2. Gram positive bacteria resistance to antibiotics.

The mechanism of resistance .to b-lactam can be found in Pseudomonas aeruginosa these mechanism include Mechanism of resistance The complex chromosomal encoded genes cause *P. aeruginosa* against various chemical agents. Different strains of P. aeruginosa with its intrinsic potential for the creation of biofilms further strengthens the resistance under different environmental factors and other mechanism like lose of porins and over expression resistance gene and b-lactamase production (Novoa et al., 2017).other study also isolate bacteria in UTI patients especially pregnant women (AlSultany, 2011) Staphylococcus is resistant it produce many toxins some studied in animal model (AlSultany, 2016) in gram positive bacteria in current study the resistane to meropenem (Elshamy & Aboshanab, 2020). A significant explanation for the rapid dissemination of AMR across bacterial communities is that plasmids or other extremely mobile genetic elements that are repeated and exchanged separately between bacterial cells and organisms are transferred to genes that impart resistance.

Table 5. Resistant of gram negative Bacteria isolate from renal failure patients to antibiotic.

BACTERIA ISOLATE	Total	ANTIMICROBIAL DRUG RESISTANCE (%)										
		ANTIBIOTIC TYPE										
		AMC	PB	CPM	ATM	CO	CAZ	GM	CIP	TOB	MEM	NET
E.coli	14	100	50	100	0%	100%	66	50%	0%	28.5%	0%	28.5
Klebsiella	5	100	100	100	40%	100%	0	100%	0%	0	0%	0
Proteus	1	100%	100	100	0	100%	0	100%	0%	0	0%	0
Pseudomonas aeruginosa	3	100	100	100	0	66%	0	33%	0%	33.3%	0%	33.3%
Serratia fonticola	1	100	100	100	0	100%	0	100%	0%	0	0%	0

Once a newly discovered antimicrobial agent is proven to be effective and is approved for therapeutic use.the current study the resistance of Staphylococcus aureus gram positive bacteria(Breijyeh e*t al.*, 2020) Inactivation of β-lactams by β-lactamases considered a significant MDR pathway another type of resistance is multidrug efflux pumps against several specific groups of antibiotics Three types of enzymes, namely acetyltransferases, adenyltransferases, and phosphotransferases, mediate resistance to aminoglycoside. These enzymes modify aminoglycosides in a chemical way. . The coding genes can be transmitted via plasmids , transposons and integrations the current study is agree with another study (Carvalhaes, Shortridge, & Sader, 2018) they are noted that meropenem. He was very successful against a wide number of Enterobacterales isolates collected from PHP and VAP over a4-year span in 31 U.S. hospitals. This collection included CRE isolates resistant to many comparator agents but mostly susceptible to. (> 99 percent). susceptible to . Meropenem was also active against isolates of P. aeruginosa which were resistant to many antipseudomonal agents and had high rates of MDR and XDR. rates. This combination agent may be considered an effective alternative for treating U.S. hospitals with HAP / VAP infections when approved by the FDA. E.coli has virulence factors LPS was studied also after extraction in animal model. (AlSultany and Jassim,2016).other researchers studying ability of some nanoparticles against pathogenic bacteria(Hathal et al.,2020).

In this study the most common types of bacteria are resistance to antibiotics (inhibitor of cell wall synthesis the reson may be that bacteria have varety of defence mechanism, including β-lactamase , which break down the β- lactam in antibiotic β-lactam , so the resistance of microbes of these antibiotics is one of the most common medical problem inflammation of urinary tract

infection in renal failer patient (Bebell, 2019) the current study disagree with(Prakash & Saxena, 2013) In 92.30 percent of cases of Enterobacter spp, nitrofurantoin was the most resistant drug followed by Ceftriaxone, Gentamycin in 53.85 percent of cases, and Cefotaxime, Tobramycin in 46.15 percent. However, the most sensitive ones were Ciprofloxacin, Moxifloxacin, Ofloxacin, Sparfloxacin, Levofloxacin, Gatifloxacin, Imipenem, Meropenem.

4. Conclusion

According to the current study renal failure is more common in male than female in Babylon province. Urinary tract infection in female more than male in renal failure patient Escherichia coli the most common bacteria isolate.

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