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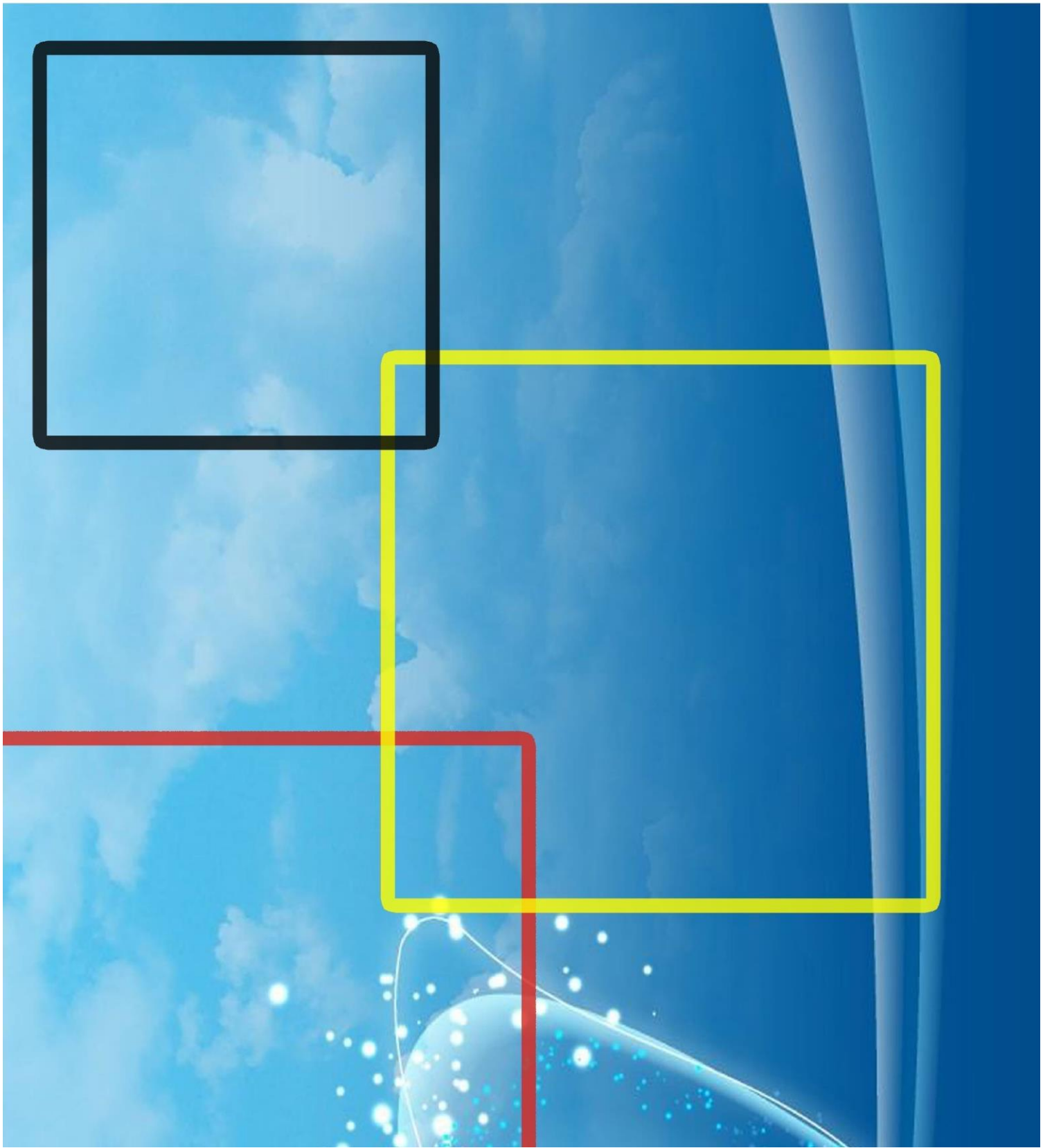

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Antioxidant and Antihyperlipidemic Effect of *Solanum Nigrum* Extract in Experimental Diabetes Model

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ABSTRACT Diabetes mellitus (DM) is a chronic metabolic non-communicable disease; it is globally considered the fifth cause of death and it has attained worldwide epidemic proportions. In our study, we aimed to investigate the diabetic effects of *Solanum nigrum* extract using the control group (C), diabetes group (D), groups given the *Solanum nigrum* extract (SN) and diabetes group + *Solanum nigrum* extract (D+SN). Our results observed the biological effectiveness of *Solanum nigrum* extract on glucose levels, significant increase serum glucose level group (D) (663±21.8 mg/dL) in comparison with C (131±9.8 mg/dL) were recorded. However, there were no significant difference in glucose level between C group (131±9.8 mg/dl) and SN group (196.14±12.1 mg/dL). Moreover, glucose level of D+SN group (484.8±40.0 mg/dL) was significantly higher than C (131±9.8 mg/dl), D (663±21.8 mg/dl) and SN groups (196.14±12.1 mg/dL). Total antioxidant status (TAS) level in D group (1.85±0.15.7) was significant when compared C group (1.28±0.17). Significant differences were observed between D group and D+SN group (1.54±0.07). However, TAS levels showed no significant difference in both SN (1.27±0.10) and D+SN (1.54±0.07) groups in comparison to the control group. Total oxidant status (TOS) level in D group (6.30±1.41) was given significant differences in comparison with control C (3.87±0.34), SN (4.87±0.80) group and D+SN (4.14±0.34) groups. In contrary, there were no significant differences between all of C, SN, D+SN groups. As a result, we can say that the *Solanum nigrum* plant extract is effective on diabetes, but it cannot lower the glucose level to normal levels, it needs to be investigated in future studies and its effects at different doses by different extraction methods.

Keywords: *Hypolipidemica agent, Streptozocin, Oxidative stress.*

ÖZ

Deneysel Diyabet Modelinde *Solanum nigrum* Ekstraktının Antioksidan ve Antihiperlipidemik Etkisi

Diabetes mellitus (DM), kronik, metabolik, bulaşıcı olmayan bir hastalıktır, dünya çapında beşinci ölüm nedeni olarak kabul edilir ve dünya çapında epidemik oranlara ulaşmıştır. Çalışmamızda kontrol grubu (K), diyabet grubu (D), *Solanum nigrum* özü (SN) ve diyabet + *Solanum nigrum* özü (D+SN) verilen gruplar kullanılarak *Solanum nigrum* ekstraktının diyabetik etkilerini araştırmayı amaçladık. Sonuçlarımız, *Solanum nigrum* ekstraktının glukoz seviyeleri üzerinde biyolojik etkinliği gözlenirken, serum glukoz seviyesinde (D) (663±21.8 mg/dL) C'ye (131±9.8 mg/dL) kıyasla anlamlı artış kaydedildi. Ancak C grubu (131±9.8 mg/dl) ve SN grubu (196.14±12.1 mg/dL) arasında glukoz düzeyi açısından anlamlı fark yoktu. Ayrıca D + SN grubunun glukoz düzeyi (484.8±40.0 mg/dL), C (131±9.8 mg/dl), D (663±21.8 mg/dl) ve SN gruplarına (196.14±12.1 mg/dL) göre anlamlı olarak yüksekti). D grubunda (1,85±0,15,7) toplam antioksidan durum (TAS) düzeyi, C grubu (1.28±0.17) ile karşılaştırıldığında anlamlıydı. D grubu ile D+SN grubu arasında anlamlı farklar gözlendi (154±007). Ancak TAS düzeyleri hem SN (1.27±0.10) hem de D+SN (1.54±0.07) gruplarında kontrole göre anlamlı farklılık göstermedi. D grubunda (6.30±1.41) toplam oksidan durum (TOS) düzeyi, kontrol C (3.87±0.34), SN (4.87±0,80) grubu ve D+SN (4.14±0.34) gruplarına göre anlamlı farklılık gösterdi. Aksine, tüm C, SN, D+SN grupları arasında anlamlı bir fark yoktu. Sonuç olarak *Solanum nigrum* bitki ekstraktının diyabet üzerinde etkili olduğunu ancak glikoz seviyesini normal seviyelere indiremediğini, farklı dozlarda ve farklı ekstraksiyon yöntemleri ile etkilerinin ileriki çalışmalarda araştırılması gerektiğini söyleyebiliriz.

Anahtar Kelimeler: *Hipolipidemik ajan, Oksidatif stres, Streptozosin.*

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic non-communicable disease; it is globally considered as the fifth cause of death and it has attained worldwide epidemic proportions. As of 2015, more than 415 million adults were investigated to have DM, and this number is predicted to elevate to 642 million by 2040 (Domingueti et al. 2016; Unnikrishnan et al. 2016). DM is a group of metabolic disorders of carbohydrate, protein and fat which can be recognized by chronic hyperglycemia (the elevation of glucose level in blood) following defects in secretion and/or action of insulin, a protein (hormone)is produced in β-cells of the pancreas (Patel et al. 2012; Ahmet et al. 2019; Söğütlü et al. 2019). Hyperglycemia is also followed by the generation of reactive species (ROS) which subsequently leads to lipid peroxidation and membrane damage and thus, plays an important role in the production of secondary complications in diabetes mellitus such as kidney, eye, blood vessel, and nerve damage. The inhibition of peroxidation chain reaction through antioxidants protect β-cells from oxidation and consequently play important roles in regulating related-metabolic activities in diabetes. Such antioxidants including phenolic compounds (tannins, flavonoids and stilbenes) and vitamin C and vitamin E can naturally be found from plant extracts preserving functions of β-cells and also preventing diabetes-induced formation of ROS (Patel et al. 2011).

In addition, extracts of several herbal medicines have been used and recommended to have potential therapeutic effects on diabetes and its complications (Tiwari and Rao 2002; Mukherjee et al. 2006). *S. nigrum* is a herb that belongs to the Solanaceae family and includes in a class of Dicotyledonae. *S. nigrum* is also known as black nightshade, garden nightshade, or blackberry nightshade (Zaidi et al. 2019; Alam et al. 2022). Extracts from *Solanum nigrum Linn* (European black nightshade) which belongs to the *Solanum* genus and Solanaceae family (Mani et al. 2022) have been reported to exhibit anti-tumor activity against different types of cancers (Xiang et al. 2016) and significant antidiabetic activities against diabetes (Poongothai et al. 2010). Due to its antipyretic - diuretic activities, this plant is extensively used as Chinese folk medicine. Recent studies revealed that extracts of *S. nigrum* exhibit antioxidant (Saibu et al. 2020), hepatoprotective (Hsu et al. 2009), antihyperlipidemic, antidiabetic (Hou et al. 2013; Sohrabipour et al. 2013) and anti-inflammatory activities (Wang et al. 2017).

The present study is carried out to evaluate the antioxidant and antihyperlipidemic activities of the extract of *S. nigrum Linn* grown in southern Iraq on the rate of diabetes in rats.

MATERIAL AND METHODS

Experimental Animals

Twenty-eight male rats weighing 200-250 g were obtained from the Experimental Medical Applicated and Research Center of Van Yuzuncu Yil University, Medical Faculty. Subjects randomly composed of seven rats each; control (C), Diabetes Mellitus without SN (D), Diabetes Mellitus with SN group given and SN given. This study was performed with Van YYU Animal Experiments Local Ethics Committee (VAN YUHADYEK) (Approval no: 2016/02, date: 25.02.2016).

Preparation of Plant Extract

Solanum nigrum L. plant was collected from northern Iraq. It has been confirmed in the herbarium of Van YYU Faculty of Science, Department of Biology (*S. nigrum L.,* Sp. PI. 186). Plant samples of Solanum nigrum were thoroughly washed under tap water, and then dried in the shade. The dried samples were finely pulverized to a powder sample. 100 g of powder was suspended in 250 ml of water for two hours and then heated to 60-65 °C for 30 minutes. The extract was collected by the separation process and this process was repeated three times. The collected extracts were put together and passed through a swab. The filtrate was evaporated at 40-50 °C in rot vapor under reduced pressure. The obtained dark semi-solid material (yield 14%) was maintained at 0-4 °C until using. A known amount of residual extract was then suspended in distilled water and administered to animals via oral (Umamageswari et al. 2017).

Experimental Design

Diabetes-induced D and DSN group rats were administered intraperitoneally (i.p.) by dissolving 45 mg/kg single dose streptozocin (STZ) (Sigma, USA) in citrate buffer at pH 4.5 (0,1 M) (Bloch and Vardi, 2005). The same amount of saline was injected into the control group. D and DSN group, 72 hours after injection of STZ, blood glucose levels were determined by means of Plus MED Accuro biosensor screener and striplines in the blood samples taken from the tail of the rats. Blood sugar levels higer than or equal to 270 mg / dl were included in the study. The rats in the SN and D+SN groups were orally administered with 250 mg/kg/day gavage of SN extract dissolved in distilled water every day (Umamageswari et al. 2017).

Control Group: Seven randomly selected rats were divided into control groups.

Group 1: Seven rats STZ solutions in this group were given 45 mg/kg IP route. (D)

Group 2: Seven rats in this group were dissolved in distilled water and the SN solution was administered orally for 25 days at 250 mg/kg/day. (SN)

Group 3: Seven rats in this group were treated with STZ solution 45 mg / kg IP, followed by 72 hours after the glucose measurement was performed. Water was added to the rinsed aqueous solution at a dose of 250 mg/kg/day for 28 days. (D+SN)

Collection of Samples

Blood samples were drawn from the left ventricle of the hearts of the animals to the glazed glass serum tubes under ketamine and rompun anesthesia after a twenty-eight-day trial. The blood samples were centrifuged at 3000 rpm for 10 minutes at + 4 °C. TAS, TOS and biochemical parameter analyzes were obtained in these samples.

Preparation of Samples

Blood samples were carefully collected from the heart. About 5 mL blood samples from each rat were withdrawn into vacutainer tubes with gel and centrifuged to (3000 rpm in 4°C) for ten minutes to separate serum, the serum was transferred to eppendorf tubes for biochemistry tests, TAS, and TOS all samples were stored at -20 ºC prior to analysis. Biochemical parameters including (glucose, high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol, triglyceride, very low-density lipoprotein (VLDL-cholesterol) were determined using kits (Architect

Plus, ci16200, by Abbott Company, USA) and biochemical auto analyzers (Cobas C311, Roche- Germany).

Statistical Analysis

The results obtained in the study were assessed using (SPSS 22.0). The results were analyzed for statistical significance using one-way ANOVA. Multiple comparisons to compare the data of groups 1-3, Raw values from all analyzes were presented as the mean±standard error of respective groups ($p<0.05$).

RESULTS

The present study was investigated to study the effect of *Solanum nigrum* extract on glucose level, total cholesterol, triglyceride, HDL, LDL, TAS and TOS on blood samples of rats. The levels of those parameters belonging to the experimental groups are shown in Table 1.

Glucose Levels

Results from table 1 represent significant increasing of serum glucose level ($p<0.05$) in diabetes group (D) in comparison with control group (C). However, there were no significant differences ($p>0.05$) in glucose level between C group and *Solanum nigrum* extract group (SN). Moreover, in regards to glucose level, results from table 1 demonstrated that (D+SN) group was significantly higher than results from all of C, and SN groups and lower than D groups.

Cholesterol Levels

In return to table 1, results showed that there were no significant differences ($p>0.05$) in cholesterol levels between C group and SN group, D group and D+SN group (Table 1). Diabetes caused significant increase ($p<0.05$) in triglycerides (Tg) level in D group in comparison to both C and SN group. In contrast, no significant differences were found between D group and D+SN group, Furthermore, triglyceride (Tg) levels showed no significant differences ($p>0.05$) between SN, D+SN and C group (Table 1). Despite recording higher levels of HDL-cholesterol in D, SN and D+SN groups in comparison to control (C group), these levels showed no significant differences ($p>0.05$) in C group compared to SN group, D group and D+SN group (Table 1). LDL-cholesterol levels demonstrated no significant difference ($p>0.05$) in C group compared to D group, SN group and D+SN group (Table 1). Results from table 1 observe that the diabetes resulted in significant increase ($p<0.05$) in VLDL-cholesterol levels in D group in comparison with control C. Whereas, SN group observed lower level of VLDL-cholesterol in comparison to control C group, this change was not significant. Similarly, the level of VLDL-cholesterol in D+SN was not significantly higher in comparison with control (Table 1).

Oxidative Stress Analysis

As a consequence of diabetes, results showed significant increase ($p<0.05$) in TAS level in D group compared to control group (C). However, results determined from respective samples observed no significant differences between D group and D+SN group. Moreover, TAS levels showed no significant difference ($p>0.05$) in both SN and D+SN groups in comparison to control, when SN administrated to diabetes rats (Table 1). Diabetes resulted in significant increase ($p<0.05$) in total oxidant status (TOS) in D group in comparison with control C, SN group and D+SN groups. In contrary, there were no significant differences ($p>0.05$) between all of C, SN D+SN groups (Table 1).

Table 1: The level of serum biochemical parameters in experimental groups.

Parameters	Control (C)	Diabetes (D)	*S. nigrum* (SN)	Diabetes + *S.nigrum* (EX) D+SN
Glucose (mg/dL)	131±9.80^{a}	663±21.80^{c}	196.14±12.10^{a}	484.80±40.00^{b}
T. Cholesterol (mg/dL)	51.50 ±1.90^{a}	59.40±3.60^{a}	53.28±2.50^{a}	60.06±3.60^{a}
Triglyceride (mg/dL)	54.66±7.10^{a}	73.57±12.70^{b}	41.42±3.60^{a}	59.66±7.80^{ab}
HDL Cholesterol (mg/dL)	38.60±2.60^{a}	41.62±3.10^{a}	42.48±1.40^{a}	45.81±3.50^{a}
LDL Cholesterol (mg/dL)	4.10±0.20^{a}	4.10±0.80^{a}	6.00±1.10^{a}	5.70±0.07^{a}
VLDL Cholesterol (mg/dL)	10.93±1.42^{a}	14.71 ±2.42^{b}	8.28±0.72^{a}	11.93±1.56^{ab}
TAS (µmol H_2O_2 Equiv/L)	1.28±0.17^{a}	1.85±0.15^{b}	1.27±0.10^{a}	1.54±0.07^{ab}
TOS (µmol H_2O_2 Equiv/L)	3.87±0.34^{a}	6.30±1.41^{b}	4.87±0.80^{a}	4.14±0.34^{a}

HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low-density lipoprotein, TAS: Total antioxidant status, TOS: Total oxidant status.

* Different letters between the lines is statistically significant ($p<0.05$). X=mean, SE=Standard error.

DISCUSSION AND CONCLUSION

High concentrations of plasma glucose lead to metabolic disorders and glucose intolerance, this metabolic abnormality is characterized by hyperglycemia (Taylor et al. 2021; Al-kuraishy et al. 2021). This condition is associated with the increase of metabolic disturbance of carbohydrate, fat, and protein enhancing the production of free radicals that follow by oxidative stress, renal failure, neurodegeneration, cardiovascular abnormalities and immune dysfunction (Hung et al. 2012).

As shown in table 1, glucose level in diabetes group (D) was significantly increased in comparison with control group (C). This result agrees with previous studies by Hung et al. (2012) which they stated that the concentrations of plasma glucose are increased in DM patients following significant deficiency in insulin. However, results showed no significant differences in glucose level between control group and *Solanum nigrum* extract group (SN). In this context, extracts of *Solanum nigrum* had no significant effect in decreasing glucose level in normal rats.

Poongothai et al. (2010) which they determined significant reduction in glucose level after administration of the aqueous extract of *Solanum nigrum* to induced diabetic rats. In addition, a relevant study considering antidiabetic activity of *Solanum nigrum* in Alloxan Induced diabetic rats by Umamageswari et al. (2017) indicated that aqueous extract of berries of this plant at 200 mg/kg/day observed significant reduction in blood glucose by 7 days, they finally concluded effective roles of berries extracts of *Solanum nigrum* as an antidiabetic activity. Tiwari and Jain (2017) studied hypoglycemic activity of *Solanum nigrum* on alloxan Induced DM in rats. In this study alcoholic extracts of leaves of this plant were used at different doses 50, 100, 200, 400 mg/kg of body weight. Their results indicated significant hypoglycemic role of this plant. Our results are in line with previous studies regarding antidiabetic activities of *Solanum nigrum*, in this context, aqueous extracts from the respective plant seems to have effective roles in regulating blood glucose levels and possesses therapeutic characteristics against hyperglycemic activities.

Hyperlipidemia can be found as a major risk factor of cardiovascular pathologies. It is confirmed that the *Solanum nigrum* plays effective roles in inhibiting H^+K^+ATPase which might subsequently serve as cardio protective regimen (Atanu et al. 2011). Lee et al. (2005) on the effect of aqueous extract of *Solanum nigrum* glycoprotein on levels of plasma lipid including total cholesterol in mice. They found the administration of this plant (20 and 40 g head body weight g^{-1}) were significantly decreased total cholesterol levels. Some researchers assessed antihyperglycaemic and antioxidant effects of leaves extract of *Solanum nigrum* in alloxan induced-diabetic rats, they found that leaves extract at 100 mg/kg was not significantly reduced the amount of VLDL in comparison to its level in normal rats (Maharana et al. 2011).

Arulmozhi et al. (2010), found that the use of the fruit extract of *Solanum nigrum* was not significantly reduced the level of triglyceride when rats treated with 250 mg/kg b.wt of this plant. A similar result was found by Gupta et al. (2009), they demonstrated that despite the reduction of triglyceride level of rats received high cholesterol diet and exposed to saponins of *Solanum nigrum* (100 mg/kg body weight p.o.), this reduction was not significant in comparison to triglyceride level in rats received normal diet. Our results showed that despite some reductions in the level of triglyceride in SN group in comparison to control C group, and in D+SN in comparison to D group, this result was not significant. In this study, the administrations of the extract of *Solanum nigrum* to respective rats were not significantly effective against those biochemical parameters in the present study.

Important oxidative parameters including Total oxidant status (TOS) and all serum antioxidants which are known as total antioxidant status (TAS). These parameters are used to monitor the progression and range of damages that resulted from oxidative stress in rat blood serum (Turkez et al. 2012). In the current study, we analyzed both TAS and TOS levels of serum in diabetic rats and we found significant differences in both parameters when compared to non-diabetic rats.

Important oxidative parameters including TOS and TAS were used to monitor the progression and range of damage results from oxidative stress in rat blood serum (Turkez et al. 2012). TAS levels were reduced, while TOS levels were increased in diabetic group. These results suggest an imbalance between antioxidant defense and free radical generation which has an important role in the progression of diabetic complication. However, TAS levels were reduced, while TOS levels were increased in diabetic group in response to administrations of *Solanum nigrum* extract. It is concluded that treatment of diabetic rats with *Solanum nigrum* decreased antioxidant factors and supported antioxidant factors. These results might improve reproductive complications as a consequence to diabetes (Beyazyıldız et al. 2013).

Due to the significant effects of our extracts on TAS and TOS, it appears that respective *Solanum nigrum* might associates with pathological states encompassing both communicable and non-communicable diseases. This indicates the need for components of this plant in our diet as potent antioxidants (Atanu et al. 2011). Based on our results, it is suggested that *Solanum nigrum* leaves glycoprotein might contributes in the regulation of radical scavenging activities including 1, 1-diphenyl-2-picrylhydrazyl radicals, hydroxyl radical, and superoxide anion (Adebooye et al. 2008).

Results from the present study reflect the contribution of *Solanum nigrum* extracts in the regulation of oxidant and antioxidant capacity and subsequently the oxidative stress harmony of diabetic rats. Moreover, the present results suggest that *Solanum nigrum* exerts its chemotherapeutic effects by modulating the antioxidant status during hyperglycemic infection.

In conclusion, the biological effectiveness of this extract can be limited at a particular level of blood glucose and might be effective when glucose level becomes out of normal range which subsequently shows medicinal contribution of *Solanum nigrum* in patients with DM. Thus, it might be worthy to conclude the anti- diabetic property of *Solanum nigrum*. This protective role might result from antioxidant and detoxifying effects of this plant as consequences of containing steroidal saponins including namely nigrumin I and II (Aali et al. 2010). Further studies elucidating mechanisms of action and exploring medicinal value of respective components of this extract can be of value. As a result, it was determined that the SN plant was statistically significant on the shaped diabetes and that the experimental diabetes level was lowered but the developing diabetes and the rising sugar could not be reduced to normal levels.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: FY
Supervision / Consultancy: ACÖ
Data Collection and / or Processing: MNF
Analysis and / or Interpretation: FY, ACÖ
Writing the Article: ACÖ, MNF
Critical Review: FY

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Marbofloksasin, Diklofenak Sodyum ve Metilprednizolonun Sistemik Endotoksemide Serum Sitokin Düzeylerine Etkisi

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ÖZ

LPS ile deneysel endotoksemi oluşturulan ratlarda marbofloksasin, diklofenak sodyum ve metilprednizolon kullanımının serum sitokin seviyeleri üzerine etkilerinin değerlendirilmesi amaçlanmıştır. Çalışmada 186 adet rat, kontrol grubu (n: 6) ayrıldıktan sonra rastgele 5 eşit gruba ayrıldı. Kontrol grubundan 0. Saat te kan örnekleri alındı. Ratlarda endotoksemi oluşturmak amacı ile intraperitoneal (IP) yolla LPS (4mg/rat) uygulandı. Endotoksemi sonrası gelişen sepsisi tedavi etmek için marbofloksasin 100 mg/kg, diklofenak sodyum 10 mg/kg, metilprednizolon 10 mg/kg dozlarında IP yolla uygulandı. İlaç uygulaması takiben 1, 2, 4, 8, 12 ve 24. saatler de tiyopental anestezisi altında kan örnekleri alınarak serum sitokin değerleri ölçüldü. Araştırmada elde edilen veriler doğal şekillenen sepsise büyük ölçüde benzerlik gösterdi. Sitokin seviyeleri incelendiğinde diklofenak sodyum ile marbofloksasin uygulamasının tek başlarına sepsisi tedavi etmede etkisinin olmadığı, ancak metilprednizolon uygulamasının tek ve kombine yapılması durumunda etkili olabileceği belirlendi. Sepsis ile yükselen sitokin düzeyleri için kortikosteroid uygulamasının tek veya antibiyotik ve NSAİİ'lerle kombine kullanılmasının faydalı olabileceği önerilmektedir.

Anahtar Kelimeler: *Diklofenak sodyum, Endotoksemi, Metilprednizolon, Sitokin.*

ABSTRACT

Effect of Marbofloxacin, Diclofenac Sodium and Methylprednisolone on Serum Cytokine Levels in Systemic Endotoxemia

It was aimed to evaluate the effects of using marbofloxacin, diclofenacsodium and methylprednisolone on serum cytokine levels in rats with experimental endotoxemia with LPS. In the study, 186 rats were randomly divided into 5 equal groups after the control group (n: 6) was separated. Blood samples were taken from the control group at 0 hour. LPS (4mg/rat) was administered intraperitoneally (IP) to induce endotoxemia in rats. To treat sepsis developing after endotoxemia, marbofloxacin 100mg/kg, diclofenacsodium 10 mg/kg, methylprednisolone 10 mg/kg were administered by IP route. Serum cytokine values were measured by taking blood samples under thiopental anesthesia at 1, 2, 4, 8, 12 and 24 hours following drug administration. The data obtained in the study showed a great deal of similarity to naturally occurring sepsis. When cytokine levels were examined, it was determined that the application of diclofenacsodium and marbofloxacin alone did not have an effect in the treatment of sepsis, but methylprednisolone application could be effective in case of single or combined application. It is suggested that corticosteroid administration alone or in combination with antibiotics and NSAIDs may be beneficial for increased cytokine levels with sepsis.

Keywords: *Cytokine, Diclofenacsodium, Endotoxemia, Methylprednisolone.*

GİRİŞ

Endotoksemi kan dolaşımında endotoksinin varlığını tanımlar. Sepsis ve septik şoklu hastalarda görülmekle beraber, deneysel olarak LPS infüzyonları, fekal nakil, eksojen bakterinin infüzyonu veya aşılanması gibi yöntemlerle oluşturulabilmektedir (Bone ve ark. 1989; Erkayman 2020; Eroğlu ve Kırbaş 2020).

Kedi ve köpeklerde sırası ile sepsis görülme oranlarının %1-5, %6-10, tedavi sonrası sağ kalma oranlarının ise %10-25, %25-50 düzeyinde olduğu rapor edilmiştir. Kedi ve köpeklerde özellikle peritonitis ve sindirim sistemi enfeksiyonlarının septik şok nedeni olduğu saptanmıştır. Ayrıca doberman, pinscher, rottweiler ve pitbull cinsi köpeklerin diğer köpeklerden daha duyarlı olabilecekleri belirlenmiştir (Otto 2007).

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Sepsis/endotoksemi buzağıların ve sığırların birçok organ ve sistemini olumsuz etkilemekle birlikte kardiyovasküler sistemin yetersizliğine de sebep olmaktadır (Akyüz ve ark. 2017; Akgül ve ark. 2019). Sepsisin yeterince şiddetli ve uzun sürmesi durumunda da septik şok ve daha sonra da çoklu organ yetmezlikleri gelişmekte ve bu aşamadan sonra da genellikle ölüm ile sonlanmaktadır (Pardon ve Depres 2018; Beydilli ve Gökçe 2019).

Birçok hayvan modelinde, bakteriyel, viral, fungal enfeksiyonlarda ve paraziter enfestasyonlarda, saldırılara karşı sitokinler, vücudun immun cevabının ilk aktörleri olarak belirlenmiştir (Özcan ve ark. 1996; Gouwy ve ark. 2005). Sitokinler; immünolojik ve inflamatuvar travmaya karşı doku cevabında ortaya çıkan, makrofajların aktive edilmesi, yangılı dokuda fibroblastların gelişmesi, kemik iliğindeki lökosit prokürsörlerinin aktivasyonu, kas yıkımlanması ve beden ısısının artışından sorumlu maddelerdir (Özcan ve ark. 1996; Tsiotou ve ark. 2005).

Başlangıçta, tipik erken proinflamatuar sitokinlerin (TNF ve IL 1) hayvanlarda organ yetmezliğini indüklediği bilinmektedir (Lelubre ve Vincent 2018). Sepsis sırasında, IL-1β, IL-6, IL-8, IL-12, IFN ve TNF-α'nın proinflamatuar sitokinlerinde bir artış sepsisteki mortalite ile ilişkilidir (Utomo ve ark, 2018).

İnflamasyon olgularında sitokin olarak ilk önce TNFα uyarılmaktadır (Sakaguchi ve Furusawa 2006; Otto 2007). TNFα ve IL-1β sinerjik etkileşim ile doğrudan veya dolaylı yollarla hemodinamik ve inflamatuvar değişikliklerin birçoğuna aracılık etmektedir (Remick 2007). TNFα'ya ek olarak IL-6 da inflamasyonda önemli sitokinlerden biridir (Gardlund ve ark. 1995). IL-6 seviyesi çoğu vakada yüksek bulunduğundan birçok organ yetmezliğinin boyutu ile ilişkilendirilmektedir. IL-6 seviyesindeki yükseklik, hasta klinik olarak iyileşene kadar devam etmektedir (Creasey ve ark. 1993; Gardlund ve ark. 1995; Rosenbloom ve ark. 1995).

İnterlökin (IL)-10, hem T hücreleri hem de makrofajlar tarafından üretilen pleiotropik bir sitokindir ve hem anti-inflamatuar hem de immünosupresif özelliklere sahiptir. IL-10, sepsis sendromlu hastaların kanında dolaşır ve artan IL-10 konsantrasyonları, olumsuz bir klinik sonuçla ilişkilendirilmiştir. Kemirgenlerde ve primatlarda yapılan deneysel çalışmalar, endojen olarak üretilen ve eksojen olarak uygulanan IL-10'un, en başta endotoksemik ve bakteriyemik şok modellerinde, inflamatuar yanıtın büyüklüğünü azaltabildiğini ve sonucu iyileştirebildiğini göstermiştir (Oberholzer ve ark. 2002).

Sepsis'in uygun antibiyotik ajanlar, sıvılar, gerektiğinde vazopresörler ve muhtemelen kortikosteroidler dışında kanıtlanmış bir farmakolojik tedavisi yoktur. Fakat sepsis patofizyolojisinin son yıllarda daha iyi anlaşılması ile birlikte beşerî alanda yeni tedavi yaklaşımları da ön plana çıkmaktadır. Monoklonal antikor tedavileri, spesifik antiendotoksin tedavileri, mezenşimal stem hücre tedavileri ve apoptotik hücre tedavileri sepsis ve septik şok durumlarında tedavide kullanılması için araştırılan konular arasındadır (Karbian ve ark. 2020; Sun ve ark. 2020).

Marbofloksasin, sadece veteriner hekimlikte kullanımı onaylanmış, gram-negatif, gram-pozitif, Mycoplasma spp. ve *Chlamydia spp*.'ye etkili florokinolon grubu bir antibiyotiktir. Farelerde LPS kaynaklı septik şokta florokinolonların koruyucu etkilerinin insanlarda da ortaya çıkabileceğini (Khan ve ark. 2000), endotoksemili koyunlarda destekleyici tedavi uygulanmasını takiben marbofloksasin için bir doz ayarlaması gerektiğini (Coşkun ve ark. 2020) bildiren çalışmalar mevcuttur.

NSAİİ'ler, araşidonik asitten prostaglandinlerin üretimini önleyerek siklooksijenazı (COX) inhibe eder. NSAİİ'lerin çok sayıda deneysel modelde yararlı etkileri vardır ve sepsis için potansiyel olarak hayat kurtarıcı bir tedavi olarak kabul edilirler (Rasooli ve ark. 2022).

Steroidlerin sepsis durumundaki etkileri üzerine yapılan çalışmalar, steroid dozu, sepsis şiddeti, kontrol grubu, mortalite oranı ve steroid uygulama zamanlaması gibi başlıklar açısından farklılıklar göstermiştir. Bununla birlikte, steroidlerin immünosupresan doğası göz önüne alındığında, sepsisin immünosupresan fazında (genellikle sepsisin geç fazında) uygulandığında zararlı olabileceği için erken tedavi de verilmesi daha etkili olabilir (Park ve ark. 2022).

Geniş etki spektrumlu antibiyotiklerden olan florokinolonların, antiinflamatuvar olarak NSAİİ'lerin ve glukokortikoidlerin tedavide kullanılabilirliği ile ilgili şekillendirilen bu araştırmada, lipopolisakkarit (LPS) ile deneysel endotoksemi şekillendirilerek sepsiste önemli rol oynayan serum sitokin seviyeleri üzerindeki etkilerinin incelenmesi, ayrıca beşerî ve veteriner sahada sepsis tedavisine katkı sağlaması amaçlanmıştır.

MATERYAL VE METOT

Araştırmada Sprague - Dawley ırkı 186 adet rat kullanıldı. Deney hayvanları Yüzüncü Yıl Üniversitesi Tıp Fakültesi Deney Hayvanları Ünitesi'nden temin edildi. Selçuk Üniversitesi Veteriner Fakültesi Etik Kurul tarafından alınan 11.04.2012 tarih ve 2012/35 numaralı izinle çalışıldı.

Araştırma kullanılan ratlardan (186 adet) önce kontrol grubu (n: 6) ayrıldı ve kontrol grubunda yer alan hayvanlara herhangi bir ilaç uygulaması yapılmadan tiyopental anestezisi (70 mg/kg IP) altında uyutularak (Lukashenko ve ark 2004) kalpten kanları alındı, sonra servikal dislokasyon yöntemi ile ötenazi edildi. Bu örneklemeden elde edilen veriler bütün gruplar için 0. (kontrol) grubu verisi olarak değerlendirildi.Geriye kalan 180 adet rat rasgele 5 eşit gruba (n: 36) ayrıldı.

Gruplar; LPS, LPS+Marbofloksasin (MAR), LPS+Diklofenak Sodyum (DS), LPS+Metilprednizolon (MPRED) ve LPS+Komibnasyon (KOMBİN) olmak üzere adlandırıldı. Bu grupta yer alan hayvanlarda sepsis oluşturmak için, LPS 4 mg/rat dozunda intraperitoneal (IP) olarak uygulandı.LPS uygulanmasının hemen ardından deney gruplarına göre Marbofloksasin 100 mg/kg (Kesteman ve ark. 2009), diklofenak sodyum 10 mg/kg (Hasani ve ark., 2011) ve metilprednizolon 10 mg/kg (Izumi ve Bakhle. 1989) dozlarında IP yolla uygulandı. İlaç uygulaması takiben her örnekleme zamanında 6 adet rat olarak şekilde 1, 2, 4, 8, 12 ve 24. saatlerinde tiopental sodyumla ratlar anesteziye alındı ve kalpten kan örnekleri aldıktan sonra servikal dislokasyon yöntemi ile ötenazi edildi.

Serum TNFα (İnvitrogen TMTNFα- α Elisa Kit, KRC3011), IL- 1β (Boster Rat IL- 1β Elisa Kit Ek 0418), IL- 6 (Raybio® Rat IL-6 Elisa Kit, ELR-IL6-001) ve IL- 10 (Boster Rat IL-10 Elisa Kit, EK0393) düzeyleri ticari kitler kullanılarak ölçüldü ve kosantrasyon tespiti ELİSA okuyucu cihazında (MWGt Lambda Scan 200, Winooski, VT, USA) yapıldı.

İstatistiksel Analiz

Tüm grupların her bir örnekleme zamanına ait veriler One-Way Anova testi ile değerlendirildi. Önemli olarak belirlen gruplar içi farkın önemliliği ise Duncan testi ile değerlendirildi (SPSS® v.19 Evaluation Versionfor Windows, IBM).

BULGULAR

Endotoksemi şekillendirilen ve ilaç uygulamaları yapılan ratlara ait bulgular (serum TNFα, IL-1β, IL-6 ve IL-10) düzeyleri tablo halinde sunuldu. Serum sitokin seviyeleri üzerinde LPS uygulamasının genel olarak yükseltici etkisi olduğu ($p<0.05$) tespit edildi. LPS uygulaması ile serum TNFα seviyesinin birinci saatten itibaren yükseldiği daha sonra diğer saatlerde azalırken sekizinci saatten sonra ise önemli düzeyde ($p<0.05$) azalarak başlangıç seviyesine döndüğü tespit edildi (Tablo 1). Birinci saatte marbofloksasin, diklofenak sodyum ve kombine uygulamalarının LPS'den farkı olmadığı ve şekillenen TNFα artışını engelleyemediği, metilprednizolon uygulamasının ise LPS'den farkı olduğu ($p<0.05$) ve diğer gruplara göre önemli oranda düşük seviyede kaldığı gözlendi. İkinci saatte metilprednizolon ve kombine ilaç uygulamasının, dördüncü saatte ise marbofloksasin, metilprednizolon ve kombine ilaç uygulamasının LPS'den farklı olduğu ($p<0.05$) belirlendi. Diğer saatlerde ise diklofenak sodyum uygulamasının gruplara göre farklı olduğu ve seviyenin yüksek kaldığı belirlendi (Tablo 1).

Tablo 1: Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında TNFα ortalamaları ve çoklu karşılaştırma testi sonuçları (pg/ml).

Table 1: TNFα averages and multiple comparison test results (pg/ml) in LPS, LPS+MARBO, LPS+DS, LPS+MPRED and LPS+COMBIN groups in endotoxemic rats.

GRUPLAR	$\overline{X}$ ±S $\overline{x}$							P
	0. saat	1. saat	2. saat	4. saat	8. saat	12. saat	24. saat	
LPS	0^{w}	515 ± 107^{bcy}	418 ± 73.8^{bx}	292 ± 74.1^{bx}	25.3 ± 16.2^{aw}	12.7 ± 7.91^{aw}	0^{w}	***
LPS+MARBO	0^{w}	373 ± 73.4^{abcx}	583 ± 114^{by}	91.1 ± 34.6^{aw}	19.0 ± 14.1^{aw}	16.3 ± 10.7^{aw}	0^{w}	***
LPS+DS	0^{w}	574 ± 123^{cy}	448 ± 116^{bxy}	226 ± 36.6^{bwx}	427 ± 81.3^{bxy}	94.4 ± 33.2^{bw}	0^{w}	***
LPS+MPRED	0^{w}	214 ± 35.2^{ay}	123 ± 29.7^{ax}	0^{aw}	0^{aw}	0^{aw}	0^{w}	***
LPS+KOMBİN	0^{w}	283 ± 62.5^{abx}	11.3 ± 5.5^{aw}	0^{aw}	0^{aw}	0^{aw}	0^{w}	***
p	-	*	***	***	***	**	-	

-; p>0.05, *; p<0.05, **; p<0.01, ***; p<0.001. ^{a,b,c}; Aynı sütun da gösterilen ortalamalar arasında farklılıklar önemlidir. ^{x,w,y}; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir. **LPS**: Lipopolisakkarit, **MAR**: Marbofloksasin, **DS**: Diklofenak Sodyum, **MPRED**: Metil Prednizolon, **KOMBİN**: Komibnasyon.

IL-1β düzeyi TNF-α ile paralel olarak yükseldiği için LPS uygulaması sonrasında IL-1β düzeyinin 2. saatten itibaren yükseldiği 8. saatten itibaren ise azaldığı ve uygulama sonunda değerinin sıfır "0" olmadığı belirlendi (Tablo 2). Birincisaatte metilprednizolonun IL-1β düzeyini sıfıra indirdiği daha sonraki saatlerde bir yükselme söz konusu olsada 2. saat dışında istatistiksel bir farklılığın söz konusu olmadığı belirlendi. LPS uygulamasında 4. saatte en yüksek düzeyde olan IL-1β seviyesinin diklofenak sodyum, metilprednizolon ve kombine ilaç uygulaması sonrası önemli düzeyde düştüğü ($p<0.05$), 8. saatte diklofenak sodyum dışındaki ilaçların LPS'den farkı olmadığı, 24. saatte ise bütün uygulamaların LPS'den farklı ($p<0.05$) olduğu tespit edildi (Tablo 2).

Tablo 2: Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında IL-1β ortalamaları ve çoklu karşılaştırma testi sonuçları (pg/ml).

Table 2: IL-1β averages and multiple comparison test results (pg/ml) in LPS, LPS+MARBO, LPS+DS, LPS+MPRED and LPS+COMBIN groups in endotoxemic rats.

GRUPLAR	$\overline{X}$ ±S $\overline{x}$							P
	0. saat	1. saat	2. saat	4. saat	8. saat	12. saat	24. saat	
LPS	0^{w}	0.70 ± 0.64^{aw}	193 ± 111^{abwx}	320 ± 82.1^{bx}	105 ± 61.4^{aw}	144 ± 39.5^{awx}	51.4 ± 18.2^{bw}	*
LPS+MARBO	0^{w}	15.5 ± 11.9^{abw}	69.3 ± 41.4^{abwx}	170 ± 80.1^{abx}	91.7 ± 43.0^{awx}	105 ± 65.3^{awx}	0^{aw}	*
LPS+DS	0^{w}	26.2 ± 13.3^{bw}	257 ± 36.5^{bxy}	127 ± 36.7^{awx}	487 ± 96.0^{bz}	317 ± 80.8^{byz}	2.64 ± 2.64^{aw}	***
LPS+MPRED	0^{w}	0^{aw}	157 ± 65.8^{abx}	28.2 ± 22.1^{aw}	20.6 ± 8.96^{aw}	0^{aw}	8.76 ± 5.66^{aw}	**
LPS+KOMBİN	0	1.38 ± 0.87^{a}	1.71 ± 1.71^{a}	22.4 ± 20.6^{a}	10.2 ± 6.39^{a}	1.79 ± 1.79^{a}	0.05 ± 0.05^{a}	-
p	-	*	*	**	***	**	**	

-; p>0.05, *; p<0.05, **; p<0.01, *** ;p<0.001. ^{a,b}; Aynı sütun da gösterilen ortalamalar arasında farklılıklar önemlidir. ^{x,w,y,z}; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir. **LPS**: Lipopolisakkarit, **MAR**: Marbofloksasin, **DS**: Diklofenak Sodyum, **MPRED**: Metil Prednizolon, **KOMBİN**: Komibnasyon.

IL-6 düzeyinde LPS uygulaması sonrası 2 ile 8. saatler arasında yükseldiği, 12 ve 24. saatte düzeyinin başlangıç seviyesi olan sıfıra düştüğü tespit edildi (Tablo 3). Birinci saatte diklofenak sodyum ve kombinasyon uygulamalarının ikisinin de IL-6 seviyesini yükselttiği ancak sadece kombinasyon uygulamasının istatistiksel olarak farklı olduğu ($p<0.05$), 2. saat ve sonraki saatlerde kombine ilaç uygulamasının IL-6 seviyesini sıfıra düşürdüğü ($p<0.05$) tespit edildi. Diğer saatler ve ilaç uygulamaları dikkate alındığında LPS uygulaması sonrası IL-6 düzeyini azaltması yönünden kombinasyon dışında, 2. ve 4. saatlerde metilprednizolonun, 8. saatte diklofenak sodyumun ve 12. saatte marbofloksasinin LPS'den farklı ($p<0.05$) olduğu tespit edildi (Tablo 3).

Tablo 3: Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında IL-6 ortalamaları ve çoklu karşılaştırma testi sonuçları (pg/ml).

Table 3: IL-6 averages and multiple comparison test results (pg/ml) in LPS, LPS+MARBO, LPS+DS, LPS+MPRED and LPS+COMBIN groups in endotoxemic rats.

GRUPLAR	$\overline{X}$ ±S $\overline{x}$							P
	0. saat	1. saat	2. saat	4. saat	8. saat	12. saat	24. saat	
LPS	0^{w}	0^{aw}	4829 ± 1336^{cx}	3468 ± 1003^{bcx}	978 ± 823^{abw}	0^{aw}	0^{w}	***
LPS+MARBO	0^{w}	0^{aw}	3817 ± 939^{bcx}	4610 ± 984^{cx}	3221 ± 1122^{bcx}	4076 ± 1979^{bx}	0^{w}	**
LPS+DS	0^{w}	5.91 ± 5.91^{aw}	3091 ± 591^{bcx}	1859 ± 657^{abwx}	5344 ± 1121^{cy}	841 ± 390^{aw}	0^{w}	***
LPS+MPRED	0^{w}	0^{aw}	1646 ± 604^{abx}	63.2 ± 29.5^{aw}	202 ± 202^{aw}	0^{aw}	0^{w}	***
LPS+KOMBİN	0^{w}	3347 ± 862^{bx}	0^{aw}	0^{aw}	0^{aw}	0^{aw}	0^{w}	***
p	-	***	**	***	***	*	-	

-; p>0.05, *; p<0.05, **; p<0.01, ***;p<0.001. ^{a,b,c}; Aynı sütun da gösterilen ortalamalar arasında farklılıklar önemlidir. ^{x,w}; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir. **LPS**: Lipopolisakkarit, **MAR**: Marbofloksasin, **DS**: Diklofenak Sodyum, **MPRED**: MetilPrednizolon, **KOMBİN**: Komibnasyon.

IL-10 düzeyinin LPS uygulaması sonrası yükseldiği, çalışma süresince de 2. saatte en yüksek seviyeye ulaştığı ve diğer saatlerde 1. saate benzer düzeyde kaldığı belirlendi (Tablo 4). Marbofloksasin uygulamasının LPS uygulamasından 2, 4, 12 ve 24. saatlerde farklı olduğu (p<0.05) IL-10 seviyesini düşürdüğü, metilprednizolon uygulamasının 8. saat dışında LPS uygulamasından farklı olduğu ve IL-10 düzeyini düşürdüğü (p<0.05), diklofenak sodyumun LPS'den 2, 4 ve 24. saatlerde farklı olduğu (p<0.05), kombinasyon uygulamasının ise 2 ve 24. saatlerde LPS'den farklı (p<0.05) olduğu ve IL-10 düzeyini düşürdüğü belirlendi. 24 saat sonra bütün uygulamaların LPS'den farklı olduğu (p<0.05) ve IL-10 düzeyini düşürdüğü tespit edildi (Tablo 4).

Tablo 4: Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında IL-10 ortalamaları ve çoklu karşılaştırma testi sonuçları (pg/ml).

Table 4: IL-10 averages and multiple comparison test results (pg/ml) in LPS, LPS+MARBO, LPS+DS, LPS+MPRED and LPS+COMBIN groups in endotoxemic rats.

GRUPLAR	$\overline{X}$ ±S $\overline{x}$							P
	0. saat	1. saat	2. saat	4. saat	8. saat	12. saat	24. saat	
LPS	0^{w}	4363 ± 965^{bcxy}	6035 ± 765^{by}	4892 ± 748^{dxy}	3373 ± 309^{abx}	5216 ± 691^{cxy}	4310 ± 713^{bxy}	***
LPS+MARBO	0^{w}	4202 ± 686^{bcx}	3669 ± 1092^{ax}	2820 ± 320^{bcx}	2546 ± 421^{ax}	2669 ± 561^{abx}	910 ± 131^{aw}	***
LPS+DS	0^{w}	2466 ± 233^{abxy}	3293 ± 193^{ayz}	826 ± 119^{awx}	4759 ± 1092^{bz}	5319 ± 1105^{cz}	1777 ± 1646^{awxy}	***
LPS+MPRED	0^{w}	1678 ± 273^{axy}	1788 ± 218^{axy}	1713 ± 229^{abxy}	2234 ± 370^{ay}	1136 ± 131^{ax}	1384 ± 273^{ax}	***
LPS+KOMBİN	0^{w}	4818 ± 1083^{cz}	2707 ± 496^{axy}	3614 ± 637^{cdyz}	5064 ± 522^{bz}	3881 ± 333^{bcyz}	1806 ± 232^{ax}	***
p	-	*	**	***	**	***	***	

-; p>0.05, *; p<0.05, **; p<0.01, ***; p<0.001. a,b,c; Aynı sütun da gösterilen ortalamalar arasında f arklılıklar önemlidir. x,w,y,z; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir. **LPS**: Lipopolisakkarit, **MAR**: Marbofloksasin, **DS**: Diklofenak Sodyum, **MPRED**: Metil Prednizolon, **KOMBİN**: Komibnasyon.

TARTIŞMA VE SONUÇ

Yapılan araştırmalarda LPS ile oluşturulan endotoksemi modelinde serum sitokin seviyeleri araştırılmıştır. Araştırmacılar LPS ile deneysel oluşturulan endotoksemi durumlarında meydana gelen değişikliklerin, doğal oluşan sepsis vakalarının sonuçları ile benzerlik gösterdiğini ve serum sitokin seviyelerinde yükselme olduğunu bildirmektedir (Howard ve ark. 1993; Biniek ve ark. 1998; Itoh ve ark. 2003; Yazar ve ark. 2004; Coimbra 2005; Yazar ve ark. 2007; Yazar ve ark. 2010; Utomo ve ark. 2018).

Araştırmalarda florokinolon grubu antibiyotiklerin deneysel sepsis üzerine, değişik dozlarda farklı sonuçlar verebileceği (yüksek doz sipropfloksasin IL-1β, IL-6 ve TNFα düzeyini düşürdüğü, düşük dozunun ise artırdığı) ve glukokortikoidlerin sepsis tedavisinde etkili olduğu bildirilmiştir (Yazar ve ark. 2004; Marik ve Lipman 2007; Yarema ve Yost 2011).

NSAİİ'ler NF-KB'nin etkinliğini engelleyerek, proinflamatuar sitokinler, iNOS ve COX oluşumunu engeller (Meduri 1999). Bu etkileri düşünülerek çalışmalar gerçekleştirilse de sepsiste kullanılması hekimler arasında tartışmalı bir konudur (Öner ve Şahin 2021). NSAİİ'ler hakkında yapılan bir çalışmada ibuprofen ve indometazin'in TNFα üretimini arttırdığı belirlenirken, sodyum salisilat'ın TNFα üretimini baskıladığı bildirilmiştir. Ayrıca nimesulidin ratlarda TNFα artışını engellediği ve diklofenak'ın ise kısmen TNFα düzeyini azalttığı saptanmıştır (Doğan ve ark. 2002). Yazar ve ark. (2007)'nın yaptığı çalışmada fluniksinmeglumin (FM) uygulanan endotoksemi indüklenmiş farelerde sitokin seviyeleri üzerinde baskılayıcı etki tespit edilirken, Yazar

ve ark. (2009) tarafından yapılan diğer bir araştırmada ise FM'in ratlarda sitokin seviyelerine etkisinin tespit edilemediği, dekzametazon'un tek başına ve kombine uygulamalarında etkili sonuçlar verdiği ve sitokin sentezinde belirgin baskılama şekillendirdiği belirtilmiştir.

Mevcut çalışmada, LPS uygulaması sonrası 1 ve 2. saatlerde TNFα (Tablo 1) ve 2-8. saatler arasında IL-1β (Tablo 2) düzeyinde artış şekillendiği belirlendi. Uygulamalar sonrasında LPS ile artışı belirlenen serum TNFα düzeyini metilprednizolon ve kombinasyon uygulaması dışında diğer ilaçların düşüremediği ($p<0.05$) belirlendi (Tablo 1). IL-1β düzeyi üzerinde, diklofenak sodyum haricinde diğer ilaçların LPS kaynaklı IL-1β yükselmesini düşürdüğü ($p<0.05$) özellikle metilprednizolon'un tek ve kombine uygulamasının daha etkili bir sonuç verdiği gözlendi (Tablo 2).

TNFα ve IL-1β'ya ek olarak IL-6 da inflamasyonda önemli sitokinlerden biridir (Gardlund ve ark. 1995). IL-6 seviyesindeki yükseklik, hasta klinik olarak iyileşene kadar devam etmektedir. IL-6 ayrıca TNFα ve IL-1β üretimini engelleyen, sTNFα reseptörünü ve IL-1 antagonistinin sirkülasyonunu indükleyen sitokindir (Creasey ve ark. 1993; Gardlund ve ark. 1995; Rosenbloom ve ark. 1995). Yazar ve ark. (2009)'nın yaptığı bir çalışmada enrofloksasinin ve FM artan IL-6 düzeini düşüremediği, dekzametazon uygulamasının hem tek başına hem de kombine uygulandığı zaman düşürdüğü rapor edilmiştir.

Mevcut çalışmada IL-6 düzeyinin LPS uygulaması sonrası 2 ile 8. saatler arasında yükseldiği, 12 ve 24. saatte düzeyinin 0 olduğu tespit edildi (Tablo 3).

Uygulamalar ve sonuçlar doğrultusunda metilprednizolon tek ve kombine uygulamasının yükselen IL-6 seviyesini etkili bir şekilde aşağıya çektiği ($p<0.05$), kombinasyon uygulamasında değerlerin sağlıklı bireylere yakın veriler olduğu tespit edildi (Tablo 3).

IL-10, vücutta en iyi bilinen antiinflamatuvar sitokin dir. Deneysel endotoksemi/sepsis modellerinde IL-10 uygulamasının ölüm oranını azalttığı bildirilmiştir (Howard ve ark., 1993). Vücut savunmasında, antiinflamatuvar sitokinlerin üretimini artırarak, proinflamatuvar cevabı dengeleyip yangıyı zayıflatabilecek IL-10 gibi ajanların klinik faydasının olabileceği belirtilmiştir (Coimbra ve ark. 2005).

Bu çalışmada IL-10 düzeyinin LPS uygulaması sonrası yükseldiği, çalışma süresince de 2. saatte en yüksek seviyeye ulaştığı ve diğer saatlerde 1. saate benzer düzeyde kaldığı belirlendi (Tablo 4). Son örnekleme zamanında IL-10 seviyesi açısından en etkili uygulamanın metilprednizolon olduğu diğer uygulamaların ise 24. saatte IL-10 seviyesini düşürmesi açısından etkili olabileceği kanaatine varıldı (Tablo 4).

Mevcut çalışmada marbofloksasin'in ve diklofenaksodyum'un genel olarak serum sitokin düzeyleri üzerine etkisi incelendiğinde, TNFα ve IL-1β seviyelerini düşüremediği saptandı. Marbofloksasinin IL-6 düzeyini düşüremediği IL-10 düzeyini ise kademeli olarak azattığı belirlendi. Diklofenak sodyumun artan IL-6 düzeyini saatlere göre değişen bir şekilde (2 ve 4. saatte azalttığı 8. saatte arttırdığı) etkilediği saptandı.

Sonuç olarak LPS ile deneysel endotoksemi şekillendirilen bu araştırmada doğal olarak gelişen sepsis durumuna benzer sitokin seviyelerinde yükselmeler gözlendi. IP yolla enjekte edilen marbofloksasin ile diklofenak sodyum uygulamaların tek başlarına sepsis tedavisinde etkisinin olmadığı belirlendi. Metilprednizolon uygulamasının sepsiste etkili olabileceği ancak tek başına kullanılamayacağı kanaatine varıldı. Kombinasyon uygulamasının %100 tedavi sağlamasa da, etkisinin göz ardı edilmeyecek derecede iyi olduğu ve sepsis ile septik şok olgularında kullanım alanı bulabileceği sonucuna ulaşıldı.

Yapılacak olan çalışmalarda diğer yangı mediyatörleri (NO, MDA vb.), aktif faz proteinleri, histopatolojik bulgular ve sağ kalım çalışmaları ile bu ilaçların sepsis üzerindeki etkisinin değerlendirilmesi ve sonuçların desteklenmesi sağlanmalıdır.

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Fikir/Kavram: ACÖ
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A Research on the Online Teaching Experiences of Ankara University Veterinary Faculty Academics

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ABSTRACT In this study, it has aimed to determine the perceptions of veterinary faculty academics of Ankara University regarding their online teaching experiences during the Covid-19 pandemic. The study has included 92 academics who provided theoretical and/or practical courses via online teaching in Ankara University Faculty of Veterinary Medicine (AUFVM). The data of the study has collected with a 21-item questionnaire. The questionnaire has basic questions such as the quality of the courses given, the active participation of the students in the courses, experienced technological problems during their education, and whether they consider online teaching effective. Academics in clinical sciences gave negative answers to the question about the sustainability of online teaching at a higher rate than the academics in basic sciences (p=0.016). Only all academics who think online teaching has no disadvantages stated that it provides effective learning (p=0.001). Academics who think online teaching provides effective teaching mostly want to continue online, while those with opposing considerations "generally" prefer to continue online or can "sometimes" continue (p<0.001). The lack of classroom interaction and technological incapacity can be considered a con of this method. As a result, it can be concluded that academics are biased towards online teaching and do not tend to prefer this method consistently.

Keywords: *COVID-19, Education, Distance, Veterinary education.*

ÖZ

Ankara Üniversitesi Veteriner Fakültesi Akademisyenlerinin Çevrimiçi Öğretim Deneyimleri Üzerine Bir Araştırma

Bu çalışmada Ankara Üniversitesi Veteriner Fakültesi akademisyenlerinin Covid-19 pandemisi sürecinde çevrimiçi öğretim deneyimlerine ilişkin algılarının belirlenmesi amaçlanmıştır. Araştırmaya Ankara Üniversitesi Veteriner Fakültesi'nde çevrimiçi öğretim yoluyla teorik ve/veya uygulamalı dersler veren 92 akademisyen dahil edilmiştir. Araştırmanın verileri 21 maddelik bir anket ile toplanmıştır. Ankette verilen derslerin kalitesi, öğrencilerin derslere aktif katılımı, eğitim sırasında yaşanan teknolojik sorunlar ve çevrimiçi öğretimi etkili bulup bulmadıkları gibi temel sorular yer almaktadır. Bulgulara göre, klinik bilimleri akademisyenleri çevrimiçi öğretimin sürdürülebilirliğine ilişkin soruya temel bilimler akademisyenlerine göre daha yüksek oranda olumsuz yanıt vermiştir (p=0.016). Yalnızca çevrimiçi öğretimin herhangi bir dezavantajı olmadığını düşünen akademisyenlerin tamamı, bu yöntemin etkili öğrenme sağladığını belirtmiştir (p=0.001). Çevrimiçi öğretimin etkili öğretim sağladığını düşünen akademisyenler eğitime çoğunlukla çevrimiçi olarak devam etmek isterken, karşıt görüşlere sahip olanlar eğitime "genellikle" veya "bazen" çevrim içi olarak devam etmeyi tercih etmiştir (p<0.001). Sınıf etkileşiminin olmaması ve teknolojik yetersizlikler bu yöntemin eksilerinden biri olarak kabul edilebilir. Sonuç olarak, akademisyenlerin çevrimiçi öğretime karşı önyargılı oldukları ve bu yöntemi sürekli olarak tercih etme eğiliminde olmadıkları sonucuna varılabilir.

Anahtar Kelimeler: *COVID-19, Eğitim, Uzaklık, Veteriner hekimliği eğitimi.*

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INTRODUCTION

The distance education model, which has become popular again with the pandemic (Franklin et al. 2021), is an education model that operates with two different working principles as direct and indirect, uses the technological infrastructure, and does not require the presence of students and lecturers in the same physical place (Wagner et al. 2021). In the direct form of distance education, which includes one-on-one interaction and direct contact, the educator and the student have the chance to communicate and interact with each other simultaneously in real time. In the direct form, it is seen that an actual course is taught remotely with technological infrastructure. On the other hand, the indirect form offers the student the chance to watch the previously recorded lectures at different times (Tsai et al. 2021; Wagner et al. 2021). The coronavirus pandemic, which emerged in Wuhan in 2019, has affected education activities drastically all over the world. Within the scope of pandemic measures, it has been reported that education at all levels has been suspended for various periods in many countries, such as for 49 weeks in Türkiye (Unesco 2020). In this process, which was suspended in line with the opinions of education and health authorities, the face-to-face/traditional education model was tried to be transformed quickly into a distance education such as online teaching (OT) and learning model (Saadeh et al. 2021).

As in the whole world, OT has been implemented in the Turkish educational system as of March 2020 in order to comply with the social distance rule as one of the precautions to prevent viral transmission during the Covid-19 pandemic (CoHE 2020a). The pandemic process has forced educational institutions to determine new strategies on how to continue veterinary training. While some preferred the hybrid method, a few veterinary schools have completely switched to online teaching and learning activities, preferring educational methods such as online lessons, small group sessions, video conferences, and pre-recorded videos via online platforms (Amanda 2020). Within that period, all first- and fifth-grade students at Ankara University Faculty of Veterinary Medicine (AUFVM) completed theoretical and practical courses remotely, and many exams were conducted remotely and online (Anonymous 2020b). To be included in OT, all academics at Ankara University had to complete the "Online Instructor Certificate Program", which includes 60 hours of theoretical and practical training. Thus, an adaptation of the lecturers to OT method has been aimed at the program (Anonymous 2020c). As of August 2020, a face-to-face and distant hybrid method has been adopted in all higher education institutions in the country (CoHE 2020b) and this hybrid method (Anonymous 2020a; Anonymous 2020b) has been continued in the 2021-2022 academic year.

This study aimed to evaluate the perceptions of AUFVM's academics regarding the practicableness and effectiveness of OT because they had officially experienced it for the first time in veterinary training.

MATERIAL AND METHODS

Approval of the study was obtained from Covid-19 Scientific Research Evaluation Commission, Republic of Türkiye Ministry of Health (Application Number: 2021-01-26T13_19_33, Date: 29.01.2021), and Ankara University Ethics Committee (Date: 12.04.2021 Decision No: 06/51).

The study was designed as a structured cross-sectional survey. A questionnaire consisting of 21 questions, including sociodemographic data, was used as the data collection tool (Table 1). The form was created using Google Forms infrastructure by making use of the previous studies (Armstrong-Mensah et al. 2020; Can and Köroğlu 2020; Di Pietro et al. 2020; Gençoğlu and Çiftçi 2020) on the subject.

The research populatio was determined as 152 academicians who provided OT due to the Covid-19 pandemic at AUVFM in the 2019-2020 and 2020-2021 academic years. Sample selection was not made since it was aimed to reach the whole population in the study. Participants were invited to the study by sending an electronic survey link to their e-mail addresses. Academics were reminded by a reminder note to participate in the questionnaire two weeks after the questionnaire link was sent for the first time. They were given two more weeks to answer the questionnaire, in this period the study was terminated when all academics who agreed to participate were reached. The completed questionnaire forms were stored anonymously in electronic media.

Data for the pilot study were collected in April 2021. The prepared form was presented to 152 academicians on a voluntarily, who formed the population of the study, and the answers of the first 20 participants who completed the form were evaluated within the scope of the pilot application. After reaching 20 participants and completing the pilot study, access to the questionnaire was temporarily terminated. Feedback was requested from the participants of the pilot study for each question and corrections were made accordingly. Within the scope of the pilot study, the questions that needed to be understood were clarified, and the answer options were revised and updated. The 20 participants in the pilot study were not included in the main study.

Key words of the study's abstract were chosen from "The Medical Subject Headings" (MeSH). Turkish key words were selected from "Türkiye Bilim Terimleri version 2.0" and written without any changes.

Statistical Analysis

After the pilot study, the data of the main study were collected between May 2021 and June 2021. The collected data were evaluated using SPSS 14.1. Descriptive statistics were calculated and shown as frequency and percentage for categorical variables. Pearson Chi-Square or Fisher's Exact Test was applied considering the distribution of expected values to cells in comparing the frequencies of categorical variables between groups. The statistical significance level was considered as $p<0.05$.

RESULTS

Among the 152 participants invited to the study; 20 academics were included in the pilot study; 92 of them voluntarily participated in the main study and no response was received from 40 of them. 57.6% of the 92 participants were male (n=53) and 42.4% were female (n=39). 23.9% of the participants were research assistants (n=22) and 76.1% were teaching fellows (professor, associate professor and assistant professor) (n=70). The sociodemographic characteristics of the academicians and the detailed frequencies and percentages of their answers to the questionnaire are given in Table 1.

The distribution of the answers given by the academics to the survey questions according to the divisions they work in is presented in Table 2. Accordingly, the distribution of the answers to the question *"Are there any students who reported that they could not attend your course's online exam due to technological/technical difficulties?"* differed statistically between the divisions where the academicians worked (p=0.047). Academics working in the Division of Food Hygiene and Technology reported that there were students who reported that they could not attend the online exam of their courses due to technological/technical difficulties at a lower level than those working in the Division of Preclinical Sciences. Regarding the use of OT in practical courses, the answer "*Definitely not applicable*" was given by the academicians of the Division of Clinical Sciences at a higher rate than the academicians of the Division of Animal Husbandry and Animal Nutrition. In addition, the answer *"Partially applicable"* was given by the academics of the Division of Animal Husbandry and Animal Nutrition at a higher rate than the academicians of the Division of Clinical Sciences (p<0.001). Finally, academics working in the Divison of Clinical Sciences gave negative answers to the question *"Would you like to continue online teaching after switching to face-to-face education again?* at a higher rate than the academics of the Division of Basic Veterinary Sciences (p=0.016).

In Table 3, the relationship between the interaction of the academics with the students during the online course and the participation of the students in the course was evaluated. Accordingly, academics who stated that questions were *never/rarely* received from students during the online course mostly found participation *insufficient*. Academics who stated that they *sometimes* get questions, mostly found the participation *insufficient and partially sufficient*, whereas academics who stated that questions were asked *often/always* mostly found the participation *partially sufficient* (p<0.001). The academics, who stated that the questions they asked during the online course were *never/rarely* and *sometimes* answered by the students, mostly reported that the participation needed to be improved. Contrary to expectations, academics who stated that the answers were *often/always* answered also noted that the involvement required to be increased (insufficient or partially sufficient, p=0.008).

The evaluations of the academics on whether OT is an effective teaching tool is presented in Table 4. The academics who found the participation of the students in the distance courses *sufficient* stated that they *sometimes* think that OT provides effective learning, while the academics who found OT *insufficient* stated that they do not think that it mostly provides *sufficient* learning (p=0.023). When OT and face-to-face education methods were compared, academics who prefer face-to-face education mostly said that OT *does not* provide or *partially* provides effective learning; on the other hand, academics who prefer OT stated that it mostly provides effective learning and *undecided* academics reported that it mostly provides *partially* effective learning (p<0.001). Although academics thought that OT has advantages, they mostly reported that it *does not* provide or *partially* provides effective learning. Unsurprisingly, academics who think that OT has no advantages stated that it *does not* provide effective learning (p=0.011). Similarly, academics who think that OT has disadvantages mostly said that it *does not* provide effective learning; while all of the academics who think that OT does not have any disadvantages stated that it provides effective learning (p=0.001). Academics who stated that OT is *definitely applicable* in practical courses mostly stated that it provides effective learning, but conversely, academicians who stated that OT should not be applied at all, mostly reported that it *does not* provide or *partially* provides effective learning (p=0.002).

Academics who think that online exams are not carried out safely stated that OT is mostly not an effective learning tool; while the academics who are *undecided* about whether online exams are carried out safely stated that OT is mostly a *partially* effective learning tool (p=0.005). Finally, academics who want to continue OT after switching to face-to-face education again said that OT is an effective teaching tool; on the contrary, academics who do not want to continue mostly said that it is not an effective teaching tool, and academicians who *sometimes* want to continue reported that it *does not* provide or *partially* provides effective teaching (p<0.001) (Table 4).

In Table 5, the willingness of the academics to continue OT after switching to face-to-face education are presented. Academics who stated that they *never/rarely* received questions from students during the online courses mostly said that they do not want to continue OT or that it could be applied *sometimes*; academics who stated that students *often/always* ask questions said that OT can *sometimes* be continued (p=0.018). Concordantly, academics who stated that the students *never/rarely* answered the questions asked by them during the online courses said that they mostly *do not* or *sometimes* want to continue OT; however, academics who stated that they are *sometimes* and *often/always* received answers from students mostly reported that OT *sometimes* could be continued (p=0.014). Even though academics who think that OT provides effective teaching mostly want to continue OT; academics who think that it does not provide effective teaching stated that they mostly do not want to continue OT or can *sometimes* continue (p<0.001). Academics who think that OT is advantageous mostly reported that OT can *sometimes* be continued; at the same time, academics who think that it is not advantageous mostly do not want to continue OT (p<0.001). Similarly, whereas, academics who think that OT is disadvantageous *mostly* stated that they *do not* or *sometimes* want to continue; academics who think that OT is not disadvantageous mostly stated that they want to continue OT (p=0.017). Academics who think that online teaching can *definitely* be used in practical courses mostly want to continue OT; however, academics who think that it cannot be used have stated that they *do not* want to continue online teaching or *sometimes* it can be continued (p<0.001). Academics who think that the assessment and evaluation method in OT should be face-to-face, mostly *do not* or *sometimes* want to continue OT; although, academics who think that it should be online and those who are *undecided* mostly stated that OT can *sometimes* be continued (p=0.021).

Table 1: Frequency and percentage of all questions in the survey.

Categories	n (%)
Gender	
Female	39 (42.4)
Male	53 (57.6)
Academic title	
Research assistant	22 (23.9)
Lecturer	70 (76.1)
Division	
Basic veterinary sciences	23 (25.0)
Preclinical sciences	24 (26.1)
Clinical sciences	21 (22.8)
Food hygiene and technology	9 (9.8)
Animal husbandry and animal nutrition	15 (16.3)
What is the quality of the courses given before the Covid-19 pandemic?	
Theoretical	10 (10.9)
Theoretical and practical	76 (82.6)
Practical	6 (6.5)
Did you provide online/distance education before the Covid-19 pandemic?	
Yes	20 (21.7)
No	72 (78.3)
Do you think you have the technological knowledge required for online teaching?	
Yes	57 (62.0)
No	2 (2.2)
Partially	33 (25.9)
Can you use your course time effectively in online teaching?	
Yes	58 (63.0)
No	8 (8.7)
Sometimes	18 (19.6)
Undecided	8 (8.7)
What would you think about your students' instant/online participation in the course you teach via online teaching?	
I find the participation of those taking the course sufficient	5 (5.4)
I find the participation of those taking the course insufficient	63 (68.5)
I find the participation of those taking the course partially sufficient	24 (26.1)
Do you get questions from your students during the online course?	
Never	4 (4.3)
Rarely	43 (46.7)
Sometimes	32 (34.8)
Often	11 (12.0)
Always	2 (2.2)
Do you get answers to the questions you ask during the online course?	
Never	5 (5.4)
Rarely	25 (27.2)
Sometimes	22 (23.9)
Often	26 (28.3)
Always	14 (15.2)
Do you experience technological/technical problems (disconnection, system not working, storage problems, etc.) while teaching online?	
Never	12 (13.0)
Rarely	40 (43.5)
Sometimes	31 (33.7)
Often	9 (9.8)
Always	-
Do you have students who reported that they could not attend your course due to technological/technical difficulties?	
Yes	50 (54.3)
No	42 (45.7)
Are there any students who reported that they could not attend your course's online exam due to technological/technical difficulties?	
Yes	49 (53.3)
No	43 (46.7)
Do you think online teaching provides effective learning?	
Yes	10 (10.9)
No	51 (55.4)
Undecided	31 (33.7)
When you compare online teaching and face-to-face education methods, which one do you prefer?	
Face-to-face education method	78 (84.8)
Online teaching method	6 (6.5)
Undecided	8 (8.7)
Do you think online teaching has some advantages?	
Yes	46 (50.0)
No	28 (30.4)
Undecided	18 (19.6)
Do you think online teaching has some disadvantages?	
Yes	82 (89.1)
No	3 (3.3)
Undecided	7 (7.6)
What do you think about the use of online teaching in practical courses?	
Definitely applicable	4 (4.3)
Definitely not applicable	47 (51.1)
Partially applicable	41 (44.6)
What method do you think should be used to assess and evaluate the course you teach via online tools?	
Face-to-face assessment	40 (43.5)
Online assessment	44 (47.8)
Undecided	8 (8.7)
Do you think that online exams are held securely (students answer questions by being honest)?	
Yes	4 (4.3)
No	67 (72.8)
Undecided	21 (22.8)
Would you like to continue online teaching after switching to face-to-face education again?	
Yes	12 (13.0)
No	27 (29.3)
Sometimes	53 (57.6)

Table 2: Distribution of answers according to the divisions.

		Divisions					p
		Basic veterinary sciences	**Preclinical sciences**	**Clinical sciences**	**Food hygiene and technology**	**Animal husbandry and animal nutrition**	
Do you think you have the technological knowledge required for online teaching?	Yes	13 (22.8)	14 (24.6)	13 (22.8)	5 (8.8)	12 (21.1)	0.781[1]
	No	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	
	Partially	9 (27.3)	10 (30.3)	7 (21.2)	4 (12.1)	3 (9.1)	
Can you use your course time effectively in online teaching?	Yes	15 (25.9)	17 (29.3)	15 (25.9)	3 (5.2)	8 (13.8)	0.181[1]
	No	1 (12.5)	3 (37.5)	2 (25.0)	0 (0.0)	2 (25.0)	
	Sometimes	6 (33.3)	3 (16.7)	1 (5.6)	5 (27.8)	3 (16.7)	
	Undecided	1 (12.5)	1 (12.5)	3 (37.5)	1 (12.5)	2 (25.0)	
What would you think about your students' instant/online participation in the course you teach via online tools?	I find the participation of those taking the course sufficient.	0 (0.0)	0 (0.0)	3 (60.0)	2 (40.0)	0 (0.0)	0.232[1]
	I find the participation of those taking the course insufficient.	17 (27.0)	16 (25.4)	14 (22.2)	5 (7.9)	11 (17.5)	
	I find the participation of those taking the course partially sufficient.	6 (25.0)	8 (33.3)	4 (16.7)	2 (8.3)	4 (16.7)	
Do you get questions from your students during the online course?	Never/rarely	10 (21.3)	10 (21.3)	16 (34.0)	4 (8.5)	7 (14.9)	0.298[1]
	Sometimes	10 (31.3)	9 (28.1)	3 (9.4)	3 (9.4)	7 (21.9)	
	Often/always	3 (23.1)	5 (38.5)	2 (15.4)	2 (15.4)	1 (7.7)	
Do you get answers to the questions you ask during the online course?	Never/rarely	4 (13.3)	10 (33.3)	10 (33.3)	3 (10.0)	3 (10.0)	0.071[1]
	Sometimes	8 (36.4)	5 (22.7)	2 (9.1)	0 (0.0)	7 (31.8)	
	Often/always	11 (27.5)	9 (22.5)	9 (22.5)	6 (15.0)	5 (12.5)	
Do you experience technological/technical problems (disconnection, system not working, storage problems, etc.) while teaching online?	Never/rarely	13 (25.0)	10 (19.2)	16 (30.8)	5 (9.6)	8 (15.4)	0.242[1]
	Sometimes	9 (29.0)	12 (28.7)	3 (9.7)	2 (6.5)	5 (16.1)	
	Often/always	1 (11.1)	2 (22.2)	2 (22.2)	2 (22.2)	2 (22.2)	
Do you have students who reported that they could not attend your course due to technological/technical difficulties?	Yes	14 (28.0)	17 (34.0)	8 (16.0)	6 (12.0)	5 (10.0)	0.080[1]
	No	9 (21.4)	7 (16.7)	13 (31.0)	3 (7.1)	10 (23.8)	
Are there any students who reported that they could not attend your course's online exam due to technological/technical difficulties?	Yes	12 (24.5)	18 (36.7)	7 (14.3)	3 (6.1)	9 (18.4)	0.047[1]
	No	11 (25.6)	6 (14.0)	14 (32.6)	6 (14.0)	6 (14.0)	

Table 2 (continued): Distribution of answers according to the divisions.

		Divisions					p
		Basic veterinary sciences	**Preclinical sciences**	**Clinical sciences**	**Food hygiene and technology**	**Animal husbandry and animal nutrition**	
Do you think online teaching provides effective learning?	Yes	6 (60.0)	2 (20.0)	2 (20.0)	0 (0.0)	0 (0.0)	0.475[1]
	No	11 (21.6)	12 (23.5)	12 (23.5)	6 (11.8)	10 (19.6)	
	Undecided	6 (19.4)	10 (32.3)	7 (22.6)	3 (9.7)	5 (16.1)	
When you compare online teaching and face-to-face education methods, which one do you prefer?	Face-to-face education method	18 (23.1)	19 (24.4)	18 (23.1)	8 (10.3)	15 (19.2)	0.092[1]
	Online teaching method	4 (66.7)	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)	
	Undecided	1 (12.5)	5 (62.5)	1 (12.5)	1 (12.5)	0 (0.0)	
Do you think online teaching has some advantages?	Yes	17 (37.0)	12 (26.1)	8 (17.4)	3 (6.5)	6 (13.0)	0.189[1]
	No	2 (7.1)	8 (28.6)	9 (32.1)	3 (10.7)	6 (21.4)	
	Undecided	4 (22.2)	4 (22.2)	4 (22.2)	3 (16.7)	3 (16.7)	
Do you think online teaching has some disadvantages?	Yes	20 (24.4)	22 (26.8)	18 (22.0)	9 (11.0)	13 (15.9)	0.831[1]
	No	1 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	
	Undecided	2 (28.6)	2 (28.6)	1 (14.3)	0 (0.0)	2 (28.6)	
What do you think about the use of online teaching in practical courses?	Definitely applicable	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001[1]
	Definitely not applicable	9 (19.1)	13 (27.7)	18 (38.3)	5 (10.6)	2 (4.3)	
	Partially applicable	10 (24.4)	11 (26.8)	3 (7.3)	4 (9.8)	13 (31.7)	
What method do you think should be used to assess and evaluate the course you teach via online tools?	Face-to-face assessment	11 (27.5)	13 (32.5)	6 (15.0)	4 (10.0)	6 (15.0)	0.582[1]
	Online assessment	9 (20.5)	10 (22.7)	12 (27.3)	4 (9.1)	9 (20.5)	
	Undecided	3 (37.5)	1 (12.5)	3 (37.5)	1 (12.5)	0 (0.0)	
Do you think that online exams are held securely (students answer questions by being honest)?	Yes	0 (0.0)	1 (25.0)	2 (50.0)	1 (25.0)	0 (0.0)	0.585[1]
	No	18 (26.9)	19 (28.4)	12 (17.9)	6 (9.0)	12 (17.9)	
	Undecided	5 (23.8)	4 (19.0)	7 (33.3)	2 (9.5)	3 (14.3)	
Would you like to continue online teaching after switching to face-to-face education again?	Yes	6 (50.0)	5 (41.7)	1 (8.3)	0 (0.0)	0 (0.0)	0.016[1]
	No	2 (7.4)	8 (29.6)	11 (40.7)	2 (7.4)	4 (14.8)	
	Sometimes	15 (28.3)	11 (20.8)	9 (17.0)	7 (13.2)	11 (20.8)	

Table 3: Online teaching and active participation in the course.

		How would you evaluate your students' instant/online participation in the course you teach via online tools?			**p**
		I find the participation of those taking the course			
		sufficient	**insufficient**	**partially sufficient**	
Do you get questions from your students during the online course?	Never/rarely	2 (4.3)	43 (91.5)	2 (4.3)	<0.001[1]
	Sometimes	3 (9.4)	15 (46.9)	14 (43.8)	
	Often/always	0 (0.0)	5 (38.5)	8 (61.5)	
Do you get answers to the questions you ask during the online course?	Never/rarely	1 (3.3)	27 (90.0)	2 (6.7)	0.008[1]
	Sometimes	1 (4.5)	15 (68.2)	6 (27.3)	
	Often/always	3 (7.5)	21 (52.5)	16 (40.0)	

Table 4: Quality of the online teaching.

		Do you think distance education provides effective learning?			**p**
		Yes	**No**	**Sometimes**	
Do you think you have the technological knowledge required for online teaching?	Yes	7 (12.3)	29 (50.9)	21 (36.8)	0.715[1]
	No	0 (0.0)	1 (50.0)	1 (50.0)	
	Partially	3 (9.1)	21 (63.6)	9 (27.3)	
Can you use your course time effectively in online teaching?	Yes	9 (15.5)	29 (50.0)	20 (34.5)	0.531[1]
	No	1 (12.5)	5 (62.5)	2 (25.0)	
	Sometimes	0 (0.0)	11 (61.1)	7 (38.9)	
	Undecided	0 (0.0)	6 (75.0)	2 (25.0)	
What would you think about your students' instant/online participation in the course you teach via online tools?	I find the participation of those taking the course sufficient.	0 (0.0)[a]	1 (20.0)[a]	4 (80.0)[b]	0.023[1]
	I find the participation of those taking the course insufficient.	7 (11.1)[a]	41 (65.1)[b]	15 (23.8)[a]	
	I find the participation of those taking the course partially sufficient.	3 (12.5)[a]	9 (37.5)[a]	12 (50.0)[a]	
Do you get questions from your students during the online course?	Never/rarely	5 (10.6)	30 (63.8)	12 (25.5)	0.3838[1]
	Sometimes	3 (9.4)	16 (50.0)	13 (40.6)	
	Often/always	2 (15.4)	5 (38.5)	6 (46.2)	
Do you get answers to the questions you ask during the online course?	Never/rarely	2 (6.7)	20 (66.7)	8 (26.7)	0.648[1]
	Sometimes	3 (13.6)	11 (50.0)	8 (36.4)	
	Often/always	5 (12.5)	20 (50.0)	15 (37.5)	

Table 4 (continued): Quality of the online teaching.

		Do you think distance education provides effective learning?			p
		Yes	**No**	**Sometimes**	
Do you experience technological/technical problems (disconnection, system not working, storage problems, etc.) while teaching online?	Never/rarely	8 (15.4)	25 (48.1)	19 (36.5)	0.504[1]
	Sometimes	2 (6.5)	20 (64.5)	9 (29.0)	
	Often/always	0 (0.0)	6 (66.7)	3 (33.3)	
Do you have students who reported that they could not attend your course due to technological/technical difficulties?	Yes	4 (8.0)	28 (56.0)	18 (36.0)	0.626[2]
	No	6 (14.3)	23 (54.8)	13 (31.0)	
Are there any students who reported that they could not attend your course's online exam due to technological/technical difficulties?	Yes	2 (4.1)	28 (57.1)	19 (38.8)	0.072[2]
	No	8 (18.6)	23 (53.5)	12 (27.9)	
When you compare online teaching and face-to-face education methods, which one do you prefer?	Face-to-face education method	3 (3.8)[a]	49 (62.8)[b]	26 (33.3)[b]	<0.001[1]
	Online teaching method	5 (83.3)[a]	1 (16.7)[b]	0 (0.0)[b]	
	Undecided	2 (25.0)[b]	1 (12.5)[b]	5 (62.5)[a]	
Do you think online teaching has some advantages?	Yes	9 (19.6)	20 (43.5)	17 (37.0)	0.011[1]
	No	0 (0.0)	22 (78.6)	6 (21.4)	
	Undecided	1 (5.6)	9 (50.0)	8 (44.4)	
Do you think online teaching has some disadvantages?	Yes	6 (7.3)	49 (59.8)	27 (32.9)	0.001[1]
	No	3 (100.0)	0 (0.0)	0 (0.0)	
	Undecided	1 (14.3)	2 (28.6)	4 (57.1)	
What do you think about the use of online teaching in practical courses?	Definitely applicable	3 (75.0)	0 (0.0)	1 (25.0)	0.002[1]
	Definitely not applicable	2 (4.3)	32 (68.1)	13 (27.7)	
	Partially applicable	5 (12.2)	19 (46.3)	17 (41.5)	
What method do you think should be used to assess and evaluate the course you teach via online tools?	Face-to-face assessment	2 (5.0)	28 (70.0)	10 (25.0)	0.061[1]
	Online assessment	8 (18.2)	18 (40.9)	18 (40.9)	
	Undecided	0 (0.0)	5 (62.5)	3 (37.5)	
Do you think that online exams are held securely (students answer questions by being honest)?	Yes	1 (25.0)	1 (25.0)	2 (50.0)	0.005[1]
	No	4 (6.0)	44 (65.7)	19 (28.4)	
	Undecided	5 (23.8)	6 (28.6)	10 (47.6)	
Would you like to continue online teaching after switching to face-to-face education again?	Yes	7 (58.3)	1 (8.3)	4 (33.3)	<0.001[1]
	No	0 (0.0)	23 (85.2)	4 (14.8)	
	Sometimes	3 (5.7)	27 (50.9)	23 (43.4)	

Table 5: Sustainability of the online teaching.

		Would you like to continue online teaching after switching to face-to-face education again?			p
		Yes	**No**	**Sometimes**	
Do you think you have the technological knowledge required for online teaching?	Yes	9 (15.8)	16 (28.1)	32 (56.1)	0.828[1]
	No	0 (0.0)	1 (50.0)	1 (50.0)	
	Partially	3 (9.1)	10 (30.3)	20 (60.6)	
Can you use your course time effectively in online teaching?	Yes	10 (17.2)	16 (27.6)	32 (55.2)	0.274[1]
	No	1 (12.5)	4 (50.0)	3 (37.5)	
	Sometimes	1 (5.6)	3 (16.7)	14 (77.8)	
	Undecided	0 (0.0)	4 (50.0)	4 (50.0)	
What would you think about your students' instant/online participation in the course you teach via online tools?	I find the participation of those taking the course sufficient.	0 (80.0)	1 (20.0)	4 (80.0)	0.357[1]
	I find the participation of those taking the course insufficient.	7 (11.1)	22 (34.9)	34 (54.0)	
	I find the participation of those taking the course partially sufficient.	5 820.8)	4 (16.7)	15 (62.5)	
Do you get questions from your students during the online course?	Never/rarely	5 (10.6)	20 (42.6)	22 (46.8)	0.018[1]
	Sometimes	5 (15.6)	7 (21.9)	20 (62.5)	
	Often/always	2 (15.4)	0 (0.0)	11 (84.6)	
Do you get answers to the questions you ask during the online course?	Never/rarely	3 (10.0)	16 (53.3)	11 (36.7)	0.014[1]
	Sometimes	3 (13.6)	3 (13.6)	16 (72.7)	
	Often/always	6 (15.0)	8 (20.0)	26 (65.0)	
Do you experience technological/technical problems (disconnection, system not working, storage problems, etc.) while teaching online?	Never/rarely	11 (21.2)	13 (25.0)	28 (53.8)	0.116[1]
	Sometimes	1 (3.2)	10 (32.3)	20 (64.5)	
	Often/always	0 (0.0)	4 (44.4)	5 (55.6)	
Do you have students who reported that they could not attend your course due to technological/technical difficulties?	Yes	4 (8.0)	16 (32.0)	30 (60.0)	0.285[2]
	No	8 (19.0)	11 (26.2)	23 (54.8)	
Are there any students who reported that they could not attend your course's online exam due to technological/technical difficulties?	Yes	3 (6.1)	17 (34.7)	29 (59.2)	0.086[2]
	No	9 (20.9)	10 (23.3)	24 (55.8)	

Table 5 (continued): Sustainability of the online teaching.

		Would you like to continue online teaching after switching to face-to-face education again?			p
		Yes	**No**	**Sometimes**	
Do you think online teaching provides effective learning?	Yes	7 (70.0)	0 (0.0)	3 (30.0)	<0.001[1]
	No	1 (2.0)	23 (45.1)	27 (52.9)	
	Undecided	4 (12.9)	4 (12.9)	23 (74.2)	
When you compare online teaching and face-to-face education methods, which one do you prefer?	Face-to-face education method	3 (3.8)	26 (33.3)	49 (62.8)	<0.001[1]
	Online teaching method	5 (83.3)	1 (16.7)	0 (0.0)	
	Undecided	4 (50.0)	0 (0.0)	4 (50.0)	
Do you think online teaching has some advantages?	Yes	12 (26.1)	4 (8.7)	30 (65.2)	<0.001[1]
	No	0 (0.0)	20 (71.4)	8 (28.6)	
	Undecided	0 (0.0)	3 (16.7)	15 (83.3)	
Do you think online teaching has some disadvantages?	Yes	8 (9.8)	27 (32.9)[b]	47 (57.3)[b]	0.017[1]
	No	2 (66.7)	0 (0.0)[b]	1 (33.3)[b]	
	Undecided	2 (28.6)	0 (0.0)[a]	5 (71.4)[a]	
What do you think about the use of online teaching in practical courses?	Definitely applicable	3 (75.0)	0 (0.0)	1 (25.0)	<0.001[1]
	Definitely not applicable	3 (6.4)	24 (51.1)	20 (42.6)	
	Partially applicable	6 (14.6)	3 (7.3)	32 (78.0)	
What method do you think should be used to assess and evaluate the course you teach via online tools?	Face-to-face assessment	1 (2.5)	15 (37.5)	24 (60.0)	0.021[1]
	Online assessment	11 (25.0)	10 (22.7)	23 (52.3)	
	Undecided	0 (0.0)	2 (25.0)	6 (75.0)	
Do you think that online exams are held securely (students answer questions by being honest)?	Yes	1 (25.0)	0 (0.0)	3 (75.0)	0.151[1]
	No	7 (10.4)	24 (35.8)	36 (53.7)	
	Undecided	4 (19.0)	3 (14.3)	14 (66.7)	

DISCUSSION AND CONCLUSION

The participation rate of the academics invited to the research was 73.6%. It is thought that this ratio will give realistic information about the reflection of the whole population of the study and the generalizability of the results. The fact that the majority of the academics (82.6%) included in the study are academic staff who teach both theoretical and practical courses may indicate that more qualified data is obtained in the evaluation of OT. In other words, the majority of the participants do not teach only theoretical or practical courses may show that their evaluations are not one-dimensional and the model is evaluated with a holistic approach.

In the study, it was determined that the clinicians do not want to carry out the practical courses with OT and they have negative thoughts about it. When clinicians and basic sciences academics are compared, it is seen that this negative idea is more striking. It is thought that the characteristic of the courses taught has an important effect on this difference of opinion. It is known that students' hands-on practices, examination, diagnosis and treatment at the bedside and experience of communication and consultation skills in practical courses make learning much more effective and easier (Mehta et al. 2021). In a study (Amanda 2020) questioning the OT experiences of trainers from various veterinary schools in different countries during the pandemic period, it is seen that some trainers emphasize the importance of hands-on practices. On the other hand, in a report discussing the effectiveness of online anatomy training of veterinarian candidates, it was stated that OT is instructive and applicable for students (Choudhary 2021). It is striking that pre-recorded videos and live applications are beneficial for successfully teaching surgical interventions to medical students. It is also demonstrated that medical students give positive feedback on surgical training with OT and this method can be used effectively in the future thanks to the developing technology (Mehta et al. 2021). In this context, it would be wrong to think that OT is only a didactic method. By making use of techniques such as video-based courses, roundtable discussions, small group sessions, case-based learning, serious games and simulations, more active use of online teaching by students can be encouraged and their learning processes can be improved.

In the study, the academics stated that they did not generally experience difficulties in participating in OT due to technological/technical reasons, while the students had. Similarly, attention has been drawn in the literature (Ahmed et al. 2020; Amanda 2020; Choudhary 2021; Mehta et al. 2021) to some technological and technical inadequacies, which may be experienced due to the inability of each student to have sufficient economic power, can be considered one of the vital disadvantages of OT.

Based on the findings of this study, the lack of active participation of students in the courses can be suggested as another disadvantage of OT. It is understood that the students' asking questions about the course is positively related to their participation in the lesson. It is observed that academics are generally not satisfied with asking and receiving questions from students. The skill of asking questions encourages active learning, keeps the interest in the lesson alive and facilitates learning. According to Tsai et al. (2021), one of the features of OT that negatively affects the academic success of students is the absence of classroom interaction. From this point of view, it can be evaluated that OT brings some difficulties in active learning, since there are only a few questions from the students in OT. Moreover, it is seen that the expected relationship, communication and cooperation between the lecturer and the student (Regmi and Jones 2020). In a study, it was concluded that face-to-face education is much more efficient in terms of working collaboratively and communication skills (Ahmed et al. 2020).

It was concluded that academics think that OT is also inadequate in cases where participation is insufficient. On the other hand, the fact that those who think OT is effective and want to use this method in the future is an indicator of the consistency between the answers. Although academics generally find students' participation in the course sufficient, it can be argued that they are not ready for OT. Moreover, when the face-to-face and distance education preferences of academics are evaluated, it is seen that 84.7% of the participants prefer the face-to-face education model. A study (Cooperman 2007) claimed that the preparation styles of the lecturers for face-to-face education and online education are very different from each other. When academics' reasons for preferring face-to-face education are justified, similar to what Cooperman (2007) mentioned, it can be argued that it would not be surprising to prefer the method they are accustomed to in terms of preparation and teaching strategies.

In the literature, when it comes to the evaluation of distance and online education, it is seen that one of the most important evaluation criteria is the student interaction with the lecturer and other students (Byrne et al. 2021). Among the most important difficulties of online exams are the problems that students may experience in ensuring their privacy and getting used to the new exam system. Another problem with online exams is ensuring exam security and preventing academic fraud. For honesty to be at the forefront, students can declare their identities before starting the exam and use microphones and cameras to ensure that the area where they will take the exam can be observed (Marín García et al. 2021). Despite these opportunities, it is considered unrealistic to control these applications by academics, who are sometimes the only instructors of the course, for exams with a large participant population. In a study, it was revealed that remotely supervised exams to ensure academic honesty cause problems in students' privacy, causing them to experience stress and anxiety (Paredes et al. 2021). Based on these data, it can be suggested to prioritize online classroom interaction and participation in the assessment and evaluation of students. As a matter of fact, according to Oncu and Cakir (2011), formative assessment is considered more appropriate instead of summative assessment in online education.

It has been determined that although questions are asked from the students to the lecturers during the courses or the questions asked by the lecturers are answered, the academicians are skeptical about the continuation of online education or they think that it can continue under some conditions. Moreover, it is clearly seen that academics associate their views on the sustainability of OT with whether it is an effective learning method and the majority of them have a negative approach to distance education being one of the basic teaching methods. However, OT has many advantages as it is a flexible method that allows students to participate wherever and whenever they want (Houlden and Veletsianos 2019; Tsai et al. 2021; Veletsianos et al. 2021; Wagner et al. 2021). For this reason, it is recommended that academics do not

ignore the features that are in favor of the students before deciding on the OT method. It is considered that conducting both distance and traditional face-to-face education together can be a much more effective method as an alternative to totally OT (Matkin 2007).

In the international literature (Bill 2007; Dhein 2007; Murray and Sischo 2007; Varnhagen and Wright 2008), there are many studies on distance education conducted from the past to the present in the fields of veterinary medicine. In recent years, the existence of studies particular to the Covid-19 pandemic draws attention (Amanda 2020; Routh et al. 2021; Mahdy and Sayed 2022). For this reason, it is thought that this study will contribute to the literature in terms of the effects of Covid-19 on veterinary medicine education.

The online teaching and learning model in the field of veterinary medicine in Türkiye is a new and open concept. Before the pandemic, in addition to some traditional courses in some veterinary faculties, it is seen that academics recorded their courses and made these recordings available to students on various social media platforms. Although it is known that academics share these videos with their students, there is no reliable data that can confirm this information. In the pre-pandemic period, no course/curriculum which is officially conducted with distance education method or any scientific research/report/document that covers the whole of veterinary education has been found in Türkiye. However, after the pandemic, a study (Aslım et al. 2023) was found that evaluated the views of veterinary students about distance education. Considering the previous study and current study, it can be claimed that these are one of the pioneering studies that proves the existence of online education in the field of veterinary medicine in Türkiye and draws a general framework by questioning its pros and cons.

Consequently, the perspectives and perceptions of academics on the new OT method were evaluated after the compulsory and sudden transition in the AUFVM due to the Covid-19 pandemic. Academics have expressed their opinion that online education generally contains technical/technological difficulties and that this method should not be used in practical courses. In addition, it has been determined that the academics have an attitude and approach not to prefer the OT method among the standard education methods in the future. Comprehensive qualitative research is needed to examine the reasons for these negative attitudes. Moreover, due to the sudden and unprepared introduction of OT in veterinary education, determining the strengths and areas that need improvement from the point of view of academics can be determined as one of the subjects of further studies.

Aslım et al. (2023) showed that most of the Turkish veterinary students (77%) thought that applied courses should be face-to-face. Besides, another study designed by the authors of current article has highlighted the experience and perceptions of students about the OT process that they are trying to adopt in the Covid-19 pandemic. In this way, it has aimed to evaluate OT to determine the perceptions of lecturers and students, compare them and make improvements as a result, not only in one way but also bidirectional.

In conclusion, this study revealed that some clinicians don't want to carry out the practical courses with OT. Insufficient participation of students and technological inadequacied can be cons of the education. It can be argued that the academics do not tend to prefer and are biased against OT.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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The second author of the study (Dr. Pınar Ambarcıoğlu) passed away due to the earthquake disaster that took place in Türkiye on February 6, 2022. Peer-review period of this article has begun after her death. This study is dedicated to Dr. Pınar Ambarcıoğlu and her family, who passed away.

AUTHOR CONTRIBUTIONS

Idea / Concept: AÜA
Supervision / Consultancy: AÜA, PA
Data Collection and / or Processing: AÜA, PA
Analysis and / or Interpretation: AÜA, PA
Writing the Article: AÜA
Critical Review: AÜA

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Investigation of the Effect of Malva Plant (*Malvasylvestris L.*) on Skin Fungus in Cattle

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ABSTRACT The purpose of this study is to determine the effect of the *Malva sylvestris L.* (MS) plant on the cutaneous fungus (Trichophytosis, Ringworm) in cattle. The research was carried out on a total of 20 cattle of 6 to 20 months of different races and genders in the Ardahan region. Animals diagnosed with Trichophytosis were divided as control (n=10) and experimental (n=10) groups. No substances were used in the control group. The extract of MS was sprayed with an atomizer onto the lesions of the experimental group. Spraying was done to cover the area where the lesions were located and wet enough. The procedure was conducted twice daily (morning and evening) and for 15 days. Starting from the pre-application (0th day) until the 36th day at intervals of two days, the lesion diameters of the cattle in the control and the experimental group were measured and recorded. At the end of the monitoring period (36th day), the diameter of the lesion was enlarged (29.20±3.58 mm) in the control group and reduced (6.60±5.16 mm) in the experimental group. This difference between the lesion diameters of the control and the experimental groups was statistically significant ($p<0.05$). In this study, it was concluded that the MS extract applied to the bovine skin fungus inhibited the growth of lesions and reduced their size. It is thought that MS extract can be used locally in the treatment of Trichophytosis in cattle.

Keywords:*Cattle, Malva sylvestris, Trichophytosis.*

ÖZ

Malva Bitkisinin (*Malva sylvestris L.*) Sığırlarda Deri Mantarı Üzerine Etkisinin Araştırılması

Bu araştırmanın amacı *Malva sylvestris L.* (MS) bitkisinin sığırlarda deri mantarı (Trikofitoz, Ringworm) üzerine etkisini belirlemektir. Araştırma Ardahan yöresinde ırk ve cinsiyetleri farklı, 6-20 aylık toplam 20 sığır üzerinde yürütüldü. Trikofitoz tanısı konan sığırlar kontrol (n=10) ve deneme (n=10) grubuna ayrıldı. Kontrol grubuna herhangi bir madde uygulanmadı. Deneme grubunda bulunan hayvanların lezyonları üzerine MS ekstresi atomizer ile püskürtüldü. Püskürtme işlemi lezyonların bulunduğu alanı kaplayacak ve yeteri kadar ıslatacak şekilde yapıldı. Bu işleme günde iki kez (sabah ve akşam) ve 15 gün boyunca devam edildi. Uygulama öncesinden (0.gün) başlayıp iki gün arayla 36. güne kadar kontrol ve deneme grubunda bulunan sığırların lezyon çapları ölçülerek kayıt edildi. Takip süresi (36. gün) sonunda kontrol grubunda lezyon çapının büyüdüğü (29.20±3.58 mm), deneme grubunda ise küçüldüğü (6.60±5.16 mm) görüldü. Kontrol ve deneme guruplarında lezyon çapları arasındaki bu fark istatistiksel olarak önemli ($p<0.05$) bulundu. Bu araştırmada sığır deri mantarı üzerine uygulanan MS ekstresi, lezyonların büyümesini durdurarak çaplarını küçülttüğü sonucuna varıldı. Sığırlarda Trikofitoz tedavisinde MS ekstresinin lokal olarak kullanılabileceği düşünülmektedir.

Anahtar Kelimeler:*Ebegümeci, Sığır, Trikofitoz.*

INTRODUCTION

Fungi cause important diseases which can be observed anywhere in the world. Dermatophytosis (Ringworm) is a fungal disorder that affects the skin surface of cattle (Hizli 2020; Abdullah et al. 2021). It is spread from cattle to humans, affecting human health (Tartor et al. 2020). In particular, animal owners, animal caregivers, technicians, veterinarians and their families are at high risk of contracting the disease. The disease can be transmitted through contact or contaminated tools and utensils (Al-Farha and Mahmood 2021). The disease is primarily caused by fungi such as Epidermophyton, Trichophyton, and Microsporium (Abdullah et al. 2021; Al-Farha and Mahmood 2021). Of these fungal genera, *Trichophyton verrucosum* is the most widespread bovine species and is transmitted to humans (Hizli 2020; Tartor et al. 2020). Skin, nails, hair and horns are most affected by the illness (Apaydin 2020).

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The course and seriousness of the disease depend on the breed, age, parasitic load, care, nutrition and immune status of the animal. Moreover, skin lesions, localization of lesions, environmental factors (humidity, temperature, and crowded environment), certain medications (like cortisol) and the virulence of the fungus determine the prognosis of the disease (Apaydin 2020; Tartor et al. 2020; Abdullah et al. 2021; Al-Farha and Mahmood 2021).

The disease causes substantial economic damage on livestock farms by causing loss of meat, milk, skin and treatment costs (Tartor et al. 2020; Abdullah et al. 2021). It affects cattle of all ages. However, the disease is more common in calves. In diseased cattle, hair loss, exuding on the skin, folliculitis, round, white-grey, thickened lesions occur. Most of these lesions occur in the head and neck. Nevertheless, it may also appear on the back, legs and other parts (Apaydin 2020; Al-Farha and Mahmood 2021).

Malva sylvestris L. (MS), a plant species belonging to the Malvaceae family, grows naturally in the Mediterranean, Asia, northern Africa and European countries. The height of the plant reaches to approximately 100-120 cm, and the length of the flower is up to 2-5 cm (Jabri et al. 2017). The plant is traditionally used in people's food (in the form of soups and salads) and in the treatment of some diseases since antiquity (Barros et al. 2010). Although all parts of the plant are used for medicinal purposes, its leaves and flowers are preferable. It is particularly used in stomach ulcers, expectorant, cough suppressant, laxative, diarrhea-relieving, muscle, skeletal and skin diseases. The plant has anti-inflammatory, anti-oxidant, anti-microbial, anti-septic and anti-cancer properties (Feizi et al. 2018). The most important chemical structure of the plant is made up of mucilage, flavonoids, tannins, tocopherols, ascorbic acid, carotenoids, and anthocyanin (Barros et al. 2010; Hajyani et al. 2015; Jabri et al. 2017).

Silver and silver chloride in the leaves of MS is believed to be effective against *Candida orthopsilos*, *Pasteurella aeruginosa,* and *Bacillus subtilis* (Feizi et al. 2018). It is also suggested that extracts from MS have anti-fungal properties on *Candida albicans* (Cardoso et al. 2012; Alizadeh et al. 2017), *C. Krusei,* and *C. Tropicalis* (Cardoso et al. 2012). In mice infected with *Candida albicans*, the watery extract of MS stimulates the immune system (Hajyani et al. 2015), and is effective against fungi such as *Alternaria alternata*, *Penicillium expansum,* and *Mucor piriformis* (Parveen et al. 2014). This proposed antifungal effect of MS increases the potential for it to be effective against fungi responsible for dermatophytosis in cattle (*Trichophyton verrucosum*, *Epidermophyton spp.*, and *Microsporium spp.*) in vivo conditions. This research was conducted to determine the anti-mycotic effect of MS extract in cattle with dermatophyte (ringworm) diagnosed by clinical and microscopic examination.

MATERIAL AND METHODS

The study has been approved by the Ethics Committee of Kafkas University (decision date 26.01.2023 and numbered KAU-HADYEK/2023-011) and Ministry of Agriculture and Forestry of Türkiye (letter dated 20.12.2022 and numbered E-29486769-325.99-8174016).

MS was collected from the Ardahan region in July 2022. These collected plants were identified by examining their morphological properties (Seçmen et al. 2011). The geographical location of the plant is between 40º 47ˡ and 42ˡˡ north latitudes, and 42º 36ˡ 32ˡˡ east longitudes (Google Earth) and its height is 2038 meters. From this area, the aboveground parts of the plant (stem, leaves, flowers, and seed) were collected and laid on a clean newspaper in such a way that they did not overlap. After that, the plants were allowed to dry in the shade and at room temperature. The plants were turned once a day to dry properly. 20 g was taken from the plant, which was found to have dried as a result of physical examination (on the 15th day after the collection day). It was ground into a mortar and placed in Mayer. Then 100 ml of boiling distillate water was added. The cap of the bottle was tightly closed and left in a water bath at 100 °C for 10 minutes. Upon completion of the waiting period, the solution was filtered using Whatman paper (No: 1) (Feizi et al. 2018). The filtrate obtained was placed in a balloon and stored at 4°C in the dark until it was used.

This research was conducted on a total of 20 cattle of different breeds and genders, between 6 and 20 months old, in the Ardahan region. Cattle suffer from dermatophytosis as a result of clinical examination (round, limited, gray-white lesions of different diameters on the skin and hair loss) were divided into control (n=10) and experimental groups (n=10). In the control and experimental groups, care was taken to spread the number of lesions and the diameter of the lesions evenly. Two lesions were found in each of the six animals in the control group, and one lesion was found in each of the four animals. Half of the animals in the experimental group had one lesion, while the other half had two.

Furthermore, two animals in the experimental group had macroscopically small lesions detected. Both groups had lesions in the head, neck, shoulder, and back region. Before beginning the study, body temperature, rumen movement, pulse, and respiratory counts were measured for all animals in the control and experimental groups. These values were found to be within the physiological limits. The control and experimental groups were formed from animals that had never received drug therapy.

The lesioned areas on all animals with suspected trichophytes in both the control and the experimental groups were thoroughly wiped with a cotton soaked in 70% alcohol. After the lesioned area dried, skin scrapings and hair samples were taken from the edges of the lesions with the help of sterile scalpels and pens, placed in a sterile petri dish and the cap was tightly closed. The samples were then brought to the laboratory. The collected samples were placed in the laboratory on 2 drops of 10% potassium hydroxide (KOH) dripped in the middle of a clean slide and the samples were lamellae-covered. The preparation is heated in a slight flame and left for 20-30 minutes at room temperature. The fungi's hyphae and arthrospores were examined under the microscope.

In addition to the microscopic examination, the samples were planted on Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) using the artificial stub method. The cultures were kept in an oven at 37 °C for 2-4 weeks and checked every two days for 2-4 weeks. The shape, structure, reproduction rate, and pigmentation status of the colonies were examined in the macroscopic examination of the reproduced cultures. Preparations were made from the cultures and stained with lactophenol cotton blue stain. Trichophyton verrucosum was identified by examining the status of the hyphae, chlamydiospores, arthrospores, and blastospore structures under the microscope (Arda 1997).

All the animals in the groups were fed in the same environment and ad libitum (with tap water and forage from the same source).

Prior to beginning the application, the lesions of all animals in the control and the experimental group were brushed with a medium hardness brush. After that, acetate paper was placed on the lesions and the diameter of the wound was drawn on the paper. The diameters were calculated using millimeter paper (Hizli 2020). No substances were used for the control group. The extract of MS was sprayed onto the lesions of the experimental group using an atomizer. The spraying was done to cover the area where the lesions were localized and to wet them sufficiently.

This procedure was carried out twice daily (morning and evening) and during the 15 days. Starting from the pre-application (0^{th} day) until the 36^{th} day at intervals of two days, the lesion diameters of the cattle in the control and the experimental group were measured and recorded.

Statistical Analysis

Data from this research were statistically evaluated using the IBM SPSS 20.0 computer program. The Shapiro-Wilk test checked the normal distribution status of the data in the groups. Since the test results showed a normal distribution, the independent sample t-test was used to compare parameters between groups. The results were presented as a mean and standard deviation. In the study, $p<0.05$ was found to be statistically meaningful.

RESULTS

The lesion diameters of the control and the experimental group, which started from the first day of application (day 0) and measured at intervals of two days, are presented below in Table 1.

Table 1: Descriptive statistics of lesion diameters of the cattle groups (mm)

Day	Control Group (n=10)				Experimental Group (n=10)			
	Min.	Max.	SD	Mean	Min.	Max.	SD	Mean
0	14.00	27.00	4.58	20.10	16.00	27.00	3.82	22.20
3	14.00	27.00	4.30	20.10	16.00	27.00	3.59	22.50
6	1500	27.00	1.31	20.50	16.00	27.00	3.64	22.20
9	18.00	27.00	3.53	22.40	17.00	26.00	3.07	21.10
12	18.00	27.00	3.52	22.70	14.00	24.00	3.24	19.90
15	20.00	30.00	3.17	23.90	14.00	24.00	2.82	18.80
18	20.00	30.00	2.95	24.60	10.00	22.00	3.94	16.40
21	22.00	30.00	2.58	25.70	8.00	20.00	4.50	14.90
24	22.00	30.00	2.58	26.30	6.00	20.00	4.37	13.60
27	22.00	32.00	3.16	27.00	3.00	20.00	4.55	11.90
30	24.00	32.00	2.83	27.70	0.00	18.00	4.74	10.60
33	24.00	32.00	2.83	27.70	0.00	16.00	4.64	9.00
36	25.00	35.00	3.58	29.20	0.00	14.00	5.16	6.60

In Table 1 above, the mean diameter of the lesion prior to the application (day 0) in the control group was 20.10±4.58 mm. In this group, the mean size of the lesions increased from day 6 and reached 29.20±3.58 mm on day 36. In the experimental group, the mean diameter of the lesion prior to the application (day 0) was 22.20±3.82 mm. In the experimental group, the lesion diameter began to decrease from the 6^{th} day and decreased to 6.60±5.16 mm on the 36^{th} day.

Table 2 compares the lesion diameters by day in the cattle groups. Table 2 shows that there is no statistical difference between the mean lesion diameter in the control and the experimental groups before applying ($p>0.05$). At the end of the follow-up period (36^{th} day), it was found that the mean lesion diameter in the experimental group decreased significantly ($p<0.05$) compared to the control group

Table 2: Lesion diameters of the cattle groups by the days

Days	Control Group (Mean ± SD)	Experimental Groups (Mean ± SD)	P value
Day 0	20.10±4.58 [a]	22.20±3.82 [a]	p>0.05
Day 36	29.20±3.58 [a]	6.60±5.16 [b]	p<0.01

[a,b]: Those with different letters on the same line were considered to be statistically significant in the p<0.05.

The control group lesion is presented in Figure 1, and the experimental group lesion in Figure 2 (arrows).

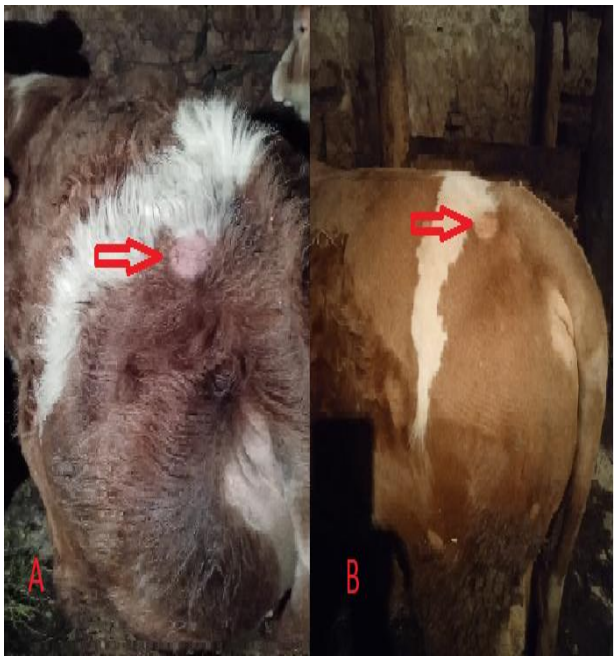

Figure 1: Appearance of lesions in the control group (**A:** Before treatment, **B:** After treatment)

Figure 1 above shows a fungal lesion on the back prior to the application (Figure 1A, arrow). In the control group, no signs of lesion improvement (reduced lesion diameter, hair growth, loss of colonies, crust, etc.) were observed in the examination performed by inspection during the follow-up period (Figure 1B).

Figure 2 below shows the fungal injuries in the experimental group. Growth and development of fungal lesions in the neck and shoulder blade area (arrows) were observed to stop at the end of the monitoring period (36th day). Moreover, although some lesions have completely disappeared, lessening was observed in some lesions (Figure 2B)

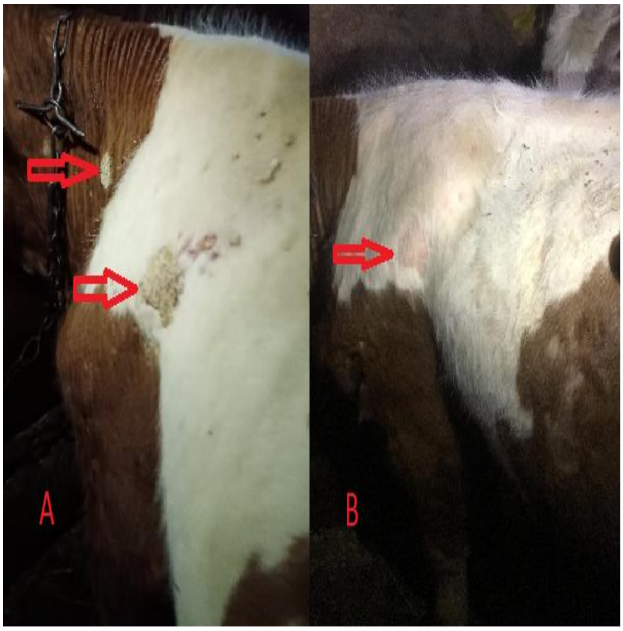

Figure 2: Appearance of lesions in the experimental group (**A:** Before treatment, **B:** After treatment)

DISCUSSION AND CONCLUSION

Ringworm disease in bovine animals is among the major diseases. The disease leads to losses of meat, milk, skin and treatment costs on farms, causing economic damage (Abdullah et al. 2021). It also threatens human health through the infection of people (Tartor et al. 2020). For this reason, the fight against disease is of great importance in terms of protecting human and animal health. Some drugs are used in the treatment of skin fungus in cattle. Griseofulvin and azoles are some of them. In the treatment of the disease, locally effective drugs are more preferred by breeders. The most important reason for this is that it is cheap and easy to apply. For this purpose, salicylic acid, benzoic acid, chlorhexidine, and iodine derivatives are most often used (Al-Farha and Mahmood 2021).

There are a lot of factors which negatively affect the fungal treatment. These include drug costs, long duration of treatment, use of low or high doses of drugs, non-use of effective drugs, antifungal resistance, drug residue in systemic treatment and various side effects (such as liver toxicity) (Hajyani et al. 2015; Jabri et al. 2017). These causes have led researchers to investigate natural, low-toxicity herbal treatment methods that can be used to treat fungi (Tartor et al. 2020).

Several studies have revealed the effects of MS. MS leaf extract has been reported to be effective against *Fusarium oxysporium*, *Rhizopus stsholonifer*, *Trichoderma sp*, *Penicillium sp* (Zohra et al. 2013), *Alternaria alternata*, *Penicillium expansum* and *Mucor piriformis* type fungi in vitro conditions (Parveen et al. 2014). Also *C. albicans*, (Cardoso et al. 2012; Alizadeh et al. 2017), *C. krusei*, and *C. tropicalis* are reported to be effective (Cardoso et al. 2012). Silver and silver chloride obtained from the leaf extract of the plant are reported to have inhibitory effects against *Bacillus subtilis*, *Pasteurella aeruginosa,* and *C. orthopsilosis* (Feizi et al. 2018). In addition to these effects, it is suggested that when administered to cancer patients before treatment with cisplatin, drug damage to the liver and kidneys is reduced, as well as anti-inflammatory and antioxidant effects (Yarijani et al. 2018).

In a study, MS is reported to increase the number of lymphocytes in mice infected with *C. albicans*, stimulating the immune system and reducing the severity of infection (Hajyani et al. 2015). In other studies, it has been observed that MS plays a protective role against oxidative stress, kidney (Saad et al. 2016), testicular and heart damage caused by Lithium carbonate applied intraperitoneal to mice (Saad et al. 2017). In addition, it is reported to be anti-inflammatory and antipsoriasis effective by reducing chronic burning edema, keratinocyte hyperproliferation and leukocyte migration on mouse ears (Prudente et al. 2017). It is reported that MS reduces paracetamol-induced liver damage (Hussain et al. 2014), kidney damage in ammonium metavanadate toxication (Marouane et al. 2011), heart muscle damage in myocardial ischemia (Zuo et al. 2017), and when applied locally on burn wound accelerates healing (Nasiri et al. 2015). It is suggested that MS stimulates the digestive tract against loperamide-induced constipation (Jabri et al. 2017), has an analgesic and anti-inflammatory effect (Seddighfar et al. 2020).

In this study, the mean diameter of the lesion prior to the application (day 0) was 20.10±4.58 mm in the control group and 22.20±3.82 mm in the experimental group. In the control and the experimental groups, the lesion diameters on day 36 was 29.20±3.58 mm and 6.60±5.16 mm, respectively (Table 1). It was observed that the size of the lesion increased over time in the control group and decreased in the experimental group. As shown in Table 2 above, the difference in the diameter of the control and treatment group lesions before application (day 0) was statistically non-significant ($p>0.05$). However, at the end of the follow-up period (day 36), the diameter of the lesion in the experimental group declined significantly ($p<0.05$) compared to the control group. In the present study, no improvement was observed in the control group at the end of the follow-up period (day 36) (Figure 1A, Figure 1B). However, in the treatment group, it was noted that while some fungal lesions were completely lost, there were signs of healing in some lesions (Figure 2B).

The results of this study are consistent with those reported by Zohra et al. (2013), Parveen et al. (2014), Alizadeh et al. (2017), Cardoso et al. (2012), and Feizi et al. (2018). This similarity may be explained by the similarity of the environmental conditions (such as temperature, humidity, oxygen, and nutrient needs) and metabolic activities required for the reproduction and development of fungal species causing dermatophytosis and other fungal species.

In this study, it was observed that MS extract applied to fungal lesions stopped the growth of lesions and stimulated healing at the end of the follow-up period (36th day). These results indicate that MS is effective against skin fungi in vivo. The results of the current study are consistent with those reported by Jabri et al. (2017), Saad et al. (2016), Saad et al. (2017), Prudente et al. (2017),

Hussain et al. (2014), Marouane et al. (2011), Zuo et al. (2017), Seddighfar et al. (2020), Nasiri et al. (2015), Hajyani et al. (2015), and Yarijani et al. (2018). In the studies conducted on plants, it is reported that the Henna plant (*Lawsonia inermis Linn*) leaves are effective against bovine skin fungus (Hizli 2020), and extract of *Aloe vera* is effective against *Trichophyton verrucosum* and *Trichophyton mentagrophytes* fungi species (Tartor et al. 2020). The results of the present study are similar to those reported by Hizli (2020) and Tartor (2020). On the basis of this similarity, it can be argued that the plants *Aloe Vera*, *Lawsonia inermisLinn,* and MS contain antifungal chemicals.

The most important chemical structure of MS is made up of mucilage, flavonoids, tannins, tocopherols, ascorbic acid, carotenoids and anthocyanin (Barros et al. 2010; Hajyani et al. 2015; Jabri et al. 2017). Flavonoids have antifungal effects by inhibiting a variety of enzymes in eukaryotic cells (Cushnie and Lamb 2005).The anti-fungal effect of the MS extract, pulverized on fungal lesions, is explained by the flavonoids contained in its structure. It may be argued that flavonoids interfere with the various enzymatic systems that *Trichophyton verrucosum* requires for reproduction and development. It is also reported that MS extract stimulates the immune system by increasing the number of lymphocytes in mice infected with C. albicans (Hajyani et al. 2015). Based on this information, MS extract is thought to accelerate recovery by stimulating the immune system in cattle with trichophytes. Also, the effect of MS on lesions may be explained by the anti-inflammatory and anti-oxidant properties of MS against fungal inflammatory reactions and oxidative damage in the skin. MS is thought to have an antioxidant effect with its vitamin C content. With this antioxidant property, it can be said that it shows its effect by protecting cell structures (such as DNA, macromolecules, and cell membrane) against oxygen groups, released as a result of inflammatory reactions. Furthermore, vitamin C supports keratin tissue and stimulates collagen synthesis. These properties are considered useful in the recovery process.

After all; the aqueous extract of MS, which was sprayed on fungal lesions in cattle diagnosed with Ringworm, had a healing effect. The use of MS extract locally is recommended to treat Trichophytosis in cattle. If the current study is supported by other studies, MS extracts can be considered as a plant-based treatment option for fungal infection.

CONFLICTS OF INTEREST

The author reports no conflicts of interest.

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The study has been approved by the Ethics Committee of Kafkas University (decision date 26.01.2023 and numbered KAU-HADYEK/2023-011) and Ministry of Agriculture and Forestry of Turkey (letter dated 20.12.2022 and numbered E-29486769-325.99-8174016).

AUTHOR CONTRIBUTIONS

Idea / Concept: ED
Supervision / Consultancy: ED
Data Collection and / or Processing: ED
Analysis and / or Interpretation: ED
Writing the Article: ED
Critical Review: ED

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Some Renal Marker Levels in Geriatric Rats

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ABSTRACT The aim of this study was to determine Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), cystatin C (Cyc-c), and interleukin-18 (IL-18) levels which are frequently used as kidney biomarkers in geriatric rats and to compare with those in young rats. For this purpose, 12 geriatric Wistar albino rats (30-36 months old) (six males and six females) and 12 (2-3 months old) (six males and six females) Wistar albino rats were used in this study. 24-hour urine samples of all groups were collected, and blood was collected for biochemical analysis under anesthesia. The mean body weight of the geriatric rats was statistically higher than the young rats ($p<0.001$). The 24-hour urine volume was also statistically higher in geriatric rats than in young ones ($p<0.01$). Serum Cyc-c ($p<0.05$), KIM-1 ($p<0.01$), glucose ($p<0.05$), total protein (TP) ($p<0.001$) and creatinine (Crea) levels in geriatric rats ($p<0.001$) were statistically higher than the young rats. However, there was no statistically significant difference in IL-18, NGAL, and urea (mg/dL) levels. In the 24-hour urine sample, although Cyc-c ($p<0.05$), Urea ($p<0.01$), Crea ($p<0.01$) and protein ($p<0.001$) levels in geriatric rats were statistically significant compared to young rats; there was no statistically significant difference in KIM-1, IL-18, and NGAL levels. As a result, in geriatric rats, the diagnosis, treatment, and prognosis must be evaluated by considering Crea, glucose, urea, TP levels, and KIM-1, Cyc-C changes. It was concluded that blood Crea, glucose, urea, TP levels as well as KIM-1, Cyc-C levels, and urinary Cyc-C levels should also be considered in future studies.

Keywords: *Biomarkers, Kidney, Rats.*

ÖZ

Geriatrik Ratlarda Bazı Böbrek Biyobelirteç Düzeyleri

Bu çalışmada ileri yaşlı ratlarda, son yıllarda böbrek belirteci olarak sıkça kullanılan neutrophil gelatinase-associated lipocalin (NGAL), kidney ınjury molecule-1(KIM-1), sistatin C (Cyc-c) ve interleukin-18 (IL-18) düzeylerinin belirlenmesi ve bu düzeylerin genç ratlardakilerle kıyaslanması hedeflenmiştir. Bu amaçla çalışmamızda 12 Adet geriatrik Wistar albino rat (30-36 aylık) (6 erkek ve 6 dişi) ile 12 adet (2-3 aylık) (6 adet erkek ve 6 adet dişi) Wistar albino rat kullanıldı. Yaşlı ratların ortalama canlı ağırlıkları genç ratlara göre istatistiksel olarak önemli derecede yüksekti ($p<0.001$). Çıkartılan 24 saatlik idrar miktarı yaşlı ratlarda gençlere göre istatistiksel olarak yüksekti ($p<0.01$). Geriatrik ratlarda Serum Cyc-c ($p<0.05$), KIM-1 ($p<0.01$), glikoz ($p<0.05$), total protein (TP) ($p<0.001$) ve kreatinin (Crea) düzeyleri ($p<0.001$) istatistiksel olarak genç ratlarınkine göre daha yüksekti. Ancak IL-18, NGAL ve Üre (mg/dL) düzeylerinde istatistiksel olarak anlamlı bir fark bulunamadı. Geriatrik ratlarda, 24 saatlik idrar örneğinde Cyc-c ($p<0.05$), üre ($p<0.01$), Crea ($p<0.01$) ve Protein ($p<0.001$) düzeyleri genç ratlara göre istatistiksel olarak anlamlı olmakla birlikte; KIM-1, IL-18 ve NGAL düzeylerinde istatistiksel olarak anlamlı fark yoktu. Sonuç olarak geriatrik ratlarda KIM-1, Cyc-C değişikliklerinin yanı sıra Crea, glucose, urea, TP düzeyleri dikkate alınarak tanı, tedavi ve prognoz değerlendirilmelidir. Ayrıca kan Crea, glukoz, üre, TP düzeylerinin yanı sıra KIM-1, Cyc-C düzeyleri ve idrar Cyc-C düzeylerinin de ileriki çalışmalarda dikkate alınması gerektiği düşünülmektedir.

Anahtar Kelimeler: *Biyobelirteçler, Böbrek, Ratlar.*

INTRODUCTION

The probability of reaching advanced age in animals also increases due to the developing technology and treatment applications. Gerontology aims to struggle with diseases that may occur with advancing age.

In humans, geriatric rat is defined at the age of 65 and above (Rosenthal and Kavic 2004; Özbek et al. 2008; Kumsar and Yılmaz 2014; Aydemir and Çetin 2019), whereas in laboratory rats, the age of 2 years and above is defined as geriatrics (Quinn 2005; Sengupta 2011; Andreollo et al. 2012). There are changes in physiological

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and biochemical values in humans compared to healthy young people in the advanced age period. With aging, organ functions decrease partially; however, it is stated that this rate of decrease differs according to both the individual and the systems (Ashworth et al. 1960; Döventaş and Döventaş 2012). Rats are significant models for humans in geriatric studies. It is reported as 16.4 rat days are equal to 1 human year between human and rat age by taking the average human lifespan of 80 years and the average laboratory rat lifespan of 3 years (Quinn 2005; Sengupta 2011; Andreollo et al. 2012).

Although the risk of disease and disability increases markedly with advancing age, early diagnosis of disability and death due to many chronic disorders helps take preventive measures (Rosenthal and Kavic 2004; Holly 2009; Kumsar and Yılmaz 2014; Aydemir and Çetin 2019).

Many organs and systems are affected by advancing age. One of them is the kidneys (Çapar and Çapar 2018). Changes in physiological and biochemical parameters are observed depending on the diseases that develop with advancing age (Gereklioğlu et al. 2007; Özbek et al. 2008; Özcan and Kapucu 2014).

Some biochemical parameters such as glomerular filtration rate (GFR) and albumin, creatinine, and urea rates are routinely evaluated in renal involvement (Başbuğan and Ağaoğlu 2018). In geriatric rats; renal 1-α hydroxylase, 1,25-(OH)2D levels, RAS – (Renin-angiotensin system); ET – (endothelin); PAF – (platelet-activating factor) – ROS (reactive oxygen species). Advanced glycation end products (AGE) (Bendayan 1998; Jerkić et al. 2001; Huang, et al. 2009). However, it has been notified that NGAL, KIM-1, Cyc-c, and IL-18 levels, which are more specific than these parameters and have been used recently, reveal renal damage early (Ichimura et al. 1998; Mori et al. 2005; Vaidya and Bonventre 2006; Faubel et al. 2007; Niemann et al. 2009; Duymaz et al. 2017; Alwan and Al-Saeed 2023; Hagiwara et al. 2023; Huang et al. 2023; Paul et al. 2023; Xing et al. 2023).

NGAL is a 25 kDa molecular weight member of the lipocalin family and is produced by inflammatory cells, adipose tissue, liver, colon, lung, and renal epithelium. The most well-known functions of NGAL are iron transport, regulation of apoptosis, infection control, structural development, and renal recovery. NGAL is a new biomarker for diagnosing very early stages of acute kidney injury (Kjeldsen et al. 1993; Yang et al. 2003; Devireddy et al. 2005, Gwira et al. 2005; Mori et al. 2005).

KIM-1 gene and protein expression, which is a type 1 cell membrane the glycoprotein cannot be detected in healthy kidneys and urine, and thus, KIM-1 mRNA is rapidly synthesized following damage. The KIM-1 protein is highly localized and produced in the apical membranes of differentiated proximal tubule cells of human and rodent kidneys after ischemia and toxic injury (Ichimura et al. 1998; Huang et al. 2023; Xing et al. 2023). High urinary KIM-1 expression has been associated with adverse clinical outcomes in patients with acute kidney injury (Liangos et al. 2007).

Interleukin-18 has been defined as a proinflammatory factor induced by interferon γ (Faubel et al. 2007). IL-18 is associated with many renal diseases (Melnikov et al. 2001; Alwan and Al-Saeed 2023). In humans, IL-18 is one of the early markers of renal tubular diseases. (Parikh et al. 2005) It has been reported that the IL-18 level is 90% specific and sensitive for diagnosing acute renal failure in humans (Melnikov et al. 2001; Faubel et al. 2007).

Another renal marker, Cyc-c, is a protease inhibitor synthesized and released from all nuclear cells (Han et al. 2002). Moreover, it is aimed to compare the change according to gender and age. Although studies related to aging in humans were found in the literature review, no similar study was found on rats.

MATERIAL AND METHODS

This study was approved by the Yuzuncu Yil University Animal Experiments Local Ethics Committee (Date: 28/01/2021; Decision number: 2021/05-17). The study was carried out at Yuzuncu Yil University Experimental Medicine Application and Research Center. In this study, 12 geriatric rats (average age of 30-36 months; 32.33 months) (6 males and six females) fed ad libitum with standard rat pellet food and ordinary tap water were used in an environment of 22-24 °C average temperature, 40-60 humidity, and reverse lighting, which was not included in any experimental study before and again, 12 Wistar albino rats (2-3 months average, 2.58 years old) (6 males and six females) fed with standard rat pellet feed and ordinary tap water ad libitum at 22-24 °C average temperature and 40-60 humidity environment, which were not included in any experimental study before, were used. Groups were formed from animals that did not show any clinical signs of disease in the animals included in the study. Feed and water consumption of the last five days and urine and stool samples of the previous 24 hours were evaluated to create a sample.

After collecting urine with a metabolic cage (Tecniplast2®), 24-hour urine samples from the animals included in the study were sacrificed with xylazine 10 mg/kg IP (2% Rompun® Bayer) and Ketamine (HCl) (10% Alfamine® Atafen) 75 mg/kg IP injectable anesthetics while the rats were under anesthesia by the high blood collection method. Serum was obtained from the obtained blood and renal damage markers NGAL (Catalog No: SG-20801, SinoGenClon Biotech), KIM-1 (Catalog No: SG-20751, SinoGenClon Biotech), Cyc-c (Catalog No: SG-720197, SinoGenClon Biotech) and IL-18 (Catalog No. SG-20281, SinoGenClon Biotech) levels were determined with species-specific ELISA kits. Furthermore, urea, creatinine, albumin (Alb), and total protein (TP) levels, which are among the routine parameters of the renal and blood glucose levels, were measured with Abbott, Architect ci16200.

Statistical Analysis

Statistical interpretation of the findings was made with the SPSS 21.0 computer package program, and the arithmetic means and standard deviations of all parameters were calculated. Kolmogorov-Smirnov normality test was performed. "One-way analysis of variance (ANOVA)" test to determine the difference between the groups, and to determine from which group the differences originate; "Duncan" test, one of the multiple comparison tests, was used. Differences at the $p<0.05$ level were considered significant.

RESULTS

The physiological activities of the young rats were normal, and their fur was shiny. Their feed and water consumption were normal, and their movements were swift and agile. While hair loss was observed in elderly rats in the geriatric category, their physiological activities were relatively slow compared to young ones.

Table 1: Live weight of rats, feed and water consumption, urine and stool amounts.

Parameters	Elderly (n:12)	Young (n:12)	p value
Live Weight (gr)	279.50±9,22	173.58±14,60	**0.001**
Water Consumption (ml/24 hours)	32.33±1.85	32.66±1,22	0.882
Feed Consumption (gr/24 hours)	15.72±1.80	17.27±0,90	0.453
Urine amount (ml/24 hours)	15.66±1.66	9.33±0,80	**0.002**
Fecal amount (gr/24 hours)	8.61±1.35	7.11±0,59	0.318

The difference between the values on the same line is statistically significant (*; p<0.05) (**; p<0.01), (***; p<0.001).

Table 2: Live weight, feed and water consumption, urine and stool amounts of rats by gender.

Parameters	Elderly Male (n:6)	Elderly Female (n:6)	Young Male (n:6)	Young Female (n:6)
Live Weight (gr)	300.67±21.19[a]	258.33±26.84[b]	179.50±13.59[c]	167.67±14.16[c]
Water Consumption (ml/24hours)	18.50±8.37 [a]	12.67±5.09 [a]	18.78±2.43 [a]	15.75±5.28 [a]
Feed Consumption (gr/24hours)	30.00±7.88 [a]	35.78±7.03 [a]	33.33±5.00 [a]	31.00±5.01 [a]
Urine amount (ml/24 hours)	13.78±2.06[a]	17.56±1.61[a]	7.11±0.48[c]	11.00±0.52[b]
Fecal amount (gr/24 hours)	11.40±5.97 [a]	5.67±3.31 [a]	8.11±2.14 [a]	5.63±2.26 [a]

Different letters on the same line are statistically significant (p<0.05).

Table 3: Serum Cyc-c, KIM-1, NGAL, IL-18, urea, creatinine (Crea), TP, Alb, and glucose levels in rats.

Parameters	Elderly (n:12)	Young (n:12)
Cyc-c (ng/mL)	41.85±4.87	35.79±7.60*
KIM-1 (pg/mL)	86.87±5.97	74.48±9.11**
NGAL (ng/mL)	0.66±0.08	0.66±0.08
IL-18 (pg/mL)	91.45±11.33	88.17±8.52
Urea (mg/dL)	46.40±8.82	45.08±3.57
Crea (mg/dL)	0.61±0.06	0.49±0.02***
TP (g/L)	67.36±4.45	58.60±1.89***
Alb (g/L)	28.27±3.13	30.30±1.05
Glucose(mg/dL)	113.38±5.73	97.50±2.52*

The difference between the values on the same line is statistically significant (*; p<0.05) (**; p<0.01), (***; p<0.001).

Table 4: Cyc-c, KIM-1, NGAL, IL-18, urea, Crea, and protein levels in urine samples of rats.

Parameters	Elderly (n:12)	Young (n:12)
Cyc-c (ng/mL)	47.02±5.68	42.49±4.11*
KIM-1(pg/mL)	83.62±7.88	85.18±8.07
NGAL (ng/mL)	0.79±0.11	0.86±0.15
IL-18 (pg/mL)	5.78±1.97	4.51±1.47
Urea (mg/dL)	3145.00±493.14	1998.10±706.99**
Crea (mg/dL)	65.82±20.41	30.05±17.00***
Protein (g/L)	50.33±15.39	27.60±10.38**

The difference between the values on the same line is statistically significant (*; p<0.05) (**; p<0.01), (***; p<0.001).

According to the results, while there was no statistical difference between the old and young rats in the consumption of Water and Feed and the amount of stool removed, statistically significant differences were found between body weight and urine volume (p<0.001 and p<0.01).

A statistically significant difference (p<0.05) was found between the mean body weights of the old and young rats according to their genders. In the comparison between 24-hour feed and water consumption, urine and stool amounts, on the other hand, there was a significant difference solely in the volume of urine.

A statistically significant difference was detected in serum Crea (p<0.001), TP (p<0.001), Cyc-c (p<0.05), KIM-1 (p<0.01), and Glucose (p<0.05) levels of old and young rats. These values were higher in the elderly than in the young.

A statistically significant difference was found between the serum Crea (p<0.001) protein (p<0.01) and Cyc-c (p<0.05) levels in the urine samples of old and young rats. These values were higher in the elderly than in the young.

DISCUSSION AND CONCLUSION

The age of 2 years and above is defined as geriatrics in laboratory rats (Quinn 2005; Sengupta 2011; Andreollo et al. 2012). There are changes in physiological and biochemical values in humans compared to healthy young people in the advanced age period. With aging, organ functions decrease partially; however, it is stated that this rate of decrease differs according to both the individual and the systems (Ashworth et al. 1960; Döventaş and Döventaş 2012).

Rats consume an average of 15-25 g (5-6 g for 100 g CA) feed and 30-45 ml (10-12 ml for 100 g CA) water per day (İde 2003). The average water and feed consumption of the rats included in this study was similar to the

researcher's statement (İde 2003) (Table 1). Basbugan and Ağaoğlu (2018) stated that male rats consume more feed and water than females in their study. However, there was a decrease in water and feed consumption compared to live weight in the older group, expressed as geriatric. It is considered that this may be due to the metabolism rate, which is affected by aging, as stated by researchers (Döventaş and Döventaş 2012).

The decrease in physiological activity that comes with aging affects feed and water consumption and the volume of urine and stool excreted. As seen in this study, the live weights of the old rats were statistically significantly higher than the young rats. This is proof that young rats are still in the developmental stage. In the comparison made according to feed and water consumption rates based on the CA determined for rats by IDE 2003, it was determined that older rats consumed less feed and water. Although there is no statistical significance between water consumption, metabolism slows down in the elderly due to aging. Nevertheless, when the urine volume excreted was examined, it was identified that the older rats produced a statistically significant volume of urine compared to the young rats (Table 2). As researchers (Döventaş and Döventaş 2012) stated, we think that this situation may develop due to the decrease in tubular uptake of water with aging.

Table 5: Serum Cyc-c, KIM-1, NGAL, IL-18, urea, Crea, TP, Alb and glucose levels in all group rats.

Parameters	Elderly Male (n:6)	Elderly Female (n:6)	Young Male (n:6)	Young Female (n:6)
Cyc-c (ng/mL)	43.28±3.20 [a]	40.65±5.69 [a]	41.70±5.71[a]	31.89±6.17 [b]
KIM-1 (pg/mL)	86.94±7.33 [a]	86.81±5.65 [a]	73.89±7.53 [b]	74.87±10.71 [b]
NGAL (ng/mL)	0.63±0.07 [a]	0.70±0.10 [a]	0.67±0.09 [a]	0.64±0.08 [a]
IL-18 (pg/mL)	95.49±13.3 [a]	88.09±9.16 [a]	90.62±11.18 [a]	86.14±5.85 [a]
Urea (mg/dL)	39.75±2.21 [a]	50.83±8.84 [b]	46.00±4.63 [a,b]	43.93±1.47 [a,b]
Crea (mg/dL)	0.64±0.06 [a]	0.58±0.54 [a]	0.47±0.16 [b]	0.51±0.02 [b]
TP (g/L)	6.46±0.43 [a]	6.96±0.32 [a]	5.90±0.24 [b]	5.82±0.13 [b]
Alb (g/L)	2.64±0.24 [a]	2.98±0.29 [b]	3.06±0.11 [b]	3.00±0.10 [b]
Glucose(mg/dL)	114.83±24.10 [a]	112.14±19.15 [a]	92.33±1.21 [b]	102.67±10.13 [a]

The difference between different letters on the same line is statistically significant ($p<0.05$).

Table 6: Cyc-c, KIM-1, NGAL, IL-18, urea, Crea, protein levels in all group rat urines.

Parameters	Elderly Male (n:6)	Elderly Female (n:6)	Young Male (n:6)	Young Female (n:6)
Cyc-c (ng/mL)	49.76±4.20 [a]	44.29±5.92 [a,b]	43.84±4.27 [a,b]	41.13±3.80 [b]
KIM-1 (pg/mL)	85.88±8.29 [a]	81.36±7.46 [a]	83.42±6.20 [a]	86.94±9.86 [a]
NGAL (ng/mL)	0.76±0.14 [a]	0.82±0.06 [a]	0.92±0.19 [a]	0.78±0.03 [a]
IL-18 (pg/mL)	6.23±2.48 [a]	5.33±1.54 [a]	5.41±1.04 [a]	3.60±1.37 [a]
Urea (mg/dL)	2899.75±435.56 [a, b]	3472.00±405.34[a]	2132.60±342.36 [b, c]	1863.60±980.9[c]
Crea (mg/dL)	55.72±18.87 [a, b]	72.56±20.00 [a]	28.32±7.64 [c]	31.48±22.93 [b, c]
Protein (g/L)	47.26±14.62 [a]	54.17±17.63 [a]	28.20±9.11 [b]	26.90±12.83 [b]

The difference between different letters on the same line is statistically significant ($p<0.05$).

Routine biochemical parameters are also affected by aging (Sorva 1992; Ham 2007). Sorva (1992) indicates that hypoalbuminemia may develop with aging. In the comparison made between serum urea, Crea, TP, Alb, and glucose levels between old rats and young rats, the amount of TP was statistically significantly higher in the elderly than in the young in this study. (Table 3). As this situation can lead to dehydration, which is one of the biggest problems of old age, as a result of the decrease in the water holding capacity and water consumption from the kidneys due to aging, it is thought that an increase in TP level in the elderly may be relative. As can be seen from the urine analysis, the urine protein ratio is statistically significantly higher in the elderly than in the young (Table 4). This result is in line with the data of Döventaş and Döventaş (2012) that there may be a decrease in TP and Alb levels and a slight increase in globulin levels due to aging. It also supports the fact that the urea, Crea, and protein ratios in the urine analysis of the elderly are statistically significantly higher than those of the youngs. The most significant factor of this determination, as stated by researchers (Ashworth et al. 1960; Döventaş and Döventaş 2012), is thought that the kidney's water-holding capacity decreases due to aging, resulting in increased urine and increased creatine level with urine, rapid urination of urea from the circulation and protein leakage of the kidney as a result of advanced aging.

Among the parameters such as Cyc-c, KIM-1, NGAL, IL-18, which have been used as kidney damage markers in recent years, KIM-1, NGAL, IL-18 are evaluated as indicators of acute inflammation. (Ichimura et al. 1998; Parikh et al. 2005; Liangos et al. 2007; Kuwabara et al. 2009; Zhang et al. 2011; Duymaz et al. 2017; Dirik et al. 2021).

It has been reported that thickening of the glomerular and tubular basement membranes with advancing age will lead to the development of hypertension and a change in the amount or quality of the glomerular filtrate (Ashworth et al. 1960).

IL-18, which is associated with many renal diseases, is one of the early markers of renal tubular disorders. IL-18 level has been reported to be 90% specific and sensitive for the diagnosis of acute renal failure in humans

(Melnikov et al. 2001; Parikh et al. 2005; Faubel et al. 2007).

In this study, no statistically significant difference was found in IL-18 levels in both serum and urine analyses of old and young rats (Table 3, Table 4, Table 5, and Table 6). It is considered that the damage in the tubules due to aging is not in the acute phase but maybe in the chronic phase and depending on reaching this period; it regresses to the same level as the young rat values.

In this study, no statistically significant difference was found in the NGAL level, a biomarker in diagnosing very early stages of acute kidney injury, both serum, and urine analysis of old and young rats (Table 3, Table 4, Table 5 and Table 6). This suggests that the damage to the kidneys due to aging is not in the acute stage or is at a chronic level.

Cystatin C is a protease inhibitor synthesized and released from all nuclear cells (Han et al. 2002). Cyc-C is filtered by the glomerulus and catabolized by proximal tubular cells due to its low molecular weight (Westhuyzen 2006; Ledoux et al. 2007). Cystatin C is not affected by the body skeletal system. It has been reported that it does not differ depending on gender and age (Grubb et al. 1992; Laterza et al. 2002; Zhang et al. 2011). In this study, these levels were 41.85±4.87 ng/mL in old rats and 35.79±7.60 ng/mL in young rats. Urine Cyc-C level was 47.02±5.68 ng/mL in the elderly and 42.49±4.11 ng/mL in young rats (Table 3, Table 4). In the light of these data, the Cyc-C level was statistically significant in both serum and urine levels ($p<0.05$) (Table 3). This situation may be an indication of changes in renal functions due to aging in rats. When evaluated in terms of gender, the level detected in young females was statistically lower than the levels of old females, old males, and young males ($p<0.05$) (Table 5 and Table 6). Although this situation does not coincide with the researchers' statement (Grubb et al. 1992; Laterza et al. 2002; Zhang et al. 2011) that Cyc-C is not affected by age and gender, the fact that there is no statistical difference between the levels of young males and older males and females supports this statement of the researchers (Grubb et al. 1992; Laterza et al. 2002; Zhang et al. 2011). We think that the reason for this difference in females may be due to individual data.

KIM-1 is a type 1 cell membrane glycoprotein. Following damage to the KIM-1 gene and protein expression, KIM-1 mRNA is rapidly synthesized. The KIM-1 protein is highly localized and produced in the apical membranes of differentiated proximal tubule cells of human and rodent kidneys after ischemia and toxic injury (Ichimura et al. 1998). Vaidya et al. (2009) reported that the level of KIM-1 could not be detected before and 6 hours after surgery, peaked at the 24th hour after surgery (4800 pg/ml) and then decreased. In this study, serum KIM-1 level was statistically higher in old rats than in young rats ($p<0.01$). No difference was detected in urine levels. The difference between the sexes was also insignificant. This is an indication that renal functions are affected in the older ones. Although the serum level is high in the older rats in the evaluation of urine analysis, it is thought that KIM-1 does not pass into the urine at a significant level. The main reason for this is that it is highly localized in the apical membranes of proximal tubule cells, as stated by Ichimura et al. (1998).

A statistically significant difference was found in Cyc-c and KIM-1 compared the renal damage markers such as serum Cyc-c, KIM-1, NGAL, IL-18 in old and young rats (Table 3). This suggests that, as researchers Ashworth et al. 1960 stated, changing physiological and metabolic effects, which reflect aging, may trigger an increase in serum Cyc-c and KIM-1 activity with a negative impact on the glomeruli. Moreover, it is considered that there may be a disruption in the catabolism of Cyc-c due to the negative changes in the functions of the tubules due to aging. It is understood that the glomerulus is negatively affected by the urine level produced by the old rats and the protein content of the urine.

In conclusion, it was determined that there are adverse effects on physiological activity and kidneys in the geriatric period. As a result of these effects, serum KIM-1, Cyc-C, creatinine, glucose, and TP levels increase. However, there is no statistically significant change in NGAL and IL-18 levels. In terms of urine analysis, it was observed that there were increases in urine Cyc-C, Crea, Protein levels in old rats. Nevertheless, no statistically significant increases in KIM-1, NGAL, and IL-18 levels. In the light of these results, it is concluded that the diagnosis, treatment, and prognosis should be evaluated by considering the serum Crea, Glucose, Urea, TP levels, KIM-1, Cyc-C levels and the changes in the urine Cyc-C, Crea, Urea and Protein levels of the patients in the geriatric period.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: YB, AFK, ENO, UÖ
Supervision / Consultancy: NY, AFK, UÖ
Data Collection and / or Processing: YB, NY, AFK, ENO, UÖ
Analysis and / or Interpretation: YB, NY, AFK, ENO, UÖ
Writing the Article: YB, ENO
Critical Review: NY, AFK, UÖ

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The Prevalence of Intestinal Parasites in Tumbler Pigeons Raised in Kırıkkale

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ABSTRACT It was aimed to investigate the prevalence of intestinal parasites in tumbler pigeons reared in Kırıkkale. One hundred and five stool samples were obtained as one sample from each cage reached. The fresh stool samples collected were taken separately in containers with caps and delivered to laboratory within approximately 2 hours. Stool samples were analyzed by Carbolfuchsin, Native-Lugol staining and Fulleborn flotation techniques. *Eimeria* spp. oocysts were sporulated in 2.5% potassium dichromate and the species were identified. Parasite species were found in 82.9% of the examined pigeon stool. In the study, *Heterakis* spp., *Ascaridia* spp., *Capillaria* spp., *Strongyle* type eggs and *Eimeria* spp., and *Cryptosporidium* spp. oocysts were detected. The number of feces infected with one parasite (32.4%) species was higher than the number of feces infected with two (22.9%), three (19.0%) and four species (8.6%). While helminth+protozoan mixed infections were detected in 40.9%, helminth parasite eggs in 13.3% and protozoan oocysts were found alone in 28.6% of the stools examined. All of the sporulated *Eimeria* oocysts were identified as *E. labbeana*. In this study, intestinal parasites were detected at a high rate in pigeons fed for hobby purposes. It has been revealed that animal owners should be informed about the issue, attention should be paid to the cleanliness and hygiene of pigeon cages, and more importance should be given to the diagnosis and treatment of intestinal parasites in these animals in order to reduce the prevalence of parasitic infections in pigeons in the region.

Keywords: *Intestine, Parasite, Prevalence, Pigeon.*

ÖZ

Kırıkkale'de Yetiştirilen Taklacı Güvercinlerde Bağırsak Parazitlerinin Yaygınlığı

Bu çalışmada Kırıkkale'de yetiştiriciliği yapılan taklacı güvercinlerde bağırsak parazitlerinin yaygınlığının araştırılması amaçlanmıştır. Ulaşılan her bir kafesten bir örnek olmak üzere 105 dışkı örneği alınmıştır. Toplanan dışkı örnekleri kapaklı kaplara ayrı ayrı alınmış ve yaklaşık 2 saat içerisinde usulüne uygun olarak laboratuvara ulaştırılmıştır. Dışkı örnekleri Karbolfuksin boyama, Native lügol boyama ve Fülleborn flotasyon tekniği ile incelenmiştir. *Eimeria* spp. ookistleri % 2.5'lik potasyum dikromat içerisinde sporlandırılarak tür teşhisine gidilmiştir. İncelenen güvercin dışkılarının % 82.9'unda parazite rastlanmıştır. Çalışmada *Heterakis* spp., *Ascaridia* spp., *Capillaria* spp. ve Strongil tip yumurtalar ile *Eimeria* spp. ve *Cryptosporidium* spp. ookistleri tespit edilmiştir. Bir tür parazitle enfekte dışkı sayısı (% 32.4), iki (% 22.9), üç (% 19.0) ve dört parazitle (% 8.6) enfekte olan dışkı sayısına göre daha yüksek orandaydı. İncelenen dışkıların % 40.9'unda helmint+protozoon miks enfeksiyonları saptanırken, % 13.3'ünde helmint yumurtaları ve % 28.6'sında protozoon ookistleri tek olarak görülmüştür. Sporlandırılan *Eimeria* spp. ookistlerinin tümü *E. labbeana* olarak teşhis edilmiştir. Bu çalışmada hobi amacıyla beslenen güvercinlerde bağırsak parazitlerine yüksek oranda rastlanmıştır. Yöredeki güvercinlerde paraziter enfeksiyonların yaygınlığını azaltmak amacıyla hayvan sahiplerinin konu hakkında bilgilendirilmesi, güvercin kafeslerinin temizlik ve hijyenine dikkat edilmesi, bu hayvanlarda bağırsak parazitlerinin teşhis ve tedavisine daha fazla önem verilmesi gerektiği ortaya konmuştur.

Anahtar Kelimeler: *Bağırsak, Güvercin, Parazit, Prevalans.*

INTRODUCTION

Pigeons involved in the Columbidae family of the Columbiformes order are used for meat production, hobby, competitions, shows and experiments (Sales and Janssens 2003; Yılmaz and Boz 2012). There are criteria for the classification of pigeons including more than 250 species based on simple differences. Pigeons are classified depending on some morphological features such as the body size, color and shape, number of feathers on the wing or tail, long sleeves, skullcap, the rose shape on the chest as well as the purpose of use and flight style (mail,

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tumbler, spinner, wader, roller, ringbeater, ornamental and singing, fleet flyer, highflier) (Yılmaz and Boz 2012). Many parasites detected in pigeons may cause poor performance, delay in development, cessation of egg production, and death in these animals (Dranzoa et al. 1999; Şenlik et al. 2005; Gül et al. 2009).

Helminths are among the important endoparasites of pigeons. The presence of *Capillaria* spp., *Ascaridia columbae*, *Heterakis* spp., *Dispharynx* spp., *Tetrameres* spp., *Syngamus* spp., *Raillietina* spp., *Cotugnia digonophora* and *Strongyle avium* has been reported in pigeons in studies conducted around the world (Şenlik et al. 2005; Gül et al. 2009; Elmajdoub and Mshiheet 2016; Mehmood et al. 2019; Ali et al. 2020; Walteros-Casas et al. 2021; Das et al. 2022; Juby et al. 2022). Helminths are primarily responsible for important clinical and subclinical infections in domestic pigeons (Ali et al. 2020).

Cryptosporidium spp. is an important protozoan parasite that causes infection in humans, mammals, and poultry. The oocysts of *Cryptosporidium* spp. were reported in a 10-day old pigeon in Turkey (Özkul and Aydın 1994). Mehmood et al. (2019) reported that they have detected *Cryptosporidium* spp. in the contents of the cloaca by 50%.

Coccidiosis is one of the most important protozoan parasites of the poultry. The presence of *Eimeria labbeana, E. columbae, E. columbarum, E. pfeifferi, E. tropicalis, E. janovyi, E. waiganiensis, E. curvata E. gourai* and *E. duculai* species in pigeons has been reported in studies conducted around the world. The presence of *E. labbeana, E. columbarum* and *E. columbae* in domestic pigeons has been reported in Turkey to date (Sarı et al. 2008; Gül et al. 2009).

A limited number of studies are conducted for detection of parasites in domestic and wild pigeons in Turkey. There was not any previous study conducted in Kırıkkale where the present study was carried out on detection of intestinal parasites of pigeons. The aim of the present study was to detect the presence and prevalence of intestinal parasites in tumbler pigeons fed for hobby and competition purposes in Kırıkkale.

MATERIAL AND METHODS

Approvals for collection of samples and the carrying out the study were obtained from Kırıkkale University Animal Experiments Local Ethics Committee (12.12.2022 dated and E-608221397-137394 numbered letter). Fecal samples of the pigeons were collected in this study by visiting the houses where tumbler pigeons were raised in Kırıkkale. One hundred and five (105) stool samples were obtained as one sample from each cage reached. A care was exercised to ensure that the samples were fresh during sample collection. The fresh stool samples collected were taken separately in plastic containers with caps and delivered to Kırıkkale University Veterinary Faculty Parasitology Department Laboratory within approximately 2 hours. Stool samples were analyzed by Carbolfuchsin staining, Native-Lugol staining and Fulleborn flotation techniques. Carbolfuchsin staining preparations were examined under a light microscope at x100 magnification; native-lugol staining preparations were examined under a light microscope at x40 magnification; and preparations prepared with Fulleborn flotation technique were examined under a light microscope at x10 magnification. The stools including the oocysts of *Eimeria* spp. detected by the Fulleborn flotation method were taken into 2.5% Potassium dichromate for sporulation of oocysts. Sporulation of these oocysts was followed by examining them daily for a week. Sporulated oocysts were identified according to their morphological features in the light of the relevant literatüre (Aboelhadid et al. 2021).

Statistical Analysis

All data were analyzed with frequency table. Infection rates are calculated as a percentage.

RESULTS

Oocysts and/or eggs belonging to at least one parasite species were found in 82.9% of the stool samples examined during the study. It was detected that stool samples positive for parasites were infected with at least one and at most four species (Table 1). A single infection was found in 32.4% of the stool samples, and a mixed infection was found in 50.5% of the samples.

Table 1: Number of parasite species detected according to stool examination.

Number of species	Number of positive samples (n)	Ratio within positives (%)	Ratio in total (%)
Single species	34	39.1	32.4
Two species	24	27.6	22.9
Three species	20	23.0	19.0
Four species	9	10.3	8.6
Total	87	100	82.9

Four different nematode eggs and oocysts belonging to two different protozoa were found in the stool samples. *Ascaridia* spp., *Heterakis* spp., *Capillaria* spp. and *Strongyle* type eggs were found among nematodes; however, the oocyst of *Eimeria* spp. and *Cryptosporidium* spp. were detected (Figure 1). Helminth+protozoan mixture, single protozoan and single helminth infections were detected, respectively in prevalence order in stool samples which were positive for parasites (Table 2).

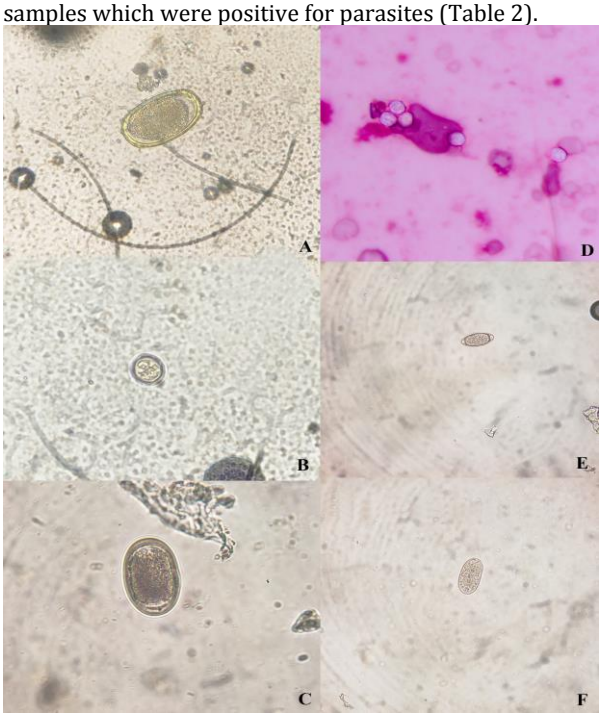

Figure 1: Parasitic eggs and oocysts detected in stool examination. A: *Ascaridia* spp. egg, B: *Eimeria* spp. oocyst; C: *Heterakis* spp. egg; D: *Cryptosporidium* spp. oocyst; E: *Capillaria* spp.; F: *Strongyle* type egg.

Table 2: Ratio of infection types detected by stool examination.

Infection type	Number of positive samples (n)	Ratio within positives (%)	Ratio in total (%)
Helminth (Single)	14	16.1	13.3
Protozoan (Single)	30	34.5	28.6
Helminth+ Protozoan (Mix)	43	49.4	40.9

The most common parasite species in stool samples examined during the study was Eimeria spp. This was followed by *Ascaridia* spp., *Capillaria* spp., *Heterakis* spp., *Cryptosporidium* spp. and *Strongyle* type eggs (Table 3).

Table 3: Parasite rates detected by stool examination in pigeon.

Parasite species	Number of positive samples(n)	Ratio within positives (%)	Ratio in total (%)
Ascaridia spp.	3	3.5	2.9
Capillaria spp.	3	3.5	2.9
Ascaridia spp.+*Capillaria* spp.	3	3.5	2.9
Eimeria spp. +*Capillaria* spp.	7	8.0	6.6
Eimeria spp.	26	29.9	24.8
Ascaridia spp. +*Heterakis* spp.	4	4.6	3.8
Eimeria spp. + *Ascaridia* spp.	7	8.0	6.6
Ascaridia spp.+ *Capillaria* spp. +*Heterakis* spp. +*Eimeria* spp.	6	6.9	5.7
Strongyle type	1	1.1	0.9
Ascaridia spp.+*Heterakis* spp. +*Eimeria* spp.	3	3.5	2.9
Ascaridia spp.+*Capillaria* spp. + *Eimeria* spp.	15	17.2	14.3
Cryptosporidium spp.,	1	1.1	0.9
Eimeria spp.+ *Cryptosporidium* spp.	3	3.5	2.9
Eimeria spp. +*Capillaria* spp. + *Cryptosporidium* spp.	2	2.2	1.9
Ascaridia spp.+*Capillaria* spp. + *Eimeria* spp.+ *Cryptosporidium* spp.	3	3.5	2.9

When the ratios of single and mixed infected feces were combined, *Ascaridia* spp. was detected by 41.9%, *Capillaria* spp. was detected by 33.3%, Eimeria spp. was detected by 68.6%, *Heterakis* spp. was detected by 12.4%, *Strongyle* type eggs was detected by 0.9, and *Cryptosporidium* spp. was detected by 8.6% (Figure 1).

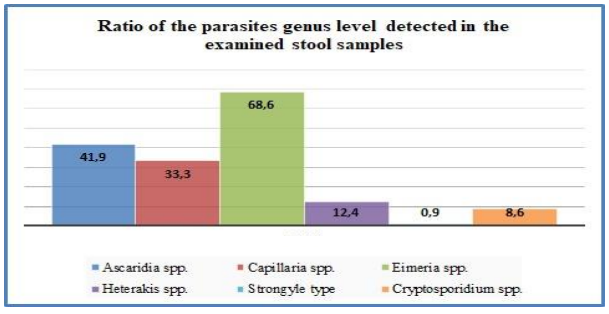

Figure 2: Ratio of the parasite's genus level detected in the examined stool samples.

All samples examined in the native-lugol staining method treated for detection of *Giardia* spp. cysts and/or trophozoites were negative for this protozoan.

DISCUSSION AND CONCLUSION

Endoparasites are infectious agents that may cause growth retardation, loss of condition and death in severe cases. Studies have been carried out to determine intestinal parasites in domestic pigeons. There is limited number of studies conducted about this subject in Turkey, and *Ascaridia* spp., *Heterakis* spp., *Capillaria* spp., *Raillietina* spp., and *Syngamus* spp. among helmiths, and *Eimeria* spp., *Isospora* spp., and *Cryptosporidium* spp. among protozoa were detected. *Eimeria* spp., and *Cryptosporidium* spp. among protozoa, and *Capillaria* spp., *Ascaridia* spp., *Heterakis* spp., and *Strongyle* type eggs among helminths were detected in this study.

Intestinal parasites were found in 82.9% of 105 pigeon feces samples examined in our study. In previous studies, this rate was between 69.16% and 87.1% in Nepal (Gurung and Subedi 2016; Adhikari et al. 2022), 81% in Indonesia (Ashfiyah et al. 2022), 84.56% in Poland (Bartosik et al. 2020); it was detected between 44.10% and 86.8% in India (Sivajothi and Sudhakara 2015; Das et al. 2022; Juby et al. 2022) and between 59.6% and 71.72% in Turkey (Sarı et al. 2008; Gül et al. 2009). It is noteworthy that intestinal parasite rates are close to each other in studies conducted in domestic pigeons around the world.

Helminth infection was found by 16.1%, protozoan infection by 34.5%, and mixed helminth+protozoan infection by 40.9% of the positive samples of this study. Sarı et al. (2008) reported in their study conducted in Niğde that 58% of domestic pigeons in which they detected parasitic agents were positive for coccidia and 42% were positive for coccidia + helminths. The mixed infection rate in stool samples analyzed in this study was 50.5%. In previous studies, Sivajothi and Sudhakara (2015) found the mixed infection rate as 31.8%, and Juby et al (2022) as 35.1% in domestic pigeons; however, Adhikari et al (2022) reported that it was 60% in pigeons fed at home, and 85.6% in those around temples. Especially the high rate of mixed parasite revealed that parasite control in pigeon breeding in Turkey and world are not done enough. This has revealed the necessity of raising the awareness of the breeders about parasitic diseases in order to reduce the presence of parasites in pigeons and to ensure that the pigeons can be reared regularly.

In studies based on fecal examination in Turkey, *Ascaridia* spp. was detected at a rate of 5.1% in Niğde (Sarı et al. 2008), and 11.03% in Van (Gül et al. 2009) in domestic pigeons. The rates revealed in the study conducted around the world were 13.58% in Poland (Bartosik et al. 2020), between 21.66% and 22.6% in Nepal (Gurung and Subedi 2016; Adhikari et al. 2022), 22% in Libya (Alkharigy et al. 2018), 42% in Indonesia (Ashfiyah et al. 2022, and between 18.60% and 33.3% in India (Sivajothi and Sudhakara 2015; Das et al. 2022). In this study, *Ascaridia* spp. was detected at a rate of 41.9%. This rate is the highest with *Ascaridia* spp. detected in domestic pigeons in Turkey which is higher than the rates reported in other countries in the world, with the exception of Indonesia (Ashfiyah et al. 2022). The cause for the higher rate in our study is considered to be due to the fact that pigeon breeders in Kırıkkale are constantly taking new pigeons to their coops in an uncontrolled manner, and

they do not perform parasite examination and anthelmintic treatment.

According to fecal examination, the rates of *Heterakis* spp. eggs detected in different provinces of Turkey were 3.7% in Niğde (Sarı et al. 2008), 6.2% in Van (Gül et al. 2009); such rates were detected by 45% in Tuban, Indonesia (Ashfiyah et al. 2022), and 2.5% in Nepal (Gurung and Subedi 2016). This rate was detected as 12.4% in the present study. *Heterakis* spp. were determined at the highest rate in this study according to the fecal examination of pigeons in Turkey. *Heterakis* spp. detected in this study is quite low compared to the rate found in Indonesia. The difference in the number of samples examined, sampling locations, the diagnostic methods used, and the farming conditions of the pigeons may be effective in the emergence of these different rates.

Capillaria spp. eggs were found in 33.3% of domestic pigeon stools examined in our study. Such rate was found as 18.62% in Van (Gül et al. 2009) and 19.9% in Niğde (Sarı et al. 2008) in other studies conducted in Turkey. The rates in the studied conducted all over the world were 32.71% in Poland (Bartosik et al. 2020), 41% in Indonesia (Ashfiyah et al. 2022), 31.67% in Nepal (Gurung and Subedi 2016), 9.30% in India; *C. obsignata* (Das et al. 2022) by 17.4%, *C. columbae* (Sivajothi and Sudhakara 2015), and *Capillaria* spp. by 19.7% (Juby et al. 2022). This rate is similar to the studies conducted around the world and in Turkey. It is considered that the differences in the results are due to the difference in the number of samples examined and the diagnostic methods used.

Adhikari et al. (2022) detected *Strongyle* spp. by 5.2%, *Strongyle avium* by 12.79% (Das et al. 2022) in India. In our study, the amount of *Strongyle* type eggs was detected as 0.9%. No other study was found in Turkey in which *Strongyle* type eggs were determined in pigeons. The results of this study revealed that *Strongyle* species of intestinal nematodes are also present in pigeons in Turkey. It was concluded that more studies should be carried out to determine the prevalence of *Strongyle* species in domestic pigeons in Turkey.

Coccidiosis is one of the most important protozoal infections of poultry. The rate of *Eimeria* spp. was detected between 59.6% and 67.58% in domestic pigeons in previous studies conducted in Turkey (Sarı et al. 2008, Gül et al. 2009). The *Eimeria* spp. rate was determined as 68.6%. This rate is similar to the rate detected in other studies conducted in Turkey. This result was higher than those detected in China by 52.8% (Dong et al. 2018), in India between 8.13% and 39.5% (Sivajothi and Sudhakara 2015; Das et al. 2022, Juby et al. 2022), in Iraq by 8.1% (Ul-Jabbar et al. 2019), in Colombia by 36% (Walteros-Casas et al. 2021) in Iran by 40.9% (Radfar et al. 2012), and lower than the rates detected in Poland as 80.86% (Bartosik et al. 2020) and Brazil by 100% (Marques et al. 2007). The high detection of *Eimeria* spp. in domestic pigeons made us think that the breeders did not pay enough attention to water and food hygiene, and that they did not comply with the quarantine conditions when new animals were taken into the poultry houses. The *E. labbeana, E. columbarum, E. columbae* and *Isospora* spp. were reported as coccidiosis strains in the studies conducted in Turkey (Sarı et al. 2008; Gül et al. 2009). All of the *Eimeria* spp. oocysts sporulated in this study were identified as *E. labbeana* according to their morphological features.

There are limited studies on the determination of *Cryptosporidium* spp. in pigeons in Turkey and the world (Sarı et al. 2008; Abreu-Acosta et al. 2009; Gül et al. 2009; Radfar et al. 2012; Li et al. 2015; Oliveira et al. 2017; Adhikari et al. 2022). The causative agent could not be detected in pigeons in Turkey based on fecal examination (Sarı et al 2008; Gül et al 2009); however, it was reported that oocysts of the agent were detected histo-pathologically in the necropsy of a 10-day old dovelet (Özkul and Aydın 1994). The rate of *Cryptosporidium* spp. was detected between 0.82% and 50% (Abreu- Acosta et al. 2009; Radfar et al. 2012; Li et al. 2015; Oliveira et al. 2017; Mehmood et al. 2019; Adhikari et al. 2022). In the stool samples examined in this study, 8.6% of *Cryptosporidium* spp. oocysts were detected. This is the first study in Turkey in which the agent was determined according to stool examination. Many reasons such as the care conditions of the pigeons, the care given to the cleanliness of the feeders and waterers, the quality of the water given to the animals may be the reason for the different results in the studies.

Consequently, this is the first study on detection of intestinal parasites in tumbler pigeons in Kırıkkale region. *Cryptosporidium* spp. was detected for the first time in Turkey by fecal examination. The highest *Ascaridia* spp., *Heterakis* spp. and *Capillaria* spp. rate has been detected in the pigeons in Turkey up to date. The higher rate of detection of intestinal parasites in pigeons fed for hobby is an indication that parasite control is not done at an adequate level. It has been revealed that animal owners should be informed about the issue, attention should be paid to the cleanliness and hygiene of pigeon cages, and more importance should be given to the diagnosis and treatment of intestinal parasites in these animals in order to reduce the prevalence of parasitic infections in pigeons in the region.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: SG, EU, BNP
Supervision / Consultancy: SG
Data Collection and / or Processing: SG, EU, BNP
Analysis and / or Interpretation: SG, EU, BNP
Writing the Article: SG
Critical Review: SG, EU, BNP

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Evaluation of Total and Lipid-Bound Sialic Acids, Trace and Macro Elements, and Some Biochemical Parameters in Dogs with Babesiosis

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ABSTRACT The present study was designed to investigate the changes at the levels of total sialic acid (TSA), lipid-bound sialic acid (LSA), trace and macroelements, and some biochemical parameters in dogs naturally infected with babesiosis. While babesiosis group consisted of seven dogs which were diagnosed with babesiosis clinically and parasitologically (ELISA), control group consisted of seven healthy dogs. Serum TSA and LSA levels in blood samples were measured spectrophotometrically by Sydow and Katapodis methods, respectively. Some biochemical parameters and macroelement measurements were performed using a modular autoanalyzer device. Trace mineral measurements were performed by ICP-MS technique. Compared to the healthy group, dogs with babesiosis had considerably higher TSA and LSA levels. Serum AST, ALP, LDH and CK enzyme activities and CRP, glucose, globulin, total bilirubin, urea, uric acid, creatinine and BUN levels of the babesiosis group significantly increased, while total protein level significantly decreased. The changes in ALT enzyme activity and triglyceride, cholesterol, HDL, LDL, HDL-CDL and ferritin levels were not statistically significant. Zinc, copper, magnesium, sodium, and potassium levels of the babesiosis group decreased significantly, while iron and chlorine levels increased significantly ($p<0.05$). Changes in calcium and phosphorus levels were not statistically significant. In conclusion, babesiosis caused significant changes in the levels of sialic acid (SA), biochemical parameters and elements in dogs.

Keywords: *Babesiosis, Biomarker, Dogs, Minerals, Sialic acids.*

ÖZ

Babesiosisli Köpeklerde Total ve Lipide Bağlı Sialik Asitler, İz ve Makro Elementler ile Bazı Biyokimyasal Parametrelerin Değerlendirilmesi

Bu çalışma doğal olarak babesiosis ile enfekte köpeklerde, total sialik asit (TSA), lipide-bağlı sialik asit (LSA), iz ve makro elementler ile bazı biyokimyasal parametrelerin seviyelerinde meydana gelen değişiklikleri araştırmak için planlandı. Klinik ve parazitolojik olarak (ELISA) babesiosis tanısı konulan 7adet köpek hasta grubunu, 7 adet sağlıklı köpekte kontrol grubuoluşturdu. Kan örneklerinde serum TSA ve LSA düzeyleri sırasıyla Sydow ve Katapodis metotları ile spektrofotometrik olarak ölçüldü. Bazı biyokimyasal parametre ve makro element ölçümleri modüler oto analizör cihazında gerçekleştirildi. İz mineral ölçümleri ICP–MS tekniği ile çalışıldı. Sağlıklı grup ile karşılaştırıldığında babesiosisli köpeklerde serum TSA ve LSA seviyelerinin önemli derecede yüksek olduğu belirlendi ($p<0.05$). Babesiosisli grupta serum AST, ALP, LDH ve CK enzim aktiviteleri ile CRP, glukoz, globulin, total bilirubin, üre, ürik asit, kreatinin ve BUN seviyelerinde önemli derecede yükselme, total protein seviyesinde ise önemli düzeyde azalma olduğu saptandı ($p<0.05$). ALT enzim aktivitesi ile trigliserit, kolesterol, HDL, LDL, TIBC ve ferritin seviyelerindeki değişikliklerin istatistiki olarak anlamlı olmadığı belirlendi ($p>0.05$). Babesiosisli grupta çinko, bakır, magnezyum, sodyum ve potasyum seviyelerinin önemli derecede azaldığı, demir ve klor seviyelerinin ise önemli derecede arttığı tespit edildi ($p<0.05$). Kalsiyum ve fosfor seviyelerindeki değişikliklerin istatistiki olarak anlamlı olmadığı belirlendi ($p>0.05$). Sonuç olarak, babesiosisin köpeklerde serum sialik asit, biyokimyasal parametreler ve elementlerin seviyelerinde önemli değişikliklere yol açtığı görüldü.

Anahtar Kelimeler: *Babeziyoz, Biyobelirteçler, Köpek, Mineraller, Sialikasidler.*

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INTRODUCTION

Canine babesiosis is a blood parasitic disease caused by various *Babesia* species (*B. vogeli, B. gibsoni, B. canis, B. vulpes and B. rossi*). The clinical symptoms of the disease include high fever, hemolytic anemia, icterus, and hemoglobinuria. The severity of these clinical symptoms varies depending on the factors related to the animal (resistance, age, breed, etc.) and the number of agents ingested (Uilenberg 2006; Crnogaj et al. 2017; Selcin and Oguz 2022). The pathogenesis of babesiosis is associated with hemolytic anemia. Babesia species increase the number of free radicals in erythrocytes and thus, cause oxidative stress. As a result of lipid peroxidation occurring in the erythrocyte membrane due to oxidative stress, erythrocytes are hemolyzed, and thus, hemolytic anemia occurs (Solano-Gallego and Baneth 2011; Crnogaj et al. 2017).

Sialic acid (SA), which is synthesized from fructose-6-phosphate in the organism, is one of the most important components of glycoconjugates (glycoprotein, glycolipid, and proteoglycan). A significant portion of SA, which is a component of membrane composition, is bound to proteins and is called as protein-bound sialic acid (PSA) while the remaining part is bound to lipid and is called as lipoprotein sialic acid (LSA).

The sum of PSA and LSA constitutes total sialic acid (TSA) (Lacin 2001). SA is also found in the last chain of several acute-phase proteins and thus, is used as an important marker of inflammatory diseases (Esmaeilnejad et al. 2020). Serum SA levels change in parallel with the damage to cells or tissues.

Important information regarding the disease's diagnosis and prognosis is provided by these alterations (Sydow et al. 1988; Taskın and Deger 2021). Along with the destruction of erythrocytes, the serum levels of SAs in the membrane structure increase (Ertekin et al. 2000). Recently, the SA level has been evaluated in the studies conducted on animals infected with hemoprotozoan parasites and it has been found that there is a close correlation between the SA level and the parasitemia rate (Esmaeilnejad et al. 2020).

Minerals are the substances necessary for the occurrence and maintenance of vitality events by participating in different structural and functional activities of the organism. Imbalances caused by abundance or deficiency of minerals cause some pathological conditions (Mert et al. 2008). Changes in the levels of mineral substances developing due to infectious diseases are also caused by decreased nutrient uptake, increased losses and impaired utilization due to the disease. Vitamin and mineral deficiencies make animals susceptible to parasitic diseases (Dede et al. 2008).

Biochemical parameters are used for diagnosis and prognosis of the disease and monitoring of treatment efficacy. With the help of biochemical parameters, it is possible to have an idea about both the physiological status of organs and tissues and the pathological conditions that occur (Turgut 2000). In hemoprotozoan diseases, significant changes occur in blood biochemical parameters as well as clinical findings in the host (Deger et al. 2005).

This study was designed to investigate the changes in the levels of TSA, LSA, trace and macroelements, and some biochemical parameters in dogs naturally infected with babesiosis.

MATERIAL AND METHODS

This study, ethics committee approval was obtained from Van Yuzuncu Yil University Animal Testing Ethics Committee on 31.01.2023 with the number 2023/03-02.

The current study was conducted on 91 stray dogs of different ages and sexes that were brought to the Animal Care and Rehabilitation Center of the Van Metropolitan Municipality from city center and surrounding districts of Van for neutering. The animals were starved the day before neutering and were given water only for the last 12 hours.

Blood samples were collected from the vena cephalica antebrachii of the dogs with at least two clinical symptoms such as high fever, icterus, weakness, increased respiration and pulse rate in 10 ml gel vacuum biochemistry tubes according to the method. The blood was centrifuged at 3000 rpm for 10 minutes and the serums were separated. The serumsobtained were screened for Babesia canis IgG antibodies using BABESIA-ELISA DOG (Afosa, Germany). Seven dogs that tested positive were included in the Babesia group. Seven dogs that were found to be healthy by clinical examination and negative by ELISA were included in the healthy group.

Biochemical Analysis

TSA analysis of the serumswas performed according to the method developed by Sydow et al. (1988). This method is based on the formation of a pink-colored complex with the Erlich reagent by SA released via hydrolysis with acid. The absorbance of the pink color forming at 525 nm is proportional to the amount of TSA. LSA analysis was performed according to the method developed by Katapodis et al. (1982). This method is based on the formation of blue-colored complexes with resorcinol reagent by sialic acids released in an acidic medium after extraction of the lipid phase. The absorbance of the blue color forming at 580 nm is proportional to the amount of LSA. The amounts of TSA and LSA were calculated by using a standard graph prepared with N-acetylneuraminic acid (NANA).

Creatine kinase (CK),alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), gamma glutamate transferase (GGT), glucose, globulin, total bilirubin, total protein, triglyceride, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total iron binding capacity (TIBC), ferritin, urea, uric acid, creatinine, blood sodium, potassium, magnesium, calcium, phosphorus, chlorine and blood urea nitrogen (BUN), measurements in the serums were performed by a modular auto analyzer (Cobas Integra 800, Roche) using a commercially available kit.

Zinc, copper, and iron measurements in the serums were performed by using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Thermo Scientific).

Statistical Analysis

SPSS (22) software was used to assess the data. Shapiro-Wilk test was used to assess whether the data were normally distributed for each parameter. Independent Samples T-test was used to determine the statistical difference between the groups. The results obtained were given as X±SD. The significance level was assessed as $p<0.05$. Sample selection was not made from the population. Seven sick dogs were included in the study. Seven healthy dogs were taken as the control group. Therefore, power analysis was not performed.

RESULTS

In the analysis of 91 serum samples collected from the stray dogs via the ELISA method, 7 (7.69%) were found to be positive for *Babesia canis* IgG antibodies. Serum TSA and LSA levels significantly increased in dogs naturally infected with babesiosis compared to the healthy group ($p<0.05$, Table 1).

Table 1: Serum total sialicacid (TSA) and lipid bound sialicacid (LSA) levels in healthy and babesiosis groups.

Parameters	Healthy group (n=7) (X ± SD)	Babesiosis group (n=7) (X±SD)	p
TSA (mg/dl)	21.79±4.09	30.08±8.27*	0.035
LSA (mg/dl)	8.44±1.28	10.17±1.06*	0.018

*p<0.05, indicates the importance between parameters on the sameline.

AST, ALP, LDH, CK and GGT enzyme activities, CRP, glucose, globulin, total bilirubin, urea, uric acid, creatinine and BUN levels significantly increased in dogs naturally infected with babesiosis compared to the healthy group ($p<0.05$).

However, the total protein level significantly decreased ($p<0.05$). The changes in ALT enzyme activity and triglyceride, cholesterol, HDL, LDL, TIBC, and ferritin levels were not statistically significant ($p>0.05$, Table 2).

Zinc, copper, magnesium, sodium, and potassium levels significantly decreased in dogs naturally infected with babesiosis compared to the healthy group, while iron and chlorine levels significantly increased ($p<0.05$).

There were no statistically significant changes in the levels of calcium or phosphorus ($p>0.05$, Table 3).

Table 2: Biochemical parameter levels of healthy and babesiosis groups.

Parameters	Healthy group (n=7) (X ± SD)	Babesiosis group (n=7) (X±SD)	p
AST (U/L)	25.71±11.05	48.28±21.76*	0.031
ALT (U/L)	29.00±9.62	32.85±8.91	0.452
ALP (U/L)	30.00±9.16	52.42±23.16*	0.045
LDH (U/L)	50.57±26.26	127.14±52.22*	0.005
CK (U/L)	9.77±5.50	32.36±12.38*	0.005
GGT (U/L)	2.18±1.25	4.34±1.32*	0.009
CRP (µg/ml)	14.14±2.79	61.57±3.20*	0.001
Glucose (mg/dl)	50.14±54.78	112.28±21.48*	0.016
Globulin (g/dl)	1.81±0.37	4.32±1.07*	0.049
Total protein (g/dl)	7.15±0.86	5.96±0.78*	0.021
Total bilirubin (mg/dl)	0.96±0.17	4.11±1.35*	0.047
Triglyceride (mg/dl)	34.85±17.33	39.14±20.59	0.924
Cholesterol (mg/dl)	219.71±88.87	170.14±52.46	0.287
HDL (mg/dl)	117.85±32.06	111.42±35.34	0.827
LDL (mg/dl)	51.00±21.80	54.00±34.80	0.270
TIBC (µg/dl)	258.40±2.17	260.28±1.71	0.051
Ferritin (µg/l)	99.70±15.71	111.91±1.40	0.785
Urea (mg/dl)	44.37±16.28	64.51±3.07*	0.045
Uricacid (mg/dl)	2.13±1.35	5.59±1.99*	0,003
Creatinine (mg/dl)	0.914±0.19	1.75±0.67*	0,001
BUN (mg/dl)	24.04±2.81	60.44±2.38*	0,005

*p<0.05, indicates the importance between parameters on the same line. Aspartate aminotransferase **(AST)**, alanine aminotransferase **(ALT)**, alkaline phosphatase **(ALP)**, lactate dehydrogenase **(LDH)**, creatine kinase **(CK)**, gamma glutamyltransferase **(GGT)**, C-reactive protein **(CRP)**, high-density lipoprotein **(HDL)**, low density lipoprotein **(LDL)**, total iron binding capacity **(TIBC)**, blood urea nitrogen **(BUN).**

Table 3: Trace and macro element levels of healthy and babesiosis groups.

Parameters	Healthy group (n=7) (X ± SD)	Babesiosis group (n=7) (X±SD)	p
Zinc (µg/g)	5.70±1.15	1.42±0.84*	0.001
Copper (µmol/L)	5.60±1.60	3.85±0.90*	0.028
Iron (µmol/L)	101.98±21.26	162.04±50.88*	0.020
Magnesium (mg/dL)	3.18±0.37	2.76±0.27*	0.032
Calcium (mg/dL)	8.18±1.37	9.33±0.59	0.063
Phosphorus (mmol/L)	1.14±0.10	1.06±0.03	0.240
Sodium (mmol/L)	148.71±3.54	135.57±1.51*	0.031
Potassium (mmol/L)	5.48±0.71	3.86±0.44*	0.044
Chlorine (mmol/L)	111.57±3.86	124.42±3.40*	0.003

*p<0.05, indicates the importance between parameters on the same line.

DISCUSSION AND CONCLUSION

Babesiosis is a protozoan blood disease caused by species of the genus Babesia. (Furlanello et al. 2005). In babesiosis, severe systemic disorders occur with the breakdown of erythrocytes. The disease causes single or multiple dysfunctions in organs such as kidneys, liver, and muscles. Many of the clinical symptoms seen in babesiosis are caused by both hemolytic anemia which occurs after hemolysis of erythrocytes and the inflammatory response (Erkılıc et al. 2019). At least two of the clinical signs, including high temperature, icterus, weakness, and faster breathing and heartbeat, were present in the dogs employed in the current investigation

SA is found on the outer surface of cell membranes in forms bound to glycoproteins and glycolipids. SA performs as a membrane receptor, participates in the control of cellular activation, and is crucial in host-pathogen interactions. SA is widely distributed in many tissues and body fluids. Therefore, SA is an important biomarker used in the evaluation of inflammatory diseases (Esmaeilnejad et al. 2020). SA levels increase significantly in metabolic, bacterial, parasitic, and viral diseases (Aytekin 2020). Previous studies reported that SA levels were significantly higher in cattle (Ertekin et al. 2000; Esmaeilnejad et al. 2020), sheep (Deger et al. 2007), and horses (Shahbazi and Hassanpour 2017) infected with different *Babesia* species compared to the control group. In a study conducted on dogs infected with *Babesia canis canis*, serum TSA levels were found to be high (Kırmızıgul et al. 2015). In the present study, serum TSA and LBSA concentrations were found to be significantly higher in the dogs with babesiosis compared to the healthy dogs. The release of SA from glycolipids or glycoproteins in the cell membrane of parasitized erythrocytes may be responsible for this increase.

Biochemical parameters are widely used to assess the clinical status of patients. Babesiosis biochemical profile changes are correlated with the degree of hypoxia and the severity of the disease (Gokce et al. 2013). In the present study, ALT, AST, ALP and GGT enzyme activities were higher in the dogs with babesiosis compared to the healthy dogs. These increases in enzyme activities are thought to be caused by the liver damage. In the studies conducted with dogs with babesiosis, ALT, AST (Furlanello et al. 2005; Yadav et al. 2011; Gonde et al. 2017; Erkılıc 2019), ALP (Furlanello et al. 2005; Crnogaj et al. 2010; Gonde et al. 2017) and GPT (Sudhakara Reddy et al. 2016) enzyme activities were found to be significantly higher than the values of the healthy dogs. However, in some studies, ALT and GGT (Crnogaj et al. 2010) and ALP, ALT, and AST (Niwetpathomwat et al. 2006) enzyme activities were found to be the same in babesiosis infections in dogs.

Skeletal muscle degeneration occurs in babesiosis (Yeruham et al. 2003). While Furlanello et al. (2005) reported that CK enzyme activity was high in the dogs infected with *Babesia*, Gokce et al. (2013) reported that CK and LDH enzyme activities were high in the dogs infected with *Babesia*. In accordance with these studies, in the current study, an increase in CK and LDH enzyme activities was found in the dogs with babesiosis. These increases might have been increased by the fact that dogs perform vigorous exercise or babesiosis causes muscle damage in dogs. Rhabdomyolysis has thus been identified as a side effect of B. canis rossi infection (Jacobson and Lobetti 1996).

In the present study, glucose levels were found to be high in dogs with babesiosis (Crnogaj et al. 2010; Yadav et al. 2011). This increase in glucose levels may be caused by increased oxidative stress and glucose mobilization. In contrast to this result, studies conducted in the dogs infected with babesiosis reported that glucose levels decreased (Keller et al. 2004; Sudhakara Reddy et al. 2016) and remained unchanged (Gonde et al. 2017).

In the present study, total protein levels significantly decreased and globulin levels significantly increased in dogs with babesiosis when compared to the healthy dogs. The decrease in the total protein level might have been caused by liver and kidney dysfunction. It is thought that it may have been formed depending on a decrease in protein synthesis due to malnutrition caused by infection. In addition, an increase in the globulin fraction in response to antigenic stimulation may be responsible for the increase in globulin level. In the studies conducted with dogs with babesiosis, it was found that total protein levels decreased (Furlanello et al. 2005; Crnogaj et al. 2010; Yadav et al. 2011; Sudhakara Reddy et al. 2016) and globulin levels did not change (Sudhakara Reddy et al. 2016; Gonde et al. 2017).

In the studies conducted in dogs infected with *Babesia canis*, it was reported that total bilirubin levels did not change (Furlanello et al. 2005; Crnogaj et al. 2010).

In contrast to the literature, total bilirubin levels significantly increased in dogs with babesiosis in the current study (Yadav et al. 2011). Extensive hemolysis of erythrocytes and liver dysfunction may be responsible for the increase in bilirubin levels.

Hemolysis of erythrocytes is a common cause of renal failure, and this causes azotemia and increased creatinine, urea and BUN in the blood plasma, which is supported bysignificantly higherlevels of urea, uric acid, creatinine, and BUN in the dogs with babesiosis when compared to healthy dogsin the current study (Sudhakara Reddy et al. 2016; Gonde et al. 2017; Erkılıc 2019). This increase might have been caused by the damage occurring in the kidneys. However, Niwetpathomwat et al. (2006) and Crnogaj et al. (2010) found that urea and creatinine levels did not change in the dogs with babesiosis.

Lipid metabolism is crucial in babesiosis due to the reason that most of the blood parasites cannot synthesize their own lipids and transfer them from host plasma (Mrljak et al. 2014). Different data are obtained on the lipid profile in *Babesia* infections in dogs. Milanović et al. (2019) found that cholesterol and HDL levels decreased significantly, while there was no significant change in triglyceride level. Eichenberger et al. (2016) detected that there was a significant decrease in triglyceride levels, while cholesterol levels did not change. Rossi et al. (2014) reported that HDL levels were significantly lower. In another study, it was found that cholesterol and triglyceride levels increased significantly in *Babesia canis* infection, HDL levels decreased, and the change in LDL levels was not significant (Mrljak et al. 2014). In the present study, changes in serum lipid profile in infected dogs were not statistically significant.

Levels of acute phase proteins (APPs) increase in the conditions such as inflammation, tissue damage, oxidative stress, malignant neoplasms, and bacterial, viral, and parasitic diseases. Thus, they are considered as important biomarkers. C-reactive protein (CRP) and ferritin levels, which are positive acute phase proteins, increase after the acute phase response (Karnezi et al. 2016). β-globulin CRP initiates the removal of pathogenic microbes or necrotic cells from the host. In the present study, increased serum CRP levelwas found to be significant in the dogs with babesiosis (Ulutas et al. 2005; Matijatko et al. 2007). This increase indicates the presence of inflammation and infection. In contrast to this result, Koster et al. (2009) reported that there was no difference between surviving and deceased dogs with babesiosisin terms of the CRP level. To the best of our knowledge, there is no study evaluating the ferritin level in babesiosis. Martinez Subiela et al. (2014) reported that ferritin level was significantly higher in the dogs with leishmaniasis when compared to healthy dogs. In contrast, Karnezi et al. (2016) found that ferritin levels did not change in the dogs with ehrlichiosis. In a study conducted in humans with babesiosis, serum ferritin levels were found to be high and it was stated that ferritin can be used as a diagnostic marker in babesiosis (Cunha et al. 2015). According to the results of the current investigation, there was no statistically significant difference in the ferritin levels between the dogs with babesiosis and the healthy dogs.

Total iron binding capacity (TIBC) is a negative acute phase protein and an indirect indicator of transferrin content. It has been reported that there is a decrease in TIBC essentially in diseases with inflammatory features (Khaki et al. 2018). In this study, it was determined that there was no statistical difference in TIBC between infected and healthy dogs (Itoh and Itoh 1992). However, Furlanello et al. (2005) found that TIBC decreased in dogs with babesiosis.

It has been emphasized that there is an indirect loss of essential body nutrients due to accelerated metabolism or consumption during the clinical course of infectious diseases (Chaudhuri et al. 2008). In previous studies, serum copper and zinc levels were found to be significantly lower in dogs with babesiosis compared to the control group (Chaudhuri et al. 2008; Teodorowski et al. 2021).

In the present study, a statistically significant decrease in serum zinc and copper levels and a statistically significant increase in iron levels were found in the dogs with babesiosis compared to the healthy dogs. Micronutrients such as copper and zinc are essential components of the antioxidant defense, which plays an important role in preventing free radical-induced damage. Copper and zinc play a role in the synthesis of various isoenzymes of SOD, the antioxidant enzyme; therefore, the decreased levels of zinc and copper in infected dogs may be caused by their increased utilization. The increase in iron levels may be caused by intravascular hemolysis of erythrocytes associated with infection. In contrast to the results of the current study, a study reported that iron levels significantly decreased in dogs with babesiosis (Chaudhuri et al. 2008).

In this study, a statistically significant decrease in phosphorus, magnesium, potassium, and sodium levels and a statistically significant increase in chlorine levels were found in the infected dogs compared to the healthy dogs. The change in calcium was not significant. Decreased serum mineral levels might have been caused by malnutrition, decreased absorption, and renal and intestinal dysfunction. Supporting the results of our study, it was found that potassium and sodium levels decreased, and chlorine levels increased with *Babesia canis* infection (Leisewitz et al. 2001; Zygner et al. 2012). In contrast to these studies, Eichenberger et al. (2016) found that potassium was higher than the reference value, while sodium, phosphate, calcium, and chlorine levels were within normal limits in dogs infected with *Babesia canis*.

The results indicated that babesiosis caused significant changes in the levels of serum sialic acid, biochemical parameters, and elements in dogs.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: UO, YD, BO
Supervision / Consultancy: UO, YD, BO
Data Collection and / or Processing: UO, BO
Analysis and / or Interpretation: UO, YD, BO
Writing the Article: YD
Critical Review: YD, BO

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Modulation of the Immune System and Hemogram Parameters by *Prunus spinosa* in Short-Term Hyperglycemia

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ABSTRACT Diabetes mellitus (DM) is one of the chronic diseases, relationship increased blood glucose level, that requires urgent global attention due to its prevalence and associated complications. DM leads to oxidative stress that plays an important role in the development of various complications in diabetes by suppressing the immune system. *Prunus spinosa* is a plant that has been used in the treatment of many diseases from past to present, thanks to its high antioxidant activity. Therefore, the present study aims to research the effect of *P. spinosa* leaf and flower mixture on immune system during the short-term diabetic condition. In the study, 56 *Wistar albino* male rats divided into 7 groups, one of which control and others six diabetic groups, were used to determine the effects of *P. spinosa* on adenosine deaminase (ADA), (xanthine oxidase) XO and myeloperoxidase (MPO) activities in the liver tissues of diabetic rats as well as on hemogram parameters. Two of these groups were given plant extract in different concentrations (25 and 50 mg/kg bw) and the results were compared with insulin, metformin and acarbose groups. The results showed that both doses administered had a modulating effect on the changing hematological parameters caused by diabetes. Treatment groups significantly decreased ADA, XO, and MPO activities compared to diabetic group. The effects of the PSE50 were found to be more effective than all other treatment. These effects of the plant in diabetesmay be due to its therapeutic immunoregulatory potential. As a result, *P. spinosa* can be a valuable resource as an adjuvant on diabetes.

Keywords: *Adenosine deaminase, Hematology, Immunity, Myeloperoxidase, Prunus spinosa, Xanthine oxidase.*

ÖZ

Kısa Süreli Hiperglisemide *Prunus spinosa* Tarafından Bağışıklık Sistemi ve Hemogram Parametrelerinin Modülasyonu

Diabetes mellitus (DM), prevalansı ve ilişkili komplikasyonları nedeniyle acil küresel dikkat gerektiren, kan glukoz düzeyi ile ilişkili kronik hastalıklardan biridir. DM, bağışıklık sistemini baskılayarak diyabette çeşitli komplikasyonların gelişiminde önemli rol oynayan oksidatif strese yol açar. *Prunus spinosa*, yüksek antioksidan aktivitesi sayesinde geçmişten günümüze birçok hastalığın tedavisinde kullanılan bir bitkidir. Bu nedenle, bu çalışma kısa süreli diyabetik durumda *P. spinosa* yaprak ve çiçek karışımının bağışıklık sistemi üzerindeki etkisini araştırmayı amaçlamaktadır. Çalışmada, diyabetik sıçanların karaciğer dokularında *P. spinosa*'nın adenozin deaminaz (ADA), ksantin oksidaz (XO) ve miyeloperoksidaz (MPO) aktiviteleri ve hemogram parametreleri üzerine etkilerini belirlemek amacıyla biri kontrol, diğerleri altı diyabetik grup olmak üzere 7 gruba ayrılan 56 Wistar albino erkek sıçan kullanıldı. Bu gruplardan ikisine farklı konsantrasyonlarda (25 ve 50 mg/kg canlı ağırlık) bitki ekstraktı verildi ve sonuçlar insülin, metformin ve akarboz grupları ile karşılaştırıldı. Sonuçlar, uygulanan her iki dozun diyabetin neden olduğu değişen hematolojik parametreler üzerinde modüle edici bir etkiye sahip olduğunu gösterdi. Tedavi grupları, diyabetik gruba kıyasla ADA, XO ve MPO aktivitelerini önemli ölçüde azalttı. PSE50'nin etkilerinin diğer tüm tedavi gruplarından daha etkili olduğu bulundu. Bitkinin diyabetteki bu etkileri, terapötik immün düzenleyici potansiyeline bağlı olabilir. Sonuç olarak *P. spinosa*, diyabet üzerinde bir adjuvan olarak değerli bir kaynak olabilir.

Anahtar Kelimeler: *Adenozin deaminaz, Bağışıklık, Hematoloji, Ksantin oksidaz, Miyeloperoksidaz, Prunus spinosa.*

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INTRODUCTION

Diabetes mellitus (DM) is a chronic endocrine disease manifested by increased blood glucose levels resulting from lack of insulin production or inefficient insulin activity (Kaikini et al. 2020). Today, the prevalence of DM is increasing severely around the world. By 2045, the world's diabetic population is expected to reach approximately 800 million, which is expected to pose serious health and economic challenges (IDF 2022). Synthetic drugs (such as acarbose, metformin, and miglitol) are generally used in the treatment of DM. Mechanisms of action of acarbose and miglitol include inhibition of the activities of carbohydrate hydrolyzing enzymes. Metformin also reduces hepatic glucose production; regulates taking and using peripheral glucose; and delays the absorption of intestinal glucose. However, patients using synthetic drugs suffer from side effects such as gastrointestinal discomfort, liver dysfunction, hypoglycemia, icterus and heart failure in humans (Dennis et al. 2019). Therefore, there is a need to investigate non-toxic plants that have strong antihyperglycemic potentials and prevent complications by strengthening the immune system. Epidemiological studies have revealed that dietary preference with plant-based foods can reduce DM complications (Sun and Miao 2020). Phenolic acids, flavonoids, anthocyanins, and proanthocyanidins in plants provide various beneficial health effects. Ascorbic acid, also known as vitamin C, is an organic acid with antioxidant properties that is involved in a series of processes occurring in living cells as one of the essential exogenous vitamins. In addition to polyphenols, ascorbic acid has been reported to reduce the risks of diabetes, atherosclerosis, cardiovascular disease, asthma and various cancer types (breast and colon cancer) thanks to its antioxidant activity (Naidu 2003). Therefore, the use of plant-based products rich in polyphenols and ascorbic acid, which can reduce DM, and its complications and contribute to the treatment, has recently received a lot of attention.

Blackthorn (*Prunus spinosa* L.) is a spiny perennial drupe belonging to the Rosaceae family, mostly grown in Europe and Western Asia. It is also known as wild plum type or sloe.It has a purple-blue-like bloom and yellow-greenish flesh. Blackthorn has a bitter taste when fresh. Therefore, it is harvested after softening by frost. The reason for this bitter taste is the high tannin content, which together with the high anthocyanin content makes these fruits rich in potential antioxidant, antibacterial and anti-inflammatory activities (Pinacho et al. 2015). Previous studies focused on the antioxidant potential of *P. spinosa* and reported that it has antidiabetic, antimicrobial and anticancer properties thanks to its antioxidant effect (Condello et al. 2019; Popovic et al. 2020). The bioactive compounds of the extract are mostly composed of phenolic acids, flavonoids and anthocyanins. Methanolic extract of *P. spinosa* flowers has been shown to be effective against glioblastoma cells by creating an antioxidant effect (Karakas et al. 2019). In addition, the polyphenols found in *P. spinosa* have antioxidant and protective propertiesagainst fibrinogen and other human plasma components (Marchelak et al. 2017). Blackthorn extracts have also been proven to be important for the release of some vital proinflammatory and anti-inflammatory factors in immune cells (Magiera et al. 2022). High levels of reactive oxygen species (ROS) and biomarkers associated with oxidative stress are observed in the blood or inflamed tissues of patients with various chronic diseases. Studies of *P. spinosa* show that it can have beneficial effects on health thanks to its high antioxidant activity (Magiera et al. 2022; Temiz and Okumus 2022). Considering these findings and the fact that *P. spinosa*isa rich source of accessible antioxidants, this study was set up to determine the effect of blackthorn flower&leaf extract on immune system markers in STZ-induced diabetic rat liver tissue.

MATERIAL AND METHODS

This study was conducted with the permission of Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee on 06.02.2020 with the number 2020/01.

Standards and Reagents

Streptozotocin (STZ), adenosine, L-ascorbic standard and sulfuric acid (H_2SO_4) were acquired from Sigma-Aldrich (Seelze).Acarbose, metformin, insulin, ketamine and xylazine were obtained from a local pharmacy. All chemicals used in the study were of analytical grade and were supplied by Merck (Darmstadt, Germany).

Plant Materials and Extraction

The leaves and flowers of *P. spinosa* used in the study were obtained from Tekirdağ city of Turkey in April 2018. The plants were quickly brought to the laboratory where were removed from foreign materials. The extract was prepared as previously described by Temiz and Okumus (2022). Briefly, flowers and leaves were lyophilized (Labconco freeze dryer 117) and then a homogeneous mixturewas formed from 50% leaves and 50%flowers. The resulting mixture was extracted at 50˚C with ethanol-water (3:1 v/v) for 3 hours using magnetic stirrer (Wisd WiseStir MSH-20D). At the end of the period, the supernatant was filtered and lyophilized. The freeze-dried *P. spinosa* extract (PSE) was kept in amber bottles under vacuum at 26°C under nitrogen atmosphere for further analysis.

L-Ascorbic acid Assay

0.1 g of the PSE was homogenized in an ice bath containing 2 mL of 4% metaphosphoric acid with a tissue homogenizer at 30.000 rpm and 30 s. The homogenate was centrifuged at 10.000 x g for 4 minutes at 4°C. Then the supernatant was filtered through a 0.45 µm PTFE filter. Measurements were performed with a HPLC system (Shimadzu LC-20 AD, Kyoto, Japan).A dC18 column (250×4.6 mm, 5 µm, Waters Atlantis) was used to determine L-ascorbic acid. The flow rate was set up as 0.7 mL/min using a mobilephase (water: H_2SO_4, pH 2.54). Detection was made at 25 °C and 244 nm. L-ascorbic acid seen in the chromatograms was defined by comparison with the retention times and standard (Lee and Coates, 1999).

Animals

This study was performed in accordance with the Guidelines for the care and use of laboratory animals issued by Van Yüzüncü Yıl University Animal Researches Local Ethics Committee and was approved by the Committee on the Ethics of Animal Experiments within that institution (decision No. 2020/01). 56 healthy male rats (Wistar albino, 200-300 g and 2-3 months of age) used in the study were obtained from Van Yüzüncü Yıl University (Turkey) Experimental Application and Research Center. Rats were keptat 22±2 °C, 50% humidity, and 12-h dark/light cycle inplastic rat cages.

Experimental Design

The rats were divided into seven groups, each consisting of eight (n=8) animals, and single dose of streptozotocin (STZ) was administered i.p. at 45 mg/kg body weight (bw). Rats with glucose levels ≥ 200 mg/dL 3 days after STZ administration were evaluated diabetic. The experimental groups formed are as follows:

Group 1: Control group (CG), 1 mL of citrate buffer was applied (i.p.).

Group 2: Diabetic group (DG), only STZ injected.

Group 3: Diabetic+PSE-25 (PSE25), 25 mg/kg (bw) PSE per day was administered by gavageto diabetic rats.

Group 4: Diabetic+PSE-50 (PSE50), 50 mg/kg (bw) PSE per day was administered by gavageto diabetic rats.

Group 5: Diabetic+insulin (Insulin) group, diabetic rats were administered 0.5 IU/kg (bw) insulin (Humulin® N Lilly, Turkey) daily (p.o.).

Group 6: Diabetic+metformin (Metformin) group, 100 mg/kg (bw) metformin (Glifor® tablets Bilim, Turkey) daily was administered to diabetic rats.

Group 7: Diabetic+acarbose (Acarbose) group, acarbose (Glucobay® tablets Bayer, Turkey) was administered to diabetic rats at a daily dose of 50 mg/kg (bw).

All groups were given food and water *ad libitum* during the 21-day experiment. Finally, the rats were anesthetized and their blood and tissue samples were collected.

Hematological parameters

Complete blood count was evaluated using an autoanalyzer (Vet-Scan HM2™ Hematology System, Abaxis, Union City, CA).

Biochemical analyses

Rat liver tissues were homogenized for 3 minutes with cold phosphate buffered saline (pH 7.4) using a homogenizer. Then, it was centrifuged at 8.570 x g for 30 min at +4 °C. The supernatants were then placed into Eppendorf tubes, and kept for further analysis at –80 °C.

Measurement of ADA Activity

ADA was measured using the method of Giusti (1974). The method is based on thegeneration of ammonia, which is directly proportional to the extinction of indophenol as a final product. The ammonia reacts with hypochlorite and phenol in an alkaline solution thereby formation of an intense blue color which is measured at 630 nm.

Measurement of XO Activity

XO was determined using the method of Prajda and Weber (1975). The XO method is based on by formation of uric acid from xanthine 37 °C. XO activity was measured at 293 nm and calculated in mmol uric acid produced per min.

Measurement of MPO Activity

MPO was analyzed by the method of Bradley et al. (1982). MPO catalyzes the conversion of hydrogen peroxide (H_2O_2) and chloride (Cl^-) to highly toxichypochlorous acid ($HOCl^-$). The produced oxygen radical (O^-) reacts with o-dianisidine dihydrochloride to form a colored compound which is measured spectrophotometrically at 460 nm.

Statistical Analyses

The findings were presented as Mean±SD. Data showed normal distribution According to result of normality test followed by Shapiro Wilk. Comparisons between of groups were conducted using the one-way ANOVA followed Tukey test. $p<0.05$ was considered to be significant. SPSS 18 statistical software package was used for statistical analyses.

RESULTS

The ascorbic acid content of *P. spinosa* used in the study was determined as 49.33 mg/100 g (not given in the table). The hematological parameters of rat treated on PSE25, PSE50 and all other groups are presented in Table 1.

Table 1: Hemogram parameters of rats.

Blood	Control	Diabetic	PSE25	PSE50	Insulin	Metformin	Acarbose
RBC (10^{12}/L)	8.9±0.5	7.8±0.3[a]	9.1±0.5[b]	9.2±0.3[b]	9.5±1.3[b]	9.1±0.5[b]	9.6±0.7[b]
Hb (g/dL)	15.6±0.4	14.3±0.5[a]	15.9±0.6[b]	16.1±0.5[b]	16.7±0.8[b]	15.6±0.9	16.5±1.2[b]
MCV (fL)	59.9±0.9	54.6±0.8[a]	59.6±1.6[b]	58.9±1.5[b]	59.3±2.0[b]	58.5±0.8[b]	58.6±1.4[b]
HCT (%)	52.7±2.0	44.0±2.9[a]	54.1±2.5[b]	54.6±2.1[b]	56.1±2.6[b]	53.2±2.6[b]	56.1±4.2[b]
MCH (pg)	17.6±0.4	17.9±10.6	17.4±0.3	17.5±0.5	17.5±0.5	17.2±0.4	17.3±0.3
MCHC (g/dL)	29.4±0.4	32.8±1.4[a]	29.0±0.6[b]	29.4±0.6[b]	29.8±0.7[b]	29.5±0.4[b]	29.4±0.6[b]
PLT (10^9/L)	272±87	720±115[a]	272±63[b]	292±53[b]	333±91[b]	301±93[b]	436±108[a,b,c,d]
MPV (fL)	7.19±0.59	8.40±0.46[a]	7.73±0.60	7.61±0.59[b]	7.89±0.16	8.04±0.38[b]	8.21±0.43[b]
WBC (10^9/L)	4.20±0.72	3.02±0.72	3.71±1.02	3.24±0.90	2.87±0.62	3.63±0.93	4.94±1.10[b,d]
LYM (%)	81.9±3.9	63.9±5.4[a]	74.2±5.2[b]	75.9±4.6[b]	65.1±7.3[a,c,d]	67.8±5.3[a]	70.9±6.7[a]
NEU (%)	15.48±2.93	29.09±5.11[a]	18.40±5.11[b]	22.88±4.26	32.68±7.57[a,c,d]	27.46±4.42[a,c]	28.43±6.72[a,c]
MO (%)	0.34±0.15	0.65±0.27	0.40±0.11	0.49±0.16	0.54±0.21	0.71±0.27[a]	0.60±0.24
EOS (%)	0.51±0.17	0.74±0.13	0.51±0.21	0.53±0.15	0.46±0.25	0.64±0.24	0.53±0.23
BAS (%)	0.23±0.09	0.48±0.23[a]	0.31±0.11	0.31±0.11	0.38±0.10	0.28±0.09[b]	0.26±0.07[b]

a: It was significantly different from control group (P<0.05). **b**: It was significantly different from Diabetic group (P<0.05). **c**: It was significantly different from PSE25 group (P<0.05). **d**: It was significantly different from PSE50 group (P<0.05). **RBC**: red blood cells; **Hb**, hemoglobin concentration; **MCV**: mean corpuscular volume; **HCT**: hematocrit; **MCH**: mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration; **PLT**: platelet; **MPV**: mean platelet volume; **WBC**: white blood cells; **LYM**: lymphocyte count; **NEU**: neutrophile; **MO**: monocytes; **EOS**: eosinophil; **BAS**: basophil.

Although the red blood cells (RBC) (P=0.039), hemoglobin (Hb) (P=0.029), mean corpuscular volume (MCV) (P=0.000), hematocrit (HCT) (P=0.000), and lymphocytes (LYM) (P=0.000) decreased markedly in diabetic group, administration of PSE25 and PSE50 importantly restored the changes in all these variables, similar to the insulin, metformin and acarbose groups. Also, mean corpuscular hemoglobin concentrated (MCHC) (P=0.000), platelet (PLT) (P=0.000), mean platelet volume (MPV) (P=0.000), neutrophile (NEU) (P=0.000) and basophil (BAS) (P=0.003) showed significant increase in diabetic rats (P<0.05).

However, PSE25 and PSE50 administration prevented further increase in these parameters near to the control range. The PLT was considerably higher in the acarbose group compared to the PSE25 (P=0.010) and PSE50 (P=0.034) groups. The LYM value was significantly lower in the insulin group compared to the control (P=0.000), PSE25 (P=0.030), and PSE50 (P=0.006) groups. However, the NEU values were higher in the insulin group compared to the control (P=0.000), PSE25 (P=0.000), and PSE50 groups (P=0.010). High lymphocyte concentration indicates a high level of immunity against pathogens. The decrease in white blood cells (WBC) and LYM may be related to the inhibition of leukocytosis from the bone marrow, which may be due to weak defense mechanism against infection.

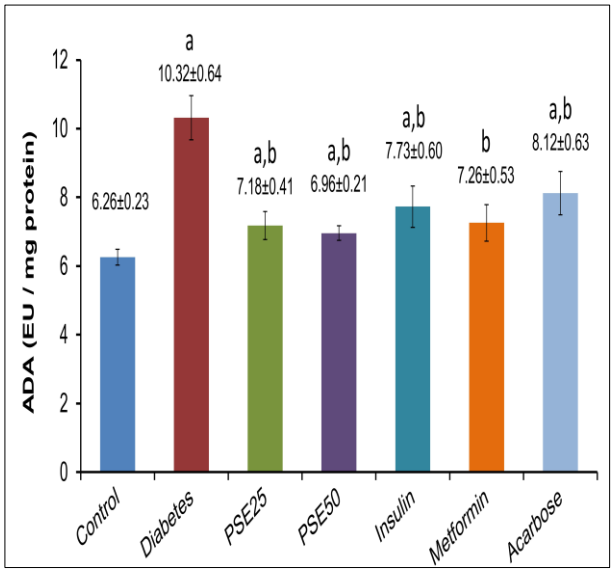

Figure 1: Liver ADA activities of rats.

a: It was significantly different from control group (P<0.05), b: It was significantly different from Diabetic group (P<0.05).

The liver ADA activities of groups are shown in Figure 1. ADA activity was highest in the diabetes group compared to all other groups. The reduction in ADA activity of the treatment groups was found to be significant compared to the control and diabetes groups (P=0.000). The ADA activity detected in the PSE50 group (6.96 EU/mg protein) was lower than the PSE25 group (7.18 EU/mg protein). Although the difference with PSE25 was not significant, the dose of 50 mg/kg PSE had a stronger effect on ADA activity. In addition, both doses (PSE25 and PSE50) showed similar activity to the insulin, metformin and acarbose groups.

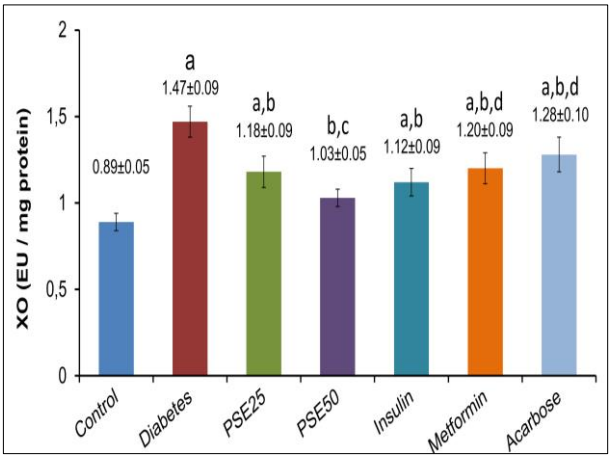

Figure 2: Liver XO activities of rats.

a: It was significantly different from control group (P<0.05), b: It was significantly different from Diabetic group (P<0.05), c: It was significantly different from PSE25 group (P<0.05), d: It was significantly different from PSE50 group (P<0.05).

In Figure 2, the XO activities of the groups are shown. The highest XO activity was found in the diabetes group (1.47 EU/mg/protein), and the lowest in the control group (0.89 EU/mg/protein). This is thought to be due to increased free radical production and oxidative stress in pathological conditions such as diabetes. The decrease in the XO activities of the groups with administered *P. spinosa* (PSE25 and PSE50) was found to be significant (P=0.000). It was determined that the PSE50 showed more XO inhibitory effect compared to the PSE25 (P=0.045). XO activities of metformin (P=0.015) and acarbose groups (P=0.000) were higher than those of the PSE50 group.

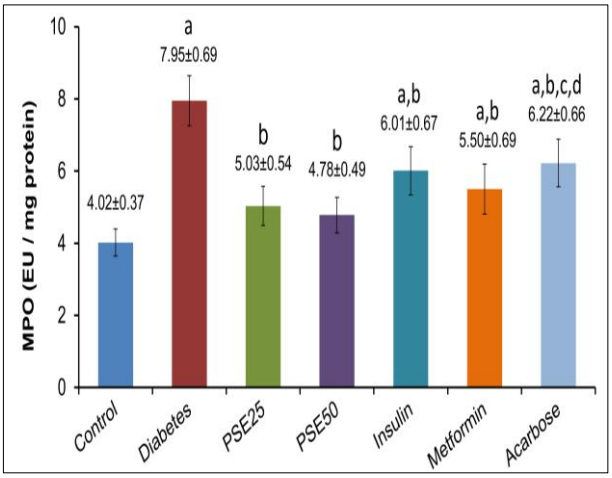

Figure 3: Liver MPO activities of rats.

a: It was significantly different from control group (P<0.05), b: It was significantly different from Diabetic group (P<0.05), c: It was significantly different from PSE25 group (P<0.05), d: It was significantly different from PSE50 group (P<0.05).

Figure 3 shows MPO activities in the liver of experimental rats. The highest MPO activity was determined in the diabetes group and the lowest in the control group. The reduction in PSE50 (P=0.000) and PSE25 (P=0.000)groups was found to be significant compared to diabetes. In the treatment methods applied, PSE50 gave a lower MPO value (4.78 EU/mg protein) compared to PSE25 (5.03 EU/mg protein) (P=0.990). The acarbose group showed higher MPO activity compared to the PSE25 (P=0.025) and PSE50 (P=0.004) groups.

DISCUSSION AND CONCLUSION

Ascorbic acid (vitamin C) is one of the exogenous vitamins and antioxidants that function in the human body. It is involved in the regulation of ROS levels and the effectiveness of other antioxidants. It is effective in stimulating the immune system by increasing the strength and protection of the organism (Bozonet and Carr 2019).In this study,the ascorbic acid result is higher than the amount determined by Kucharska and Sokol-Lętowska (2008) (20-30 mg/100 g) and by Jablonska-Rys et al. (2009) (21.94 mg/100 g). It is thought that this difference is due to the analysis method applied, the soil and climate characteristics of the plant, and the use of the fruit part in the analysis. However, Sikora et al. (2013) determined the amount of vitamin C in *P. spinosa*fruit to be 131.64 mg/100 g dw. As a result, it was determined that the vitamin C content of *P. spinosa* leaves and flowers was close or higher values than the fruit.

Blood is the main transport medium with basic physiological functions in the transport of gases (O_2 and CO_2), nutrient components, and many metabolites. It also includes various immune cells that defend in pathogenic conditions. Evaluation of hematological parameters is a very common routine in clinical trials to determine a person's health status. In this way, it allows the interpretation of the blood-related functions of the consumed substances (Yakubu et al. 2007). In diabetic rats, the RBC count was found to be significantly reduced due to non-enzymatic glycosylation of the RBC membrane protein, which is directly related to the hyperglycemic state ($P<0.05$). Because high glucose level causes to the formation of toxic products, which leads to reduced bone marrow production, low hemoglobin production by affecting the shape of erythrocytes (Singh and Shin 2009). Similarly, pancreatic damage is linked to the occurrence of diabetes. Demolition of pancreatic islet cells results in decreased insulin secretion and increased blood glucose. This increased blood glucose causes oxidative stress and can alter hematological parameters such as HCT, MCV, WBC and LYM (Baltzis et al. 2018). In this study, a decrease in RBC, HCT, MCV, WBC and LYM occurred in the diabetes group. The values found in the PSE25 and PSE50 groups were close to the control group. Similarly, *Prunus spinosa* administration significantly increased Hb, HCT, MCV, and MCH levels against tartrazine toxicity (Balta et al. 2019). Also, it can be concluded that the applied plant extract may have provoked the production of erythropoietin and increased the stem cells in the bone marrow to produce RBC. These results can be featured to the antidiabetic effect of the plant extract (Temiz et al. 2021), and its protection against damage to blood cells of diabetic rats.

In addition, vitamin C in the structure of the plant suppresses the formation of free radicals by preventing oxidative stress. In this way, the cellular antioxidant system is significantly stimulated and the destructive effect on cells is reduced (Gegotek and Skrzydlewska 2022).

Polymorphs include neutrophils, eosinophils, and basophils, and these structures play an important role in immune defense processes. Neutrophils are the main leukocytes and act as the first line of defense. The decrease of neutrophils causes a decline in functional activity, resulting in impaired immunity. In diabetic rats, a decrease of polymorphs is usually observed (Konsue et al. 2017). However, in this study, it was determined that there was an increase in the diabetes group compared to the control group. A similar situation has been found in previous studies where there was a marked increase in the percentage of neutrophils in STZ-induced diabetic rats compared to animals treated with buffer citrate (Mahmoud 2013). Contrary to the current study, Balta et al. (2019) found that *Prunus spinosa* increased PLT and LYM%, as well as decreased MO% and NEU% against tartrazine administration. This contradiction may be due to the fact that diabetes includes many complex conditions.

ADA is an enzyme of the purine degradation pathway and catalyzes the hydrolytic conversion of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine, respectively. ADA activity is increased in some infectious diseases affecting the immune system. It has been observed that the activity of this enzyme decreases after the treatment of diseases affecting immunity (Bauerle et al. 2011). Therefore, measurement of ADA enzyme activity may be clinically useful in the treatment and follow-up of some immune diseases. It was reported in many studies that ADA activity increased in diabetes (Dayani et al. 2022; Temiz 2023). It probably has a strong relationship between ADA and fasting plasma glucose, i.e., elevated ADA activity is related to reduced glucose uptake in diabetes. However, plant phenolics and flavonoids are compounds that strongly inhibit ADA activity (Egba et al. 2022). Recently, it was reported that *Prunus cerasus* may be beneficial to adenine and oteracil potassium induced hyperuricemia through reduction of ADA activity (Li et al. 2020). Durak et al. (2004) showed that some plant extracts resulted in a significant inhibition of ADA activity in diseased tissues thanks to its high phenolic components. Similar to these results, it can be said that the decrease in the ADA activity of the applied plant extract is due to the high phenolic component content of *P. spinosa* (Temiz and Okumus 2022).

XO is a complex iron-sulfur flavoprotein that catalyzes the hydroxylation of hypoxanthine to xanthine and finally to uric acid. XO is an important precursor for superoxide anion in different tissues in many disease states. It is expressed mainly in the liver and intestine. Itis also important in the pathogenesis of diabetes-associated vascular dysfunctions (Desco et al. 2002). Its inhibition is of great importance for reducing free radicals and ROS.The XO inhibitory potential of plant products used in the treatment of many diseases has been proven by many studies (Mandal et al. 2018; Bhat et al. 2019). This feature is related to the phenolic and flavonoid contents of plants, which are characterized by their antioxidant capacity (Peter and Gandhi 2017). In addition, it has been reported that ascorbate(the form of vitamin C) is effective in reducing XO activity in preventing or reducing reperfusion injuries in stimulated neutrophils (Dwenger et al. 1992). It was found that *Prunus amygdalus* treatmentsignificantly down-regulated XO activity in Fe-nitrilotriacetate (Fe-NTA) toxicity (Pandey et al. 2018). Besides, Yi et al. (2012) showed that *Prunus mume* fruitcould mediate the hypouricemic effect by inhibiting XO activity in the liver.Similar to our results, it was determined in the literature that XO activity was the highest in diabetic rats, and this activity decreased after positive developments in the treatment method (Olugbuyi et al. 2022).

MPO is a hemoprotein associated with many inflammatory events and cardiovascular diseases. The MPO is secreted from leukocytes in response to oxidative stress and plays a crucialfunction in the immune system. In blood, MPO concentrations are measured as a marker of neutrophil initiation and degranulation (Soehnlein 2009). The current results are in line with previousstudies (Aseer et al. 2015;

Olugbuyi et al. 2022) in which diabetes led to elevation of inflammatory status. Phenolic acids and flavonoids are responsible forquenching and/or scavenging the ROS produced in increasing amounts under stress, as well as giving the plants a bitter and sour taste.In this way, it plays a role in regulating the activity of many enzymes. In a previous study by Tabart et al. (2012) on anti-inflammatory capacity, it was stated that the plant extract used could scavenge the ROS produced by neutrophils and inhibit the activity of MPO. Pandey et al. (2018) has stated that *Prunus amygdalus* treatment inhibited the level of MPOas a measure of antioxidant and anti-inflammatory potential during Fe-NTA toxicity.The results obtained from our study were found to be compatible with similar studies.It is thought that the MPO inhibitory activity of *P. spinosa* is due to its phenolic content and high ascorbic acid content, as indicated in its ADA and XO activities.

As a result, *P. spinosa* extract supported to regulate hematological parameters of STZ-induced diabetic rats. In addition, PSE25 and PSE50 groups were found to be a source of ADA, XO,and MPO inhibitors close to insulin, metformin and acarbose in the liver tissues of diabetic rats. Results indicate that *P. spinosa* extract may have an effective potential in protecting the immune system in diabetes.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: MAT, EO
Supervision / Consultancy: MAT
Data Collection and / or Processing: MAT, EO
Analysis and / or Interpretation: MAT, EO
Writing the Article: MAT, EO
Critical Review: MAT, EO

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The Effect of Short-Term or Long-Term Progesterone-Based Estrus Synchronization on Fertility Parameters in Tuj Ewes outside the Breeding Season

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ABSTRACT The objective of this study was to investigate the impact of estrus synchronization on certain fertility parameters in Tuj ewes during the non-breeding season, using short-term (ST) or long-term (LT) progesterone (P4)-impregnated sponge and equine chorionic gonadotropin (eCG). Forty-eight clinically healthy Tuj ewes were selected and divided into three groups: Group I (G1, n=15), Group II (G2, n=18), and Group III (G3, n=15). G1 and G2 received hormonal treatment with a P4-impregnated sponge inserted into the vagina on day 0, and 500 IU eCG was administered intramuscularly five or ten days later, respectively. The sponges were removed from the vagina seven days after insertion for G1 and 12 days after insertion for G2. G3 did not receive any hormone treatment. All groups were exposed to the ram 24 hours after sponge removal. Estrus was monitored every six hours for five days, and pregnancy was confirmed by transrectal ultrasonography. The estrus rate, estrus onset time, and pregnancy rate were significantly different between G1 and G3 and between G2 and G3 ($p<0.05$). However, there was no difference between the treatment groups (G1 and G2) in terms of fertility parameters ($p>0.05$). In conclusion, ST or LTP4-impregnated vaginal sponge treatment was equally effective on fertility parameters in Tuj ewes during the non-breeding season.

Keywords: *Estrus synchronization, Medroxyprogesterone acetate, Pregnancy rate, Sheep.*

ÖZ

Üreme Mevsimi Dışında Tuj Koyunlarında Kısa Süreli veya Uzun Süreli Progesteron Temelli Östrus Senkronizasyonunun Fertilite Parametrelerine Etkisi

Bu çalışmanın amacı, kısa (ST) veya uzun süreli (LT) progesteron (P4)-emdirilmiş sünger ve kısrak koriyonik gonadotropin (eCG) kullanarak östrus senkronizasyonunun Tuj koyunlarında bazı fertilite parametreleri üzerindeki etkisini araştırmaktır. Klinik olarak sağlıklı 48 Tuj koyunu seçildi ve üç gruba ayrılmıştır: Grup I (G1, n=15), Grup II (G2, n=18) ve Grup III (G3, n=15). G1 ve G2, P4-emdirilmiş sünger vaginaya yerleştirilerek hormon tedavisi aldı ve sırasıyla beş veya on gün sonra 500 IU eCG intramusküler olarak uygulandı. Süngerler, G1 için yerleştirme tarihinden yedi gün sonra ve G2 için yerleştirme tarihinden 12 gün sonra vaginadan çıkarıldı. G3 herhangi bir hormon tedavisi almadı. Tüm gruplara, sünger çıkarılmasından 24 saat sonra koç katımı yapıldı. Östrus, beş gün boyunca her altı saatte bir izlendi ve gebelik transrektal ultrasonografi ile doğrulandı. Östrus oranı, östrus başlangıçzamanı ve gebelik oranı G1 ve G3 ve G2 ve G3 arasında anlamlı şekilde farklıydı ($p<0.05$). Fakat tedavi grupları arasında (G1 ve G2) arasında fertilite parametreleri açısından fark tespit edilmedi ($p>0.05$). Sonuç olarak, kısa veya uzun dönem P4-emdirilmiş vaginal sünger tedavisi, Tuj koyunlarında üreme sezonu dışında fertilite parametreleri açısından eşit derecede etkilidir.

Anahtar Kelimeler: *Gebelik oranı, Koyun, Medroksiprogesteron asetat, Östrus senkronizasyonu*

INTRODUCTION

Seasonally polyestrous sheep exhibit multiple estrous cycles that occur only during a specific season of the year, provided that pregnancy does not interrupt the cycles. In sheep, cyclic activity begins as the amount of darkness in a day increases from late summer to early autumn, and they become sexually receptive (Bartlewski et al. 2011; Abecia et al. 2012). There are various methods for exogenous progesterone (P4) administration, with the most common being the use of an intravaginal device or oral feed additives that slowly release P4. Currently, in small ruminants, medroxyprogesterone acetate (MAP), fluorogestone acetate (FGA), and intravaginal P4-releasing devices [such as controlled internal drug release (CIDR)] are widely used for both in-season and out-of-season

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estrus synchronization purposes (Kuru et al. 2018; Biehl et al. 2019; Kuru et al. 2022; Arya et al. 2023).

Progesterone and its analogues are widely used for induction and synchronization of estrus in sheep. In this method, P4-impregnated sponges or CIDRs are inserted intravaginally for 12-14 days. The sheep will exhibit estrus approximately 30-48 hours after the removal of the P4-impregnated device. To achieve higher fertility parameters, equine chorionic gonadotropin (eCG) should be administered on the day of P4 withdrawal. In recent years, short-term (ST) P4 protocols have also been developed in small ruminants. In this approach, P4-impregnated sponges or CIDR are inserted into the vagina for a period of 5-7 days, which is shorter than the half-life of the corpus luteum in the ovaries. The response to ST treatments can be similar to that of long-term (LT) treatments (Abecia et al. 2012; Kuru et al. 2018; Garoussi et al. 2020; Hameed et al. 2021; Kuru et al. 2022). However, we did not come across any studies investigating the effect of short-term P4-based estrus synchronization on fertility parameters in Tuj ewes outside the breeding season in our literature review.

The aim of this study was to investigate the effect of ST (7 days) or LT (12 days) intravaginal P4 sponge and eCG treatment on some fertility parameters in Tuj ewes during the non-breeding season.

MATERIAL AND METHODS

This study was conducted following the approval of the Local Ethics Committee for Animal Experiments of Kafkas University (KAÜ-HADYEK 2019/130, Decision date: 24.10.2019), Kars, Türkiye.

Animals

Forty-eight clinically healthy Tuj ewes aged between 2 and 4 years, weighing between 40 and 50 kg, with a body condition score (BCS) ranging from 2.5 to 3.5 (1=emaciated, 5=obese), and having completed the postpartum period without any complications were included in the study. Furthermore, eight Tuj rams (n=8) were used for mating during estrus detection.

Nutrition

Sheep were pastured in the fields during the daylight hours and then returned to the barn in the evening throughout the study period. During synchronization, the sheep were fed with dry hay and barley when they returned from the pasture. In addition to the regular feeding during pregnancy, we added 0.4 kg of concentrated feed per sheep per day, which contained 16% crude protein and 2700 kcal of metabolizable energy (Hasel Koyun Yemi®, Hasel Yem, Türkiye) (Table 1). Water and mineral licking buckets were provided *ad libitum* during the study period.

Estrus Synchronization Protocol

The estrus synchronization procedures in the study were carried out during the non-breeding season (March-May). Prior to synchronization, the sheep were divided into three groups, balanced in terms of age, BCS, and weight.

Group I (G1, n=15, Short-term P4 + eCG, 7-day group): A P4-impregnated sponge (60 mg medroxyprogesterone acetate, MAP, Esponjavet®, Hipra Animal Health, Türkiye) was inserted into the vagina on day 0. On the fifth day, 500 IU eCG (Oviser®, Hipra Animal Health, Türkiye) was injected intramuscularly. The sponges were removed from the vagina on day 7, and 24 hours later, mating with the ram was performed (Figure 1).

Group II (G2, n=18, Long-term P4 + eCG, 12-day group): A P4-impregnated sponge (60 mg, MAP) was inserted into the vagina with a special applicator on day 0. On the tenth day, 500 IU eCG was injected intramuscularly. The sponges were removed from the vagina on day 12, and 24 hours later, mating with the ram was performed (Figure 1).

Group III (G3, Control, n=15): No intervention was performed in this group, and mating with the ram was performed at the same time as the other groups (Figure 1).

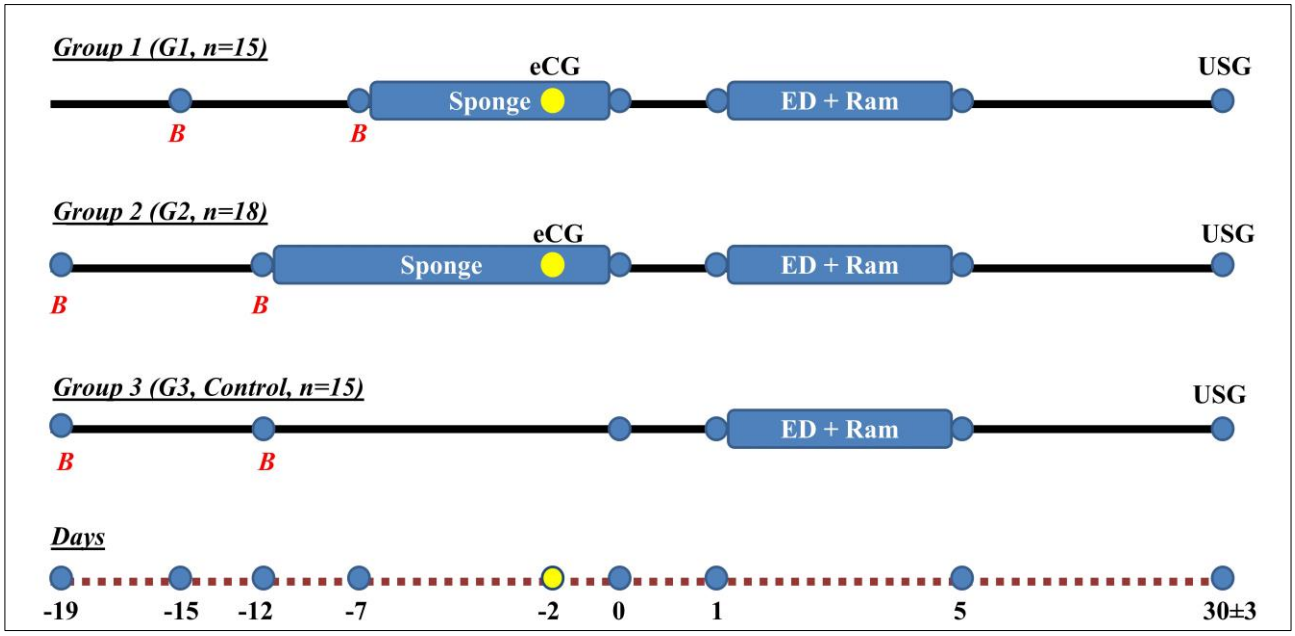

Figure 1: Estrus synchronization procedures and blood sampling days in the groups. '**eCG**: Equine chorionic gonadotropin, **USG:** Ultrasonography, **ED**: Estrus detection, **B:** Blood.

Estrus Detection and Mating

Estrus detection was initiated 24 hours after removal of the sponges in all groups. Heat was observed every 6 hours for 5 days. Ewes that allowed ram mating were accepted in estrus and recorded. Ewes that mated two or three times were separated from the group and placed in a separate area with rams.

Pregnancy Diagnosis

Pregnancy diagnosis was performed using transrectal ultrasonography (5-7.5 MHz, SonoSite Vet 180Plus®, SonoSite, USA) 30±3 days after sheep were mated. The diagnosis of pregnancy was confirmed by visualizing the embryo.

Blood Sampling and Processing

Blood samples were collected from the jugular veins of ewes 7 days prior to sponge treatment and on the day of intravaginal sponge insertion. Samples were collected using a holder (BD Vacutainer®, Becton, Dickinson and Company, USA) and a holder needle (BD Vacutainer®) into 8.5 mL vacuum tubes with gel (BD Vacutainer®). After collection, the tubes were centrifuged at 3000 rpm for 15 min using a centrifuge (NF 400R®, Nüve, Türkiye). Serum samples were then transferred to microcentrifuge tubes and stored at -18 °C until P4 concentration was determined.

Serum Progesterone Analysis

Serum P4 concentration was determined using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Bioassay Technology Laboratory, China) specifically designed for sheep. The ELISA kit was read with an ELISA plate reader (Epoch®, Biotek, USA). The standard range of the commercial kit was between 0.05 ng/mL and 15 ng/mL, with a sensitivity value of 0.027 ng/mL.

Fertility Parameters and Lamb Yield Traits

Fertility parameters, including time of onset of estrus, duration of estrus, estrus rate, pregnancy rate, conception rate, lambing rate, twinning rate, fecundity, lamb yield, and survival rate were determined. The relevant parameters and traits were calculated according to the formulas (Kuru et al. 2017; Kuru et al. 2022; Yılmaz et al. 2022). To determine the survival rate of lambs, a follow-up was conducted for 30 days after delivery.

Statistical Analysis

In the study, the mean±standard error (SE) of estrus onset time, estrus duration, and P4 concentration were provided. Estrus onset time and duration, which were determined to have a normal distribution according to the Kolmogorov-Smirnov test, were compared using one-way ANOVA and post-hoc Tukey Honestly Significant Differencetest. Two-way analysis of variance (ANOVA) was conducted to analyze the data for the measured P4 concentration in the groups, including group effect, time effect, and group × time interactions.

Tukey's multiple comparisons test was used for pairwise comparisons of group days and between-group days. For the comparison of estrus rate, pregnancy rate, conception rate, lambing rate, twinning rate, fecundity, and lamb yield, the Pearson chi-square test or Fisher's exact test was used. All analyses were performed using SPSS® (Version 26.0, SPSS Inc./IBM Group, Chicago, IL, USA) and GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). Differences between groups were considered significant at $p<0.05$ after statistical analysis.

RESULTS

Serum Progesterone Concentration

Serum P4 concentration was less than 1 ng/mL in all groups 7 days before treatment and on the day of sponge insertion (Figure 2). In one ewe from G2, serum P4 concentration was higher than 1 ng/mL. This ewe was excluded from the study as it showed cyclic activity.

Table 1: Characteristics of concentrate feed.

Nutrients	**%**
Crude protein	16
Crude cellulose	10
Crude fat	3.2
Crude ash	9
Insoluble ash in HCL	1
Molasses	5
Salt	1
Vitamins	**IU/kg**
Vitamin A	15000
Vitamin D	5000
Vitamin E	30000
Minerals	**%**
Calcium	1.6
Phosphorus	0.4
Sodium	0.4
Trace minerals	**mg**
Manganese	50
Copper	10
Iron	50
Iodine	800
Zinc	50

Fertility Parameters

Both short- and LT treatments using P4-impregnated sponges (G1 and G2) resulted in significantly higher rates of estrus induction compared to the control group (G3). The estrus rates were 73.33%, 70.59%, and 15.38% in G1, G2, and G3, respectively (p=0.001, Figure 2).

Estrus onset time was also significantly different between the groups, with 36.5±2.76 hours in G1, 32.9±1.36 hours in G2, and 55.0±5.0 hours in G3 (p=0.002). Estrus onset time was statistically different between G1 and G3 (p=0.006), and between G2 and G3 (p=0.001). However, estrus duration did not differ statistically between the groups (p=0.991, Figure 2).

Outside the breeding season, P4-impregnated sponge treatment (G1 and G2) increased the pregnancy rate compared to the control group (G3), with pregnancy rates of 46.67% in G1, 58.82% in G2, and 6.67% in G3 (p=0.007). ST or LTP4-based estrus synchronization did not have a statistically significant effect on pregnancy rate (p=0.492). There was a statistically significant difference in pregnancy rate between G1 and G3 (p=0.035) and between G2 and G3 (p=0.002, Figure 2).

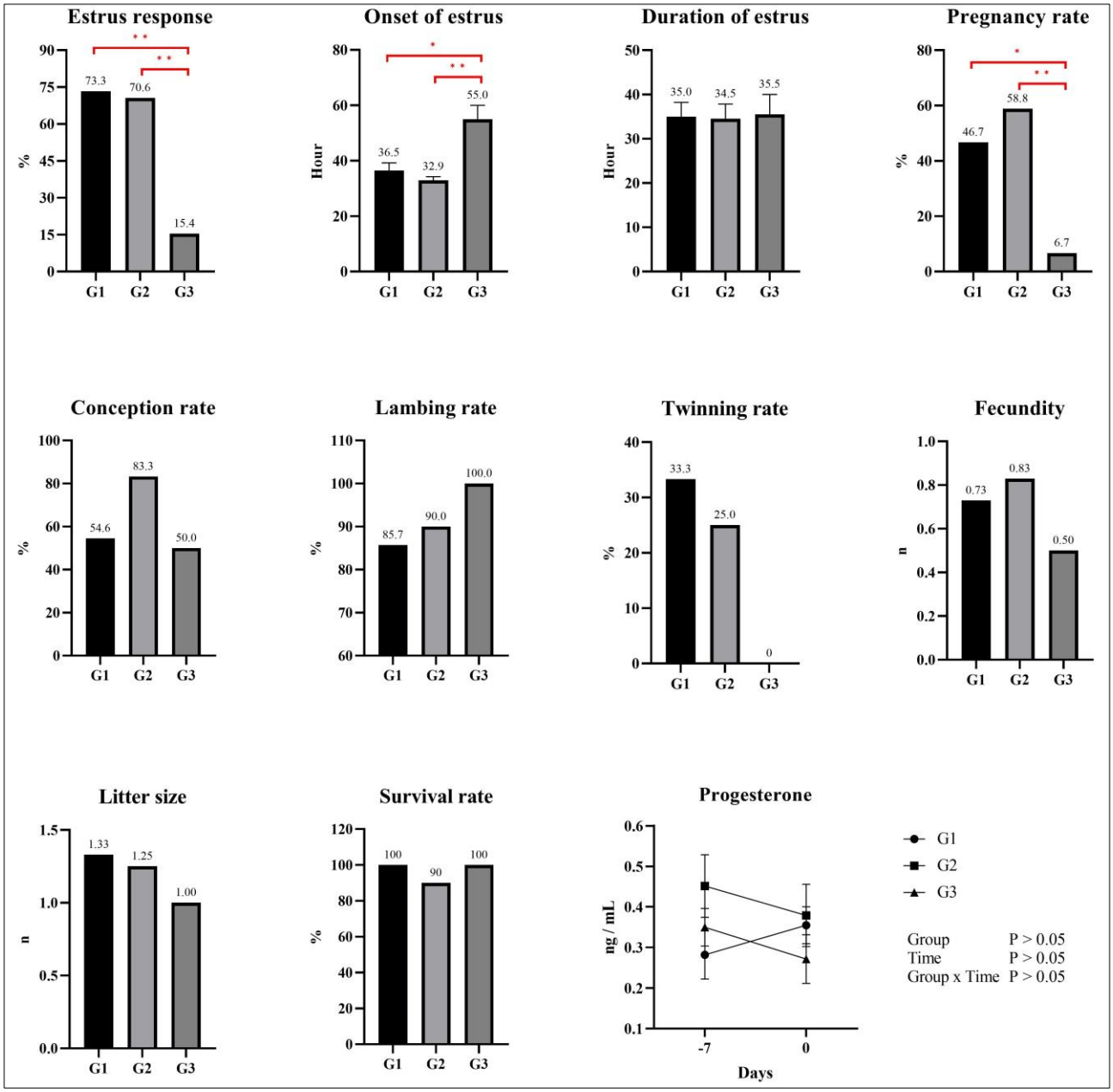

Figure 2: Changes in fertility parameters among groups.

Serum progesterone concentration in the groups before 7 days of treatment and on the day of sponge insertion. According to Pearson's chi-squared test, there was a statistically significant difference in estrus response between G1 vs G3 and G2 vs G3 (p=0.001). Short- or long-term progesterone-impregnated sponge treatments did not have a significant effect on estrus rates. Post-hoc Tukey HSD test revealed a statistically significant difference in estrus onset time between G1 vs G3 and G2 vs G3. Pearson's chi-squared test showed a statistically significant difference in pregnancy rate between G2 vs G3 (p=0.002). Fisher's exact test demonstrated a statistically significant difference in estrus rates between G1 vs G3 (p=0.035). G1 refers to the group receiving short-term (7-day) progesterone and equine chorionic gonadotropin (eCG) treatment, G2 refers to the group receiving long-term (12-day) progesterone and eCG treatment, and G3 refers to the control group. The asterisks indicate statistical significance between groups, *: p<0.05 and **: p<0.001.

Long-term (12 days) P4 treatment had a positive effect on conception rate, but there was no statistically significant difference in conception rate between the groups (p=0.285). Lambing rates did not differ statistically between the groups (Figure 2) (p=0.901).

STP4 treatment had a positive effect on twinning rate, but there was no significant difference in twinning rate between the groups (p=0.788). LTP4 treatment appeared to increase fecundity numerically, but there was no statistically significant difference between the groups (p=0.917, Figure 2).

Progesterone and eCG treatment had a positive effect on twinning rate, which in turn had a positive impact on lamb yield. Litter size in G1, G2, and G3 was 1.33, 1.25, and 1, respectively (p=0.981). Lamb survival did not differ statistically between the groups (p=0.622, Figure 2).

Raw materials of concentrate feed include barley, corn, wheat bran, sunflower seed meal, cottonseed meal, molasses, minerals, salt, calcium carbonate, and vitamins.

DISCUSSION AND CONCLUSION

The Tuj ewes are one of the local breeds of Türkiye, which is raised in the Northeastern Anatolia region. Particularly, the Tuj ewes raised in Kars and its surrounding areas is categorized as a breed with a fatty thigh among sheep breeds. This breed, which has delicious meat, has adapted to the harsh and tough climatic conditions and geography of its region (Aksoy et al. 2001). Despite the adverse

environmental conditions, various synchronization protocols have been applied to stimulate estrus both during and outside the breeding season in Tuj ewes (Ozturkler et al. 2003; Gungor et al. 2007; Kaçar et al. 2008; Kaya et al. 2013; Kamiloğlu et al. 2017). However, literature research has not found any studies on estrus synchronization treatment using ST P4-containing intravaginal sponges in Tuj ewes outside the breeding season.

This study aims to determine the effect of short (7-day) or long (12-day) intravaginal P4-impregnated sponges with eCG on some fertility parameters in Tuj ewes outside the breeding season.

Different synchronization protocols using P4 or its analogues for 12 or 14 days in Tuj ewes can result in estrus rates varying from 50-100% outside the breeding season (Gungor et al. 2007; Kaçar et al. 2008; Kaya et al. 2013). The study revealed estrus rates of 73.33%, 70.59%, and 15.38% in G1, G2, and G3, respectively (p=0.001). Moreover, no statistically significant difference in estrous response was observed between the ST and LT groups. The obtained findings align with the previously reported research (Gungor et al. 2007; Kaçar et al. 2008; Kaya et al. 2013) data pertaining to Tuj ewes, as described above, with the exception of the control group.

In our study, the induction of estrus with P4-impregnated sponges and eCG outside the breeding season was quite remarkable. The results of this study indicate that the use of a ST (7-day) P4-impregnated sponge treatment in Tuj ewes outside the breeding season yielded a similar estrus response to the LT (12-day) treatment, suggesting the efficacy of this protocol in Tuj ewes. As a high estrus response is crucial in affecting other fertility parameters, this finding is of particular importance. Given the notably low estrus response in the control group, the use of P4+eCG treatment may be necessary to achieve a satisfactory estrus response in Tuj ewes during a non-breeding season.

Progestogens are commonly used in small ruminants for estrus synchronization outside the breeding season. Injection of eCG one or two days prior to sponge removal increases fertility (Kuru et al. 2018). Following sponge removal, the onset of estrus in Tuj ewes during studies conducted outside the breeding season occurs within a range of 34-59 hours (Gungor et al. 2007; Kaçar et al. 2008; Kaya et al. 2013). Kuru et al. (2017) and Kuru et al. (2020) reported that in the local breed of sheep known as Pirlak, estrus onset occurred within 34-45 hours or 31-38 hours following sponge removal. However, ST P4 treatment may lead to a delayed onset of estrus compared to LT treatment (Özyurtlu et al. 2011; Texeira et al. 2016; Kuru et al. 2022), although some studies have reported no statistical difference between the two protocols (Ataman et al. 2006; Amer and Hazzaa 2009). In our study, the group that received ST P4 exhibited a numerically later onset of estrus compared to the LT group. Furthermore, combined P4 and eCG treatment resulted in a more synchronized onset of estrus compared to the control group.In our study, the onset of estrus differed significantly between the groups, with 36.5±2.76 hours in G1, 32.9±1.36 hours in G2, and 55.0±5.0 hours in G3 (p=0.002). Furthermore, there was no statistically significant difference in the onset of estrus between our ST and LT protocols. According to Özyurtlu et al. (2011), the estrus onset occurred around 53 and 41.5 hours following the ST and LT P4 treatments, respectively. Similarly, Texeira et al. (2016) reported that the estrus initiation happened at 46 and 32 hours for the respective protocols. In contrast, Kuru et al. (2022) observed estrus starting at 40.5 and 32.9 hours after the ST and LT treatments, while Ataman et al. (2006) and Amer and Hazzaa (2009) determined the onset of estrus at 46.3 and 45.6 hours, and 37.4 and 32.9 hours, respectively. These findings indicate conflicting results among different studies, likely attributed to variations in protocols used and the animal populations under investigation. Moreover, the discrepancies in previous studies might also be influenced by the differences in progestin (P4) treatment durations and dosages employed.

The duration of estrus in Tuj ewes after synchronization outside the breeding season can vary between 23 to 42 hours (Gungor et al. 2007) and 32 to 36 hours (Kaya et al. 2019). In sheep, there may be no difference in estrus duration after ST or LT P4 treatment outside the breeding season (Özyurtlu et al. 2011; Texeira et al. 2016), or LT P4 treatment may lead to a shorter estrus duration (Sareminejad et al. 2014). In the present study, we observed results (35-35.5 hours) that align with the previously reported duration of estrus in Tuj ewes from earlier investigations (Gungor et al. 2007; Kaya et al. 2019). Furthermore, our examination revealed that the administration of both ST and LT P4 protocols did not exert any significant influence on the duration of estrus.

Currently, the aim of many estrus synchronization protocols is to increase fertility rates and subsequently improve pregnancy rates. This not only results in economic gains but also allows for the most efficient use of breeding stock. Previous studies have shown that LT (9-14 days) P4 treatment outside the breeding season resulted in pregnancy rates of 46.6% (Kaya et al. 2013),46.7% (Kaya et al. 2019), and 50% (Kaçar et al. 2008) in Tuj ewes. ST P4 treatment in ewes outside the breeding season can either be advantageous (Sareminejad et al. 2014), disadvantageous (Silva et al. 2021), or similar (Özyurtlu et al. 2011; Texeira et al. 2016; Kuru et al. 2022) in terms of pregnancy rates compared to LT treatment. In our investigation, the pregnancy rates were recorded as 46.67% in G1, 58.82% in G2, and 6.67% in G3. Moreover, we observed that the pregnancy rate among Tuj sheep synchronized with P4 outside the breeding season in our study demonstrated a noteworthy similarity with the rates reported in the existing literature (Kaçar et al. 2008; Kaya et al. 2013; Kaya et al. 2019).Additionally, there were no statistically significant differences in both pregnancy and conception rates between the groups receiving short or LT P4 treatment. However, the pregnancy and conception rates in G2 were numerically higher, which was an interesting finding. Although statistically similar pregnancy rates were observed with both ST and LT P4-impregnated sponge treatment, a short protocol may be preferred in Tuj ewes to alleviate the stress caused by intravaginally inserted devices (Kuru et al. 2015; Kuru et al. 2018).

There is currently no study on the effect of ST P4 treatment on lambing rate in Tuj ewes outside the breeding season, but the use of LT P4-impregnated sponges has resulted in lambing rates ranging from 46.6% to 100% (Kaçar et al. 2008; Kaya et al. 2013; Kaya et al. 2019). In addition, previous studies on ewes have shown that ST or LT P4 treatment did not have a significant effect on lambing rate (Ataman et al. 2006; Amer and Hazzaa 2009; Özyurtlu et al. 2011). In our study, the lambing rate was 85.7% and 90% after ST or LT P4 treatment, respectively, and consistent with the literature (Ataman et al. 2006; Amer and Hazzaa 2009; Özyurtlu et al. 2011), the

duration of P4 treatment did not have a significant effect on lambing rate in Tuj ewes.

Tuj ewes have a low rate of twinning and rarely give birth to triplets both during and outside the breeding season. Many studies have shown that the genetic capacity of Tuj ewes is not suitable for triplets or higher order multiple births (Aksoy et al. 2001; Öztürkler et al. 2003; Kaçar et al. 2008; Kaya et al. 2013; Kamiloğlu et al. 2017). In our study, the twinning rate was 33.3% and 25% after ST or LT P4 treatment, respectively. Although the rates were statistically similar between groups, it can be said that ST P4 treatment had a positive effect on the twinning rate. Similarly, this situation also had a positive effect on litter size, and litter size in G1 was numerically higher than in G2, although there was no statistically significant difference. When future comprehensive studies determine how ST or LT P4 treatments affect follicular dynamics and hormone levels related to follicular development in Tuj ewes outside the breeding season, the effects on both twinning rate and litter size will be better understood.

In our previous studies, we found that various P4-based estrus synchronization protocols at different durations outside the breeding season had no effect on lamb survival rates. These rates ranged from 86.67% to 100%, as reported in our studies (Kuru et al. 2017; Kuru et al. 2020). However, our current study shows that lamb survival rates during the first month after birth were quite high, ranging from 90% to 100%. This indicates that ST or LT P4-containing synchronization protocols can be used in Tuj ewes to meet the market demand for lamb.

Although statistical analysis did not reveal any significant difference, it may be preferable to use ST protocols that result in high litter size and survival.

In conclusion, the use of short (7 days) or long (12 days) P4-containing intravaginal sponges with eCG treatment effectively stimulated estrus and achieved a more synchronized onset of estrus with higher pregnancy rates in Tuj ewes outside the breeding season compared to the control group. However, there was no superiority of either short or long intravaginal sponge treatments in terms of the investigated fertility parameters. Therefore, ST protocols are preferred in synchronization protocols using P4-containing sponges in Tuj ewes outside the breeding season. This would allow the sheep to be less affected by vaginitis and stress caused by prolonged sponge use. Additionally, increasing lamb production with such synchronization protocols during high-demand periods in the market may be more advantageous for farmers.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: ET, MK
Supervision / Consultancy: MK
Data Collection and / or Processing: ET, BBK, MK
Analysis and / or Interpretation: ET, BBK, MK
Writing the Article: ET, MK
Critical Review: ET, BBK, MK

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Hyperglycemic Effect of Dietary Boron in Rats with Experimental Diabetes Mellitus Induced by Streptozotocin

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ABSTRACT In this study, the effect of boric acid (BA) on blood sugar levels, vitamins and minerals in Streptozotocin (STZ)-induced diabetes in rats was investigated. In the study, 48 male Wistar albino rats (200-220 g) were divided into 6 groups, no special treatment was administered to Group1, experimental diabetes was induced by administering STZ (45 mg/kg) through intraperitoneal (IP) injection to other groups. Group 2 diabetes; Group 3 (6 U/kg insulin) insulin; Group 4; 250 ppm, group 5; 500 ppm and group 6 1000 ppm BA mixed with their feed. Blood glucose levels of all groups were quantified from blood taken from the tail vein every week. At the end of study, the rats were sacrificed and their blood was taken. The serum levels of vitamins A, E, and minerals were studied. When compared with other groups, blood glucose levels of groups 4, 5 and 6 were found to be increased ($p<0.05$). When compared to the baseline values, it was found that Vitamin A in the group 2 and 4, vitamin E in the group 4, Cu in the group 4, 5, 6, Zn, Mg and Na in the group 2 decreased and Fe in the group 2 increased. While Ca and P decreased in groups 5 and 6, no change was observed in Al in all groups. As a result, it has been observed that boron has a hyperglycemic effect when evaluated together with vitamins and minerals in diabetic rats. Whether boron is a suitable agent in the treatment of diabetes should be evaluated with further studies.

Keywords: *Boron, Diabetes mellitus, Rat, Mineral, Streptozotocin, Vitamin.*

ÖZ

Streptozotosin ile Deneysel Diyabet Oluşturulan Ratlarda Diyetteki Borun Hiperglisemik Etkisi

Bu çalışmada ratlarda Streptozotosin (STZ) ile oluşturulan diyabetes mellitüste borik asidin (BA)'in kan şeker, vitamin ve mineral düzeyleri üzerine etkisi araştırıldı. Sunulan çalışmada 48 adet erkek Wistar albino rat (200-220 g) 6 gruba ayrıldı, Grup 1'e özel bir tedavi uygulanmadı, diğer gruplara intraperitonal (İP) 45 mg/kg STZ uygulanarak deneysel diyabet oluşturuldu. Grup 2 diyabet grubu; Grup 3 (6 U/kg insülin) insülin grubu; Grup 4, 5 ve 6'nın yemlerine sırasıyla 250, 500 ve 1000 ppm BA katıldı. Tüm grupların kan glikoz seviyesi haftalık olarak ölçüldü. Çalışmanın sonunda sıçanlar anestezi altında kan örnekleri alındıktan sonra sakrifiye edildi. Elde edilen serum örneklerinden A ve E vitamin ile bazı mineraller ölçüldü. Kan glikoz düzeylerinin 4, 5 ve 6. gruplarda arttığı belirlendi ($p<0,05$). Başlangıç değerleri ile karşılaştırıldığında 2 ve 4. grupta A vitamini, 4. grupta E vitamini, 4, 5 ve 6. gruplarda Cu, 2. grupta Zn, Mg, Na ve Fe düzeylerinin azaldığı tespit edildi. 5. ve 6. gruplarda Ca ve P azalırken, tüm gruplarda Al'de değişiklik gözlenmedi. Sonuç olarak, borun diyabetik sıçanlarda vitamin ve mineral düzeyleri ile birlikte değerlendirildiğinde hiperglisemik etkiye sahip olduğu gözlendi. Bor'un diyabet tedavisi için uygun bir ajan olup olmayacağı ileri çalışmalarla değerlendirilmelidir.

Anahtar Kelimeler: *Bor, Diabetes mellitus, Mineral, Streptozotosin, Sıçan, Vitamin.*

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder that affects more than 400 million people across the world, causing impairments in carbohydrate, fat and protein metabolism due to absolute or relative insufficiency of insulin hormone secretion and/or insulin action (Regazzi 2018; Sacan et al. 2021). DM is an illness that requires lifelong continuous monitoring and treatment, impairs the quality of life of the patient due to its acute and chronic complications, and causes the death of thousands of people every year across the world due to chronic complications

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such as hypoglycemia, ketoacidosis, hyperglycemic hyperosmolar nonketotic coma, nephropathy, neuropathy, myocardial infarction, and atherosclerosis throughout the course of the disease (Domingueti et al. 2016). Although the underlying cause of DM complications is not fully elucidated, a great many studies have reported role of oxidative stress and lipid peroxidation. Lipid peroxidation and oxidative stress damage tissues by causing dysfunction of pancreatic β cells (Coban et al. 2015; Singh et al. 2022). Since free radicals increase and radical-binding systems decrease in DM, diabetic patients need antioxidants more. Natural vitamins (A, E, C) are used as antioxidants to hinder the detrimental effects of lipid peroxidation and oxidative stress (Asmat et al. 2016). Changes in antioxidant status in DM may be a result of nutritional status due to inadequate intake or excessive loss or may cause excessive consumption of these antioxidant vitamins in an environment where the oxidative load may be high (Zatalia and Sausi 2013).

Trace elements are essential for the body functions, growth, and physiology of the organism. Studies show that there is a relationship between glucose homeostasis and trace elements. Chronic hyperglycemia significantly changes the level of certain trace elements. Besides, the change in the level of trace elements increases the oxidative stress in DM and increases the occurrence of diabetes complications (Bahtıyar and Hacıoglu 2019; Krol et al. 2016). In a study conducted by the American Diabetes Association in 2007, the estimated cost of diabetes in the world was determined to be 174 billion dollars (Khalil 2017). Since diabetes is a socioeconomic burden in many countries and the medications used in its treatment cause multiple adverse effects in patients, the discovery and research of alternative hypoglycemic agents (herb, mineral, vitamin) play a vital role (Cakir et al. 2018; Tan et al. 2019).

Recent in vitro and epidemiological studies show that boron applications have significant effects on human health. Boron is found in nature as borax, colemanite, and boric acid, which is in group III-A in the periodic system with atomic number 5. Boron is an element that plays key roles in hormonal metabolism (insulin, estrogen, testosterone, calcitonin), cell membrane functions, minerals (Ca, P, Mg), lipid and carbohydrate metabolism, energy use, immune system functions, and enzymatic reactions (Bolt 2020; Wang et al. 2021; Ozel et al. 2022). Boron changes the permeability of the cell membrane to bioactive substances, the functions of membrane enzymes, and the affinity of receptors by forming complexes with polysaccharides, sugars, adenosine-5-phosphate, riboflavin, pyridoxine, pyridine nucleotides, phosphoinositides, dehydroascorbic acid, glycolipids and glycoproteins from organic compounds carrying cis-hydroxyl groups (Nielsen 2014; Hunter et al. 2019). It has also been reported that B compounds may have an inhibitory effect on important cellular components such as the proteasome, protease, and peptidase, by binding electron carriers such as NAD and hydroxyl group to the macromolecule side chain such as serine residues in protein structures (Cebecı et al. 2022). It has been suggested that boron is effective in the central nervous system and endocrine system functions by affecting vital structures for the organism such as magnesium, calcium, nitrogen, copper, glucose, reactive oxygen, triglyceride and estrogen in the life cycle of humans (Breydo 2013). Boric acid makes up 98.4% of the boron in the blood, and boron was mentioned as boric acid in most of the studies and pure boric acid (17.5% boron) was used as a boron source (Karimkhani et al. 2021). The lowest observed adverse effect level (LOAEL) and no-observed-adverse-effect level (NOAEL) for boric acid were found to be 78 and 55 mg BA/kg/day (13 and 10 mg/kg/day for boron), respectively. Higher doses cause developmental toxicity in mice, rats, and rabbits (Bolt et al. 2020; Sevım and Kara 2022).

In this study, the impact of boron administered as boric acid (4.1, 8.2, and 15.0 mg boron/kg) on weekly blood glucose levels, some vitamins and minerals in rats with diabetes mellitus induced by STZ (streptozotocin) was researched.

MATERIAL AND METHODS

Experimental Animals

In the study, 48 male 2-3 months old Wistar albino rats, with a bodyweight of 200-220 g were used which were obtained from the experimental animal unit of Van Yuzuncu Yil University. The rats were fed with standard or boric acid-enriched pellet feed in rooms at 22±2 ℃ illuminated with a 12-hour light, and 12-hour dark cycle. Before inducing experimental diabetes (except for the control group), the rats were fasted for 12 hours with free water intake, then released and housed in standard plastic cages with standard or boric acid-enriched feed and water intake. Before launching the study, approval was obtained from YYU Animal Experiments Local Ethics Committee (YUHADYEK) (adopted with its decision dated April 19th, 2012, and numbered 2012/03-04).

Preparation of Boric Acid Enriched Feed

Boric acid feed material to be used in the feeding of rats during the experiment was obtained from YYU Medical Faculty Health Research and Education Center Experimental Research Unit as standard pellet feed (Bayramoglu Feed Industry, Erzurum). Feed content is given in table 3. The feeds were ground in Ika Werke 2000 electric mill and passed through a 2 mm sieve in the laboratory of the Department of Animal Nutrition and Animal Nutrition Faculty of Veterinary Medicine, Van YYU. The amount of boric acid used in the experiment was prepared as 250, 500, and 1000 ppm (0.025%, 0.05%, 0.1% respectively) per 1 kg feed. Boric acid solutions of different concentrations were prepared in deionized water with the help of a magnetic stirrer and mixed homogeneously with the pellet powder. It was made into pellets and dried in the laboratory.

Induction of Diabetes in Rats with STZ

To induce diabetes, 45 mg/kg STZ (Vargas et. al 2023) solution prepared phosphate in citrate buffer (0.1 M. Ph: 4.5) was injected intraperitoneally (IP) into all rats except the control group. Before launching the study, rats in the other groups, except the control group, were fasted for 12 hours with free water intake. One week after STZ injection, the blood glucose levels of rats were determined by tail vein using a glucometer. Those with a level of >200 mg/dl were considered diabetes and included in the experiment.

Experiment Protocol

They were divided into 6 groups as follows, with 8 experimental animals in each group. On the 7th, 14th, 21st, 28th, 35th, and 42nd days of the experiment, blood was drawn from the tails of the rats with the help of a scalpel, and the blood glucose levels in the rats were quantified using a Glucometer.

The rats in Group 1 were the control group and fed with normal water and standard pellet feed.

The rats in Group 2, the diabetic group in which diabetes was induced by STZ, were fed with normal water and standard pellet feed.

The rats in Group 3, whose diabetes was induced by STZ and treated with insulin, were fed with normal water and standard pellet feed, and daily insulin at a dose of 6 U/kg was administered as IP.

The rats in the Group 4 were given normal water and pellet feed containing 250 ppm boric acid for 28 days.

The rats in the Group 5 were given normal water and pellet feed containing 500 ppm boric acid for 28 days.

The rats in the Group 6 were given normal water and pellet feed containing 1000 ppm boric acid for 28 days (Fail 1991; Scialli et al. 2010).

Blood Tests

Blood was drawn from the heart on the first and last day of the experiment, in accordance with the technique, by administering 50 mg/kg ketamine and 10 mg/kg xylazine IP for anesthesia. Blood samples were taken into gel biochemistry tubes, kept for 30 minutes, and centrifuged at 3000 rpm to separate the serum. The obtained material was stored in a refrigerator at -80 °C until analysis. In the subsequent stage, vitamin A and E levels were determined in Thermo Finnigan HPLC, and mineral levels were determined in AAS (Atomic Mass Spectrometry) and ICP-MS device, and the change between groups was analyzed statistically.

Chromatographic Analysis

Quantification of vitamins A and E: First, 200 µl of 5% sodium chloride, then 400 µl of ethanol was added to 200 µl of serum to precipitate the proteins in the sample and mixed in a vortex. 700 µl of n-hexane was added to this mixture and centrifuged at 3500 rpm for 10 minutes. In this way, fat-soluble vitamins A and E were extracted into the n-hexane phase. The combined n-hexane phases obtained by repeating this extraction process twice were evaporated to dryness under nitrogen gas. Residual methanol in the tube was dissolved into a mixture of DCM (50:50) ready for Chromatographic identification. For the determination of vitamins, A and E, C18 ODS-2 column, a mobile phase consisting of a mixture of methanol and water (97:3) degassed in an ultrasonic water bath was used. The flow rate of the mobile phase was set as 1.05 ml/min and the injection amount was set as 20 µl. A more sensitive fluorescent detector (excitation: 325 nm, emission: 480 nm) was preferred for the quantification of vitamins A and E) (Karatas et al., 2008).

Mineral Levels Analysis

Blood mineral analyzes were performed at YYU Central Research Laboratory Training and Research Center with ICP-MS and AAS devices. For analysis, serums were diluted with deionized water (100-fold diluted for ICP-MS and 10-fold for AAS). Results were presented in ppm.

Statistical Analysis

Descriptive statistics for the features studied were expressed as mean and standard deviation. Regarding these characteristics, a Repeated Measured Analysis of Variance was performed to determine whether there were differences between the study groups in terms of weeks and times (day zero - last day). Tukey's test was used to identify different groups and weeks following the Analysis of Variance. The statistical significance level was taken as 5% in the calculations and the SPSS statistical package software was used for the calculations.

RESULTS

Blood glucose results

There was no difference between the groups in the 1st week blood sugars ($p>0.05$). The blood glucose levels and standard errors of the study groups are shown in Table 1. Animals injected with STZ (groups 2, 3, 4, 5, 6) had blood glucose levels > 200 mg/dl one week later (week 2). It was determined that blood glucose levels increased (group 2, 3, 4, 5, 6) compared to the control group ($p<0.05$). It was determined that upon giving different doses of boric acid-containing feed (3rd, 4th, 5th, and 6th weeks) the blood glucose levels of the animals in Groups 4, 5, and 6 increased compared to the Groups 1, 2, and 3 ($p<0.05$) (Table 1).

Variation Between Vitamin and Mineral Levels

In our study, at the end of the experiment, it was observed that the serum vitamin A level of the 2 and 4 groups decreased significantly compared to the baseline values, and the serum vitamin E level of the Group 4 was lower than the baseline values ($p<0.05$). A significant decrease was observed in Cu levels compared to baseline values in Groups 4, 5, and 6, and in serum Zn, Mg, and Na levels in Group 2 ($p<0.05$), whereas increase was observed in serum Fe levels of group 2 ($p<0.05$). Besides, a significant decrease was observed in serum Ca and P levels in Groups 5 and 6, while no significant change was observed in serum Al in all groups (Table 2).

DISCUSSION AND CONCLUSION

Considering the fact that it affects many people, its treatment, complications, the rapid increase in the number of diabetes patients and the burden it brings to the national economy, DM is considered one of the most important problems in the health world. Intense efforts are being made to develop new strategies to achieve better metabolic control in diabetes and reduce diabetes-related complications (Domingueti et al. 2016; Darenskaya et al. 2021).

Vitamins and minerals are involved in biological processes, which are essential for the physiological functions of the body, as cofactors in the structure of enzymes, and in the formation of proteins and enzymes. Some trace elements control vital biological events by binding to receptor sites in the cell membrane or changing the shape of the receptor to prevent certain molecules from entering the cell. Trace elements and vitamins play a key role in DM and its complications as antioxidants (Kaur et al. 2021). It is well known that some inorganic trace elements such as chromium, zinc, copper, iron, sodium, potassium and nickel play a significant role in the maintenance of normoglycemia by activating the β-cells of the pancreas (Narendhirakannan et al. 2005). It has been reported that a change occurs in the metabolism of some minerals (Mg, Cu etc.) in DM and there is a relationship between this change and the progression of the disease (Tamrakar and Kachhawa 2016). Boron has notable effects on carbohydrate, lipid, protein, and enzyme metabolism, vitamins, trace elements, and immune and hormonal systems in humans and animals. Moreover, studies on the effects of boron on oxidative stress, which is of great importance in the complications of DM, have increased in recent years. Physiological amounts of boron regulate both energy substrate usage and mineral metabolism (Ozyarim and Coban 2021; Rahman et al. 2021).

Table 1: Descriptive statistics for glucose and comparison results of groups.

	1. group	2. group	3. group	4. group	5. group	6. group	*p*
1st week	93.00±8.00 a	93.57±11.80 a	93.57±11.80 a	104.25±13.37 a	100.00±13.26 a	95.14±12.15 a	0.359
2nd week	100.00±14.00 b	384.38±71.42 a	349.00±98.28 a	369.13±35.00 a	317.50±101.60 a	400.90± 69.27 a	0.001
3rd week	104.50±11.43 d	414.13±54.00 c	112.43±23.55 d	536.14±66.08 ab	447.00±148.29 bc	600± 0.01 a	0.001
4th week	95.13±7.31 d	438.80±64.16 c	104.00±24.30 d	500.00±85.45 b	371.43±97.00 bc	600 ± 0.01 a	0.001
5th week	90.25±5.00 d	414.17±54.41 c	130.43±29.01 d	536.57±59.22 ab	456.00±123.53 bc	600 ± 0.01 a	0.001
6th week	98.00±12.00 c	471.00±100.00 b	97.43±11.23 c	536.57±59.22 ab	491.43±125.49 b	600 ± 0.01 a	0.001

In the weeks with $P<0.05$, the difference in glucose change between the groups is significant. (a, b, c →) The difference between the group averages with different letters in the same line is significant.

1. group (control group) was fed with normal water and standard pellet feed.
2. group (the diabetic group with 45 mg/kg STZ, in whom diabetes occurred) was fed with normal water and standard pellet feed.
3. The group (DM+ insulin group in which 6 U/kg insulin was administered every day as IP) was fed with normal water and standard pellet feed.
4. group (DM + 250 ppm boric acid) was given normal water and pellet feed containing 250 ppm boric acid for 28 days.
5. group (DM+500 ppm boric acid) was given normal water and pellet feed containing 500 ppm boric acid for 28 days.
6. The group (DM+1000 ppm boric acid) was given normal water and pellet feed containing 1000 ppm boric acid for 28 days.

Table 2: Mineral and vitamin levels of the groups on the first and last day.

	1. group		2. group		3. group		4. group		5. group		6. group	
	Day Zero	The last day	Day Zero	The last day	Day Zero	The last day	Day Zero	The last day	Day Zero	The last day	Day Zero	The last day
Vitamin A	0.15±0.07	0.13±0.04	0.17±0.06	0.07±0.01 #	0.12±0.04	0.10±0.01	0.12±0.03	0.08±0.02 #	0.13±0.01	0.11±0.02	0.11±0.02	0.10±0.01
VitaminE	0.07±0.03	0.06±0.02	0.08±0.03	0.08±0.04	0.07±0.03	0.06±0.02	0.09±0.02	0.05±0.04 #	0.08±0.02	0.04±0.01	0.04±0.01	0.03±0.03
Cu	21.00±7.00	21.00±11.00	15.30±4.00	14.00±4.00	16.43±5.00	12.38±2.19	19.00±4.00	11.62±1.70 #	16.35±2.26	11.21±1.24 #	17.39± 4.11	12.14±2.04 #
Zn	24.00±6.02	23.48±5.00	33.36±6.00	23.27. ±8.00 #	15.31±3.10	26.18±2.00 #	24.00±.5.55	21.00±2.00	24.15±4.00	22.00±2.00	23.31±2.00	21.00±2.21 #
Fe	110.28±10.46	110.24±8.34	99.00±5.00	113.00±10.00#	111.56±14.15	112.00±14.27	111.00±11.00	107.00±11.36	110.00±13.44.	111.00±14.32	109.00±12.00	109.00±13.21
P	464.00±121.26	428.51±53.40	437.00±59.51	400.16±53.00	460.00±30.27	454.30±11.20	458.24±34.00	442.37±34.51	438.31±121.1	367.29±142.00#	423.00±±35.00#	370.00±37.31#
Na	100.24±3.44	97.65±4.00	98.32±5.40	93.00±3.00 #	96.47±5.00	95.00±4.00	99.31±4.00	96.13±4.00	97.00±5.00	94.50±3.52	97.00±4.50	95.29±4.00
Ca	15.19±4.07	16.09±2.19	16.00±0.08	14.60±3.00	16.00±2.00	16.00±1.00	17.00±0.09	15.60±2.41	17.00±0.06	14.35±1.48 #	17.00±1.01	14.11±1.10 #
Mg	11.00±1.48	10.22±2.00	11.02±2.22	7.80±4.29 #	10.00±2.00	10.45±1.39	11.00±3.00	8.00±3.00	10.46±3.00	9.41±1.37	10.00±2.00	8.38±1.42
Al	19.00±1.11	19.43±0.24	19.07±0.26	19.09±0.17	19.15±0.43	19.00±0.70	19.00±0.43	19.26±0.71	19.00±1.10	19.51±0.17	19.28±1.11	19.38±0.10

#: The difference from day zero is significant ($p<0.05$)
1. group (control group) was fed with normal water and standard pellet feed.
2. group (the diabetic group with 45 mg/kg STZ, in whom diabetes occurred) was fed with normal water and standard pellet feed.
3. The group (DM+ insulin group in which 6 U/kg insulin was administered every day as IP) was fed with normal water and standard pellet feed.
4. group (DM + 250 ppm boric acid) was given normal water and pellet feed containing 250 ppm boric acid for 28 days.
5. group (DM+500 ppm boric acid) was given normal water and pellet feed containing 500 ppm boric acid for 28 days.
6. The group (DM+1000 ppm boric acid) was given normal water and pellet feed containing 1000 ppm boric acid for 28 day

In the study, the impact of boron administered as boric acid on blood glucose levels, some vitamins (A, E), and minerals (Cu, Zn, Fe, P, Na, Ca, Mg, Al) in STZ-induced (45 mg/kg) DM was investigated.

It is well-documented that boron has an effect on insulin and serum glucose levels, and it is reported that boron can form a complex with the hydroxyl group in the structure of glucose and alter the blood glucose level (Muz et al. 2022). Although it was revealed in some studies that boron administered as gavage to diabetic rats decreased blood glucose levels compared to the control group (Cakır et al. 2018; Coban et al. 2015). In other studies, it was emphasized that boron added to the diet in rats decreased the insulin concentration without changing the plasma glucose concentration (Bakken and Hunt 2003; Kucukkurt et al. 2015). Hunt et al. (1997) reported that boron intake in chickens with vitamin D deficiency increased plasma glucose concentration, Dissordi et al. (2017) reported that the amount of physiological boron added to the normal water of diabetic rats increased blood sugar (Hunt et al. 1997; Dessordi et al. 2017). In this study, our results were like those of Hunt et al. (1997) and Dissordi et al. (2017). In the present study, blood glucose levels of the rats were measured one week after 45 mg/kg STZ was given to the other groups, except for Group 1, and because all rats' blood glucose levels were above 200 mg/dl (except for the control group), all of them were considered diabetic in the 2nd week and work started. When the feed containing boric acid was given to Groups 4, 5, and 6, the blood glucose level increased compared to Groups 1, 2, and 3. We think that the fact that boron raises the blood glucose level in diabetic rats is caused by the fact that boron triggers polyphagia by forming a complex with the cis-hydroxyl group in the glucose structure and inhibits the glycolytic pathway by reversibly reducing the activity of NAD or FAD, which are main substrates of glycolysis (Hunt et al. 1997; Geyıkoglu and Turkez 2007).

Dubey (2020) and Rad (2022) stressed that in the relationship between diabetes and its complications with reactive oxygen species, the antioxidant defense system is impaired to protect beta cells in the pancreas and glucose tolerance might also change. Studies have revealed a decrease in vitamin A and E levels in plasma in DM (Yuztaş and Degeryoruk 2014; Dubey et al. 2020; Rad et al. 2022). Thus, in our study, it was found that vitamin levels decreased compared to baseline values in all groups fed with boric acid, but only vitamin A in Group 2 and 4, and vitamin E in Group 4 decreased significantly ($p<0.05$). It can be suggested that there is a decrease in vitamin E and A levels due to the increase in free radical production and decrease in radical binding systems in long-term impaired glucose tolerance since diabetics need more antioxidants in oxidative stress. In minerals, serum copper (Cu) levels were significantly lower in Groups 4, 5 and 6 compared to the baseline values. Cu is an essential element for the activity of antioxidants in long-term impaired glucose tolerance. It has been reported that the decrease in plasma Cu level in DM is due to denaturing of antioxidant enzymes and oxidation of free oxygen radicals (Cho et al. 2007; Martirosyan et al. 2020; Sonkar et al. 2021). We are of the opinion that the decrease in mineral Cu level in Groups 4, 5, and 6 is due to denaturation or inhibition of antioxidant enzymes, given the fact that boric acid also exacerbates hyperglycemia in DM. Zinc (Zn) is required for insulin production in the pancreas and storage of insulin in vesicles. Moreover, it mediates insulin-induced glucose uptake into these cells by increasing the expression of Zn finger protein 407 and Zn-alpha-2-glycoprotein, glucose transporter type 4 (GLUT 4) protein in adipocytes and skeletal muscles (Bahtıyar and Hacıoğlu 2019). Bahtiyar and Hacıoğlu reported that serum Zn levels decreased in DM patients compared to the control group. When the data of our study were examined, it is noticed that although there was a decrease in serum Zn levels in Groups 2, 4, 5, and 6, the decrease in Groups 2 and 6 was statistically significant. It is seen that there is a significant increase in Group 3. We consider that the data we obtained support the view that a decrease in Zn level may occur due to a decrease in gastrointestinal absorption and/or hyperzincuria (Sanjeevi et al. 2018; Sonkar et al. 2021) while the increase in Group 3 is due to the daily administration of 6 U/kg insulin and the Zn in the structure of insulin. It has been reported that serum Fe levels increase in DM (Sha et al. 2021). In the presented study, serum Fe levels increased in groups 3, 5 and 2 compared to the initial values, but the increase in group 2 was significant. This increase is thought to be since Fe electrons act as electron donors and acceptors in biological systems and that Fe plays a role in the pathogenesis of diabetes as a prooxidant (Hansen et al. 2017; Skalnaya et al. 2017; Martirosyan et al. 2020). Although serum Na level decreased in groups 4, 5 and 6, it was not found to be statistically significant ($p>0.05$). At the end of the experiment, serum Na levels in group 2 decreased significantly compared to the initial values. Hyperglycemia has a diluting effect on electrolyte concentrations by regulating the internal environment for osmotic diuresis. A decrease in serum Na levels has been observed in DM due to osmotic diuresis (Siddiqui et al. 2014). Hakkı et al (2013) reported that boron increases the calcium and phosphorus levels in the bones in rabbits, Naghii and Samman (1996), and Khaliq et al. (2018) reported that the urinary excretion of calcium and phosphorus decreases with the addition of boron to the diet in menopausal women, and phosphorus and calcium are stored in the bones to reduce the possible bone loss, while Dupree et al. (1994) reported that the addition of boron to the diet in rats with vitamin D deficiency increased the absorption of calcium and phosphorus balance from the serum, and Dessordi et al.(2017) found that boron supplementation in the diet of diabetic rats decreased the serum Ca level of boron compared to rats with normal diet and control group (Dupree et al. 1994; Naghii and Samman 1996; Hakkı et al. 2013; Khaliq et al. 2018). In the study, a significant decrease was found in the serum Ca and P levels in Groups 5th and 6th compared to the baseline values. The serum P and Ca levels might have decreased because of the decrease in urinary excretion of B Ca and P and the contribution of these minerals to bone storage. Dessordi et al. (2017) Cakır et al. (2018) and Wang et al. (2021) observed a decrease in serum magnesium (Mg) levels in diabetes due to polyuria (Dessordi et al. 2017; Çakır et al. 2018; Wang et al. 2021). In the presented study, serum Mg levels decreased in groups 2, 4, 5 and 6 compared to the baseline values, but the decrease was found to be significant only in Group 2. The decrease in the 2nd group was due to polyuria and, as Abdelnour et al. suggested, the decrease in the 4^{th}, 5^{th} and 6^{th} groups with boron was not significant due to the storage of boron in the bones by decreasing urinary Mg excretion (Abdelnour et al. 2018).

Considering all the data obtained, in conclusion, it was determined that boron mineral did not lower blood sugar in rats with DM, on the contrary, it had a hyperglycemic effect by increasing it. It has been determined that boric acid reduces serum vitamin levels in diabetics, decreases

bone loss that may occur by increasing the absorption of calcium and phosphorus balance from serum in mineral levels, and the decreases in Cu, Zn, Na, and Mg levels resulted from the long-term impaired glucose tolerance. Besides, it was determined that the increased Fe level was caused by the decrease in the insulin level of iron stores in diabetes, and no change occurred at the mineral Al level. In future studies, it is recommended to investigate the hyperglycemic effect of boron by considering its impact on vitamins and minerals.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest for this study.

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AUTHOR CONTRIBUTIONS

Idea / Concept: NAA, HÖ, GO
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Data Collection and / or Processing: NAA
Analysis and / or Interpretation: NAA, HÖ, GO
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Investigation of *Escherichia coli* O157:H7 and *Salmonella* Bacteriophages in Cattle Fecal Sources

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ABSTRACT *S.* Typhimurium and *E. coli* O157:H7 are the most important foodborne pathogens forming bacterial biofilms that contribute to their virulence, antimicrobial resistance, and surface survival, causing severe food poisoning outbreaks worldwide. Bacteriophages are antibacterial agents that are increasingly used to control foodborne pathogens, and they also play a role in the solution against the development of antibiotic resistance. In addition, bacteriophages can be found in wastewater, natural and animal wastes, and foodstuffs. Aim of this study to determine the purification and lytic effects of *Salmonella* spp. and *E. coli* specific phages circulating in our country, which can effectively combat common *Salmonella* spp. and *E. coli* infections in our country and the world by using samples taken from the cowshed. In this study, 3 *S.* Typhimurium and 1 *E. coli* O157:H5 bacteriophages were isolated, and their lytic activities were determined. As a result, it is thought that the lytic activities of *S.* Typhimurium and *E. coli* O157:H7 bacteriophages purified from Aksaray province in this study can shed light on the treatment of *S.* Typhimurium and *E. coli* O157:H7 infections and prevention studies in the food industry.

Keywords: *Bacteriophage, E. coli O157:H7, Fecal, Purification, S.Typhimurium.*

ÖZ

Sığır Fekal Örneklerinden *Salmonella* spp. ve *Escherichia coli* O157:H7'ye Özgü Bakteriyofaj Varlığının Araştırılması

Salmonella Typhimurium *(S.* Typhimurium*) ve Escherichia coli (E. coli)* O157:H7, virülanslarına, antimikrobiyal dirençlerine ve yüzeylerde hayatta kalmalarına katkıda bulunan bakteriyel biyofilmler oluşturan ve dünya çapında ciddi gıda zehirlenmelerine neden olan önemli gıda kaynaklı patojenlerdir. Bakteriyofajlar, gıda kaynaklı patojenleri kontrol etmek adına her geçen gün artarak kullanılan antibakteriyel maddeler olup ayrıca antibiyotik direncin gelişmesine karşı çözümde rol oynamaktadır. Ayrıca bakteriyofajlar atık sularda, doğada ve hayvansal atıklarda olabileceği gibi birçok gıda maddesinde bulunmaktadır. Bu çalışmanın amacı sığır altlıklarından alınan dışkı örnekleri kullanılarak ülkemizde ve dünyada sık rastlanan *Salmonella* türleri. ve *E. coli* infeksiyonları ile mücadele etmede etkili olabilecek, ülkemizde sirküle olan *Salmonella* spp. ve *E. coli* spesifik spot test ile fajların purifikasyonu ve litik etkilerinin belirlenmesi amaçlanmıştır. Bu çalışmada 3 tane *S.* Typhimurium ve 1 tane *E. coli* O157:H7 bakteriyofajları izole edilerek, litik aktiviteleri belirlenmiştir. Sonuç olarak bu çalışmada Aksaray ilinden pürifiye edilen *Salmonella* Typhimurium ve *E. coli* O157:H7 bakteriyofajlarının tespit edilen litik aktiviteleri ile *Salmonella* Typhimurium ve *E. coli* O157:H7 kaynaklı enfeksiyonların tedavisi ve gıda endüstrisinde korunma çalışmalarına ışık tutabileceği düşünülmektedir.

Anahtar Kelimeler: *Bakteriyofaj, E. coli O157:H7, Fekal, Pürifikasyon, S. Typhimurium.*

INTRODUCTION

Salmonella spp. and *E. coli* are zoonotic foodborne pathogens of worldwide importance (EFSA 2021). Salmonella spp. is estimated to cause 93 million enteric infections and 155,000 deaths worldwide yearly (Ritter et al. 2019). Although *E. coli* is found in the gastrointestinal tract of animals and humans, it has many pathogenic serogroups (Wasteson 2001; Zhu et al. 2022). *E. coli* O157:H7 is often isolated in cattle and poultry products (Schoeni and Doyle 1994; Naylor et al. 2005). In addition, it is reported that *Salmonella* and *E. coli* can adhere, colonise and form biofilms on various food surfaces and fresh products, exacerbating their dangers (Zhu et al. 2022).

In the one health context, *Salmonella* spp. and *E. coli* have been reported to have complex routes of transmission

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involving the environment, animal reservoirs and water. Foodstuffs, especially livestock and poultry, are considered the most common sources of *Salmonella* spp. *and E. coli* infections in humans (Yim et al. 2011; Li et al. 2018).

The most prevalent pathogen that still poses a significant threat to the public's health by causing gastroenteritis, enteric fever, infectious diarrhea, and sepsis is *S.* Typhimurium (Ao et al. 2015; Jeon and Ahn 2021). The issue is getting national and international due to the rapid appearance and spread of *Salmonella*, which is resistant to the antibiotics. Due to the limited antibiotic regimens, multidrug-resistant *Salmonella* infections can lead to treatment failure (Wang et al. 2019; Wolput et al. 2022). Therefore, the pharmaceutical sector has long focused on creating new antibiotics. The rate at which multidrug-resistant bacteria developed resistance to newly discovered antibiotics, however, was much slower than the rate at which bacteria developed antibiotic resistance (Hede 2014). Therefore, alternative treatments are urgently required to treat and stop infections resistant to several drugs (CDC 2019).

It has been confirmed use of bacteriophages for treatment and prevention in disease struggles for resistant bacteria in drugs used in treatments. Based on the experience and current results of bacteriophage applications against bacterial infections in countries where alternative therapies have been approved, many scientists and companies have come to believe that the use of phages to treat and prevent bacterial diseases will be successful (Kutateladze and Adamia 2010). Felix-O1 (FO1) phage belonging to *Salmonella* spp. was first described in the 1930s (Ata 2018). It has been reported that they can also be found in many natural foodstuffs, especially in animal wastes and wastewater. *Salmonella* spp. and *E. coli* phages can be isolated from poultry extracts, poultry and farm environments, and wastewater treatment plants. Since the bacteriophages are generally non-toxic and bacteria-specific and affect many microorganisms, the recent resistance of bacteria to the antibiotics has brought phages to an important place in this field (Atterbury et al. 2007).

The life cycle of the phage consists of several steps, including attachment, penetration, replication, virion assembly, and release. Phage adsorption to the surface of host cells is an essential step in species-specific interaction (Rakhuba et al. 2010). The phages can nonspecifically recognise receptors on the host cell surface and then specifically bind to them (Guglielmotti et al. 2012). Although there are many studies of *Salmonella* spp. and *E. coli* bacteriophages in many countries, specific phage studies for *Salmonella* spp. and *E. coli* species in Türkiye are very few, and they are generally purified from poultry litter (Ata 2018; Sahin et al. 2020). Therefore, study aimed to determine the purification and lytic effects of phages that may be effective in controlling zoonotic infections, which are essential for human and animal health, from faeces samples taken from litter in cattle farms.

MATERIAL AND METHODS

Bacterial Strain and Culture Conditions

This study was approved by Aksaray University Experimental Animal Practice Center Ethics Committee (Aksaray, Türkiye) (RPN: 2023/5-18).

All litter samples in this study were taken from healthy cattle farms in Aksaray province and delivered to the laboratory within 24 hours at +2 – 8 °C under cold chain conditions. To examine the lytic activity of infective phages against *Salmonella* spp. and *E. coli* O157:H7 taken in this study, a total of 50 stool samples were obtained from litters of 38 cows and 12 calves from 50 different farms with the help of transportable swabs. The samples delivered to the laboratory were diluted 1:10 with physiological saline. After the debris part precipitated, it was centrifuged at 13,000g at 4 °C for 10 minutes, and the supernatant parts were passed through a 0.2 µm filter and used as a phage source (Sambrook et al. 1989; Higgins et al. 2005). *Salmonella and E. coli* strains were obtained from field strains from Aksaray and Yuzuncu Yil University Veterinary Faculty Veterinary Microbiology Culture Collections to determine the lytic activities of phages.

Bacteriophage Isolation

Salmonella spp. phages were purified by direct isolation and modified double layer agarose method by enriching with *Salmonella* Typhimurium, *S.* Dublin, *S. enterica*, *E. coli*, and *E. coli* O157:H7 field isolates. For direct isolation, samples were mixed with a 1:10 magnesium-salt buffer (50 mM Tris-HCl [pH: 7.5], 0.1 M NaCl, eight mM MgSO4) followed by filtration through a 0.45 mm bottle-top filter and a two-step procedure through a 0.2 mm syringe attached filter. After the filtered filtrate was thickened at 55 °C by adding 0.7% Nutrient agar, passages of 4 ml to 1.5% TSA agar were performed and then incubated at 37 °C. For the phage enrichment method, the obtained filtrates used for direct isolation were mixed with Nutrient-broth (Oxoid Ltd., London, England) at a ratio of 1:10 and then 1 mL of bacterial strain was added. After 16 hours of incubation at 37°C, it was subjected to a spot test and modified double-layer agarose method (Switt et al. 2013). For these processes, culture supernatants were obtained by concentrating at 7500 x G for 15 minutes using an ultrafiltration unit with ten kdA Vivaspin® (Sartorius), following manufacturer's recommendations.

Lytic Activity Test

The lysis activity of each of the four bacteriophage isolates was evaluated in the specific host and other hosts. The cross-lytic activities of the phages were determined by assuming that the structure of the isolates could vary depending on the serotype, origin, region, and year differences. First, the lysis activities of bacteriophages were standardized in their hosts, and then their activities in other hosts were determined. For this purpose, spot test and double layer agarose method were used (Holmfeldt et al. 2007).

In the spot agar method, the target bacteria were adjusted to $2x10^4$ cfu/bacteria and spread on Nutrient Agar, and 15 µl phage dilutions were dropped on it. The numbers are determined according to the McFarland method (McFarland 1907). After the plates were left to dry for 30 minutes at room temperature, they were incubated at 37 °C for 18 hours, and the diameters of the lytic activities were evaluated. Lytic activity after various phage dilutions; was recorded using a scale ranging from no fragmentation (-), blurred point (+), clear point (++), and diffuse clear point (+++) (D'Andrea et al. 2020).

In the double-layered agarose method, 100 µl of phage and 300 µl of target bacterial culture were mixed in a sterile bottle, added to 4ml molten LB agar at 44-47 °C, and poured onto the plates.

After incubation, plaque formation and lytic activity were evaluated (Sahin et al. 2020).

RESULTS

In this study, 50 samples, 38 of which were collected from cows and 12 from calf litters, were used to examine the lytic activity of infective phages against *Salmonella* spp. and *E. coli* O157:H7. Of these, bacteriophage activity was determined against *S.* Typhimurium in only three samples and *E. coli* O157:H7 in 1 sample. Two of the samples showing lytic activity with *S.* Typhimurim host bacteria were from calves, and one from cows; the sample showing lytic activity with *E. coli* O157:H7 was obtained from samples taken from cows (Table 1). No lytic activity was detected in *S.* Dublin and *S. enterica* strains. While determining the lytic activity of bacteriophages, McFarland, and *E. coli* O157:H7 and *S.* Typhimurium waters were adjusted to 2×10^4 and controlled on Nutrient Agar. *E. coli* O157:H7 10^{-6}; phages were obtained, in which lytic activity continued until the dilutions of *S.* Typhimurium 10^{-8}, *S.* Typhimurium 10^{-4}, and *S.* Typhimurium 10^{-4}.

Table 2 shows the lytic activities of four bacteriophages in their possible hosts. Accordingly, it was determined that Phage-1 (*E. coli* O157:H7) showed lytic activity in *the E. coli* O157:H7 strain as expected and showed the highest lytic activity with +++ at 10^{-2}. It was determined that it did not give any action in hosts belonging to *Salmonella* species. In other phage's (Phage-2, Phage-3 and Phage-4) (*S.* Typhimurium), it was determined that it gave lytic activity in its born host, *S.* Typhimurium, but did not show lytic activity in *S.* Dublin, *S. enterica* and *E. coli* hosts. The lytic activities of phage-2 were determined as +++ in 10^{-2} and 10^{-4}, ++ in 10^{-6} and + in 10^{-8}. The lytic activities of Phage-3 and Phage-4 were determined with ++ at 10^{-2}.

Dilutions of 10^0, 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} of bacteriophages were evaluated by spot test using 10 µl by double seeding. It was determined that they gave the best lytic activity in 10^{-2} and $10^{-4,}$ shown (Figure 1).

Table 1: Farm, source, year and regions of the four isolated bacteriophages.

	Farm	Source	Year	Region
Phage 1	3	Dairy cow	2023	Central Anatolia
Phage 2	5	Dairy cow	2023	Central Anatolia
Phage 3	7	Calf	2023	Central Anatolia;
Phage 4	8	Calf	2023	Central Anatolia;

Table 2: Lytic activities of four isolated bacteriophages in *S.* Typhimurium, *E. coli* O157:H7, *S.* Dublin, *S.* enterica, *E. coli* (Field strain) bacteria.

	S. Typhimurium				*E. coli* O157:H7				*S. Dublin*	*S. Enteritica*	*E. coli* (Field strain)			
	10^{-2}	10^{-4}	10^{-6}	10^{-8}	10^{-2}	10^{-4}	10^{-6}	10^{-8}			10^{-2}	10^{-4}	10^{-6}	10^{-8}
Phage 1	-	-	-	-	+++	++	+	-	-	-	+	-	-	-
Phage 2	+++	+++	++	+	-	-	-	-	-	-	-	-	-	-
Phage 3	++	+	-	-	-	-	-	-	-	-	-	-	-	-
Phage 4	++	+	-	-	-	-	-	-	-	-	-	-	-	-

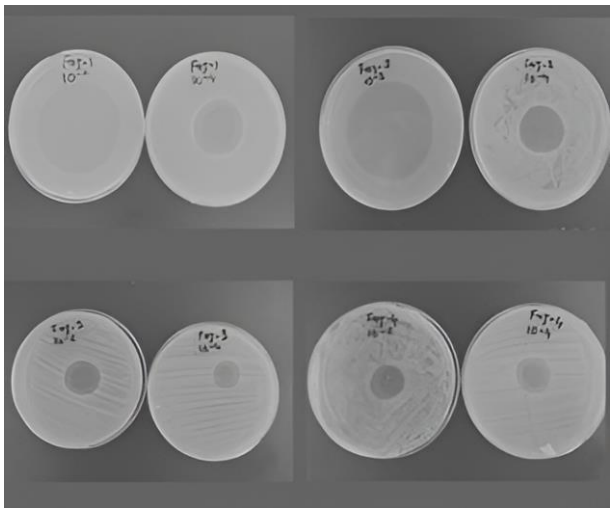

Figure 1: Spot test of lytic activities of isolated *E. coli* O157:H7(Phage-1) and *S.* Typhimurium (Phage-2, Phage-3, Phage-4) bacteriophages.

DISCUSSION AND CONCLUSION

Salmonella spp. and *E. coli* O157:H7 are zoonotic agents that can cause essential epidemics in human and animal health. Especially in recent years, due to the increase in multi-antibiotic resistance, alternative treatment strategies need to be developed (Banin et al. 2017). Recently, the phages have gained renewed attention as potential therapeutic tools due to their specificity (Garrido-Maestu et al. 2019; LeLièvre et al. 2019; Thung et al. 2019). It is vital that phage can be used as an alternative treatment because it is host specific and has less toxicity (Bourdin et al. 2014). It is very promising because it is less economical and because the bacteria rarely develop resistance to phages. It has been reported that bacteriophages cause protection and therapeutic effect in the study of infections caused by many bacteria (Petsong et al. 2019).

The bacteriophages are frequently isolated from poultry farms, poultry meat and faecal samples. Studies in cattle

holdings are rare. Phages can be found in various hosts, and each bacteriophage may have different lytic activity. Bacteriophages isolated from multiple sources are very important in terms of diversity. It has been reported that other *Salmonella* phages were isolated in 45 of 160 stool samples collected from healthy and sick cattle (*S.* Enteritidis bacteriophage 64.4%, *S.* Typhimurium 8.9%). Still, lytic activity did not occur in *E. coli* (Dueñas et al. 2017). Similarly, there are other isolated and purified studies against *Salmonella enterica* serovars (McLaughlin et al. 2006; Akhtar et al. 2014; Duc et al. 2020; Lu et al. 2022; Olsen et al. 2022).

E. coli O157:H7 is a flora bacterium found in the gastrointestinal tract of animals is abundant in faeces. Therefore, it easily contaminates sewage, wastewater, soil, and food. As a result, various wastewater, such as sewage, is known to be the best source for isolating phage that naturally infects *E. coli* O157:H7 (Viazis et al. 2011). Some studies reported that *E. coli* O157:H7 was isolated and purified similarly (Viazis et al. 2011; Litt and Jaroni 2017; Duc et al. 2020).

In a study conducted in the provinces of Nigde, Aksaray, Ankara and Kayseri in Türkiye, it was reported that *S.* Typhimurium was isolated and purified in 33 of 92 samples, and *S.* Enteritidis was isolated in 56 of them (Yildirim et al. 2018a). In another study from the same region, *E. coli* O157:H7 was isolated and purified in 37 of 92 samples (Yildirim et al. 2018b). It was emphasized that phages purified specifically against *S.* Typhimurium and *S.* Enteritidis, and *E. coli* O157:H7, which show lytic activity against *Salmonella* species from foodborne pathogens, can be used as a possible alternative to chemical antimicrobials against these pathogenic bacteria. In another study, it was reported that 13 *S.* Typhimurium and 22 *S.* Enteritidis bacteriophages were isolated and purified in Istanbul (Ang-Kucuker et al. 2000).

In conclusion, this study evaluated *Salmonella* species and bacteriophage specific to *E. coli* O157:H7 strains from litter samples collected from Aksaray province. The collected samples detected phages with lytic activity in 6% *S.* Typhimurium and 2% *E. coli* O157:H7 ratio. Faeces collected from the litters were used as a source for obtaining bacteriophages. These results might lead to phage therapy, food industry, and prevention studies for *Salmonella* species and *E. coli* O157: H7.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: GSG, KA
Supervision / Consultancy: GSG, KA
Data Collection and / or Processing: GSG, KA
Analysis and / or Interpretation: GSG, KA
Writing the Article: KA, GSG
Critical Review: KA, GSG

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Çankırı'nın Farklı Bölgelerinde Üretilen Balların Antibakteriyel, Antioksidan Özellikleri ve Bazı Kimyasal Parametreleri

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ÖZ

Bal, yüksek besleyici özelliği olan aktif bileşenlerince zengin bir besin kaynağıdır. Balın antibakteriyel, antioksidan aktiviteleri ile kalite kriterleri arıların bulundukları coğrafik konuma, iklime, bitki florasına, çevresel şartlara göre değişiklik göstermektedir. Yapılan bu çalışmanın amacı, Çankırı ilinin farklı bölgelerindeki balların antibakteriyel ile antioksidan aktiviteleri ve bazı kimyasal parametrelerinin belirlenmesidir. Çankırı ilinin 7 farklı ilçesine ait köylerden toplam 14 adet bal örnekleri toplanmıştır. Toplanan bal örneklerinin nozokomiyal enfeksiyon etkenlerinden geniş spektrumlu beta laktamaz üreten *Escherichia coli* ve metisilin dirençli *Staphylococcus aureus*'a karşı antibakteriyel aktivitesi disk diffüzyon ve broth mikrodilüsyon yöntemleri ile analiz edilmiştir. Antibakteriyel aktivite testleri sonucunda tüm balların *S.aureus* ve *E.coli* bakterilerine karşı 21.5 mg/mL ile 42.5 mg/mL aralığında farklı minimum inhibitör konsantrasyon değerlerine sahip olduğu ve minimum bakterisidal konsantrasyon değerlerinin 42.5 mg/mL ve daha üst değerde olduğu belirlenmiştir. Yapılan analizler sonucunda balların fenolik madde toplam miktarları 36-84 µg/mL aralığında bulunmuştur. Balların nem oranları % 15.40- % 20.80, elektrik iletkenliği 0.18-0.34 mS/cm, pH değerleri 3.4-4.4, diastaz sayısı ortalama 17.5, en yüksek prolin miktarı 944.42mg/kg olarak bulunmuş, renk parametreleri olan L*, a* ve b* ortalama değerleri sırasıyla 44.31, 1.42 ve 18.92 olarak bulunmuştur. Sonuç olarak, Çankırı yöresine ait balların farklı konsantrasyonlarda antibakteriyel aktiviteye sahip olduğu, fenolik içerik ve antioksidan aktivitenin paralellik gösterdiği, kimyasal parametreler açısından Türk Gıda Kodeksi Standartlarına uygun olduğu saptanmıştır.

Anahtar Kelimeler: *Antibakteriyel aktivite, Antioksidan aktivite, Bal, Kimyasal parametre.*

ABSTRACT

Honey Produced in Different Regions of Çankırı Antibacterial, Antioxidant Properties and Some Chemical Parameters

Honey is a food source rich in active components with high nutritional properties. Antibacterial, antioxidant activities and quality criteria of honey vary according to the geographical location, climate, plant flora and environmental conditions. This study aimed to determine antibacterial and antioxidant activities and some chemical parameters of honey from different regions of Çankırı province. A total of 14 honey samples were collected from villages in 7 different districts of Çankırı province. The antibacterial activity of the collected honey samples against extended spectrum beta lactamase producing *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* was analyzed by disc diffusion and broth microdilution methods. As a result of antibacterial activity tests, it was determined that all honeys had different minimum inhibitory concentration values between 21.5 mg/mL and 42.5 mg/mL against *S.aureus* and *E.coli* bacteria and minimum bactericidal concentration values were 42.5 mg/mL and higher. As a result of the analyses, the total amount of phenolic substances in honey was found in the range of 36-84 µg/mL. Moisture ratios of the honeys were 15.40%-20.80%, electrical conductivity was 0.18-0.34 mS/cm, pH values were 3.4-4.4, diastase number was 17.5, the highest proline amount was 944.42 mg/kg and the average values of color parameters L*, a* and b* were 44.31, 1.42 and 18.92 respectively. As a result, it was determined that the honeys of Çankırı region had antibacterial activity at different concentrations, phenolic content and antioxidant activity were parallel, and the chemical parameters were by the Turkish Food Codex Standards.

Keywords: *Antibacterial activity, Antioxidant activity, Honey, Chemical parameter.*

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GİRİŞ

Bal, bal arıları tarafından bitki özleri veya bitki üzerinde yaşayan böcek salgılarının toplanmasıyla özel maddelerle birleştirilerek peteklerde depolanarak olgunlaşan doğal bir arı ürünüdür (Tarım Orman Bakanlığı 2020). Bal besleyici özelliğinin yanında eski dönemlerden bu yana sağlık alanında özellikle cerrahi yaralarda kullanılan antibakteriyel özelliği olan bir ürün olmuştur. Günümüzde de ilaç kullanımından kaynaklı yan etkilerin görülmesinden dolayı doğal ürünlere yöneliş söz konusudur. Bu anlamda doğal tedavi yöntemlerinde apitrerapi ilk sıralarda yer almaktadır. Balın içeriğinde farklı proteinler, vitaminler, oligasakkaritler (fruktoz, glukoz), enzimler, lipitler, mineraller, fenolik asitler gibi birçok bileşik bulunmaktadır (Bogdanov ve ark. 2002). Arıların bulunduğu bitki örtüsü, bölgedeki bitki florasının nektar tipi, coğrafik konum, yükseklik, ısı farklılığı, hasat zamanı gibi birçok özellik balın kalite ve içeriğini etkiler. Botanik kaynakların farklılığına bağlı olarak da hasat edilen ballarda farklı renk, koku ve tat gözlemlenebilmektedir (Karlıdağ ve ark. 2021).

Balın içeriğinde bulunan enzimlerden glukoz oksidaz enzimi glukozu parçalayarak ortamda hidrojen peroksit ile glukonik asit oluşmasına sebep olur, oluşan hidrojen peroksit ve asit ortam balın antibakteriyel özellik kazanmasını sağlar (Çakıcı ve Yassıhüyük 2013). Balın antioksidan özelliği ise daha çok içerdiği fenolik bileşikler, E vitamini ve askorbik asitten kaynaklanmaktadır (Parlakpınar ve Polat 2021). Bal antioksidan özelliği sayesinde birçok hastalığa sebep olan kimyasal reaksiyonlar sonucu oluşan, nitrik oksit radikalleri hidroksil, süperoksit, reaktif oksijen gibi bileşiklerin oluşumunu azaltır veya engelleyerek etkisini gösterir (Halliwel 2003; Yaylacı ve ark. 2007). Balın antibakteriyel ve antioksidan aktivitesine lizozim, fenolik asit ve flavonoidler katkıda bulunur. Bu özellikleri sayesinde yaralı bölgede granülasyon doku oluşumunu uyararak yara iyileşmesini hızlandırabilir. Ayrıca yara izinin azalmasına yardımcı olabilir. Antioksidan aktivitede rol alan fenolik bileşikler üzerine yapılan çalışmalar bazı bakterilerin gelişimini engellediğini göstermiştir (Davidson ve ark. 2005). Son zamanlarda sentetik antioksidan olarak kullanılan maddelerin olumsuz yan etkileri nedeniyle bitkilerin antioksidan özellikleri üzerine yapılan çalışmalar önem kazanmıştır (Gülçin ve ark. 2006). Günlük diyette bitkisel ürünler ile bitki orjinli bal gibi bileşiklerin yer alması gün geçtikçe artmakla birlikte fonksiyonel gıda terimi de önem kazanmaya başlamıştır.

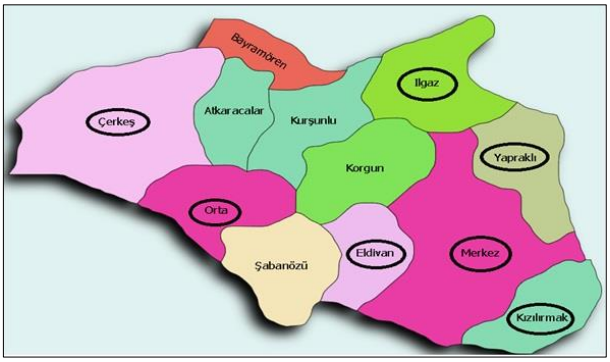
Balın kimyasal özelliklerinden nem oranı; balın yapısını etkilediği için %20'nin altında olan ballarda fermantasyon oluşmadığı için uzun süre dayanıklılıklarını korurlar (Çetin ve ark. 2014; Tarım Orman Bakanlığı 2020). Balın elektriksel iletkenlik özelliği daha çok balın orijinini belirlemek için kullanılan önemli bir parametredir. Elektriksel iletkenlik balın içeriğindeki mineral, protein, şeker oranına bağlı olarak salgı balını çiçek balından ayıran bir özelliktir. Türk Gıda Kodeksi Bal Tebliğine (TGKBT) (Tarım Orman Bakanlığı 2020) göre elektrik iletkenliği değerleri çiçek balında en fazla 0.8 mS/cm, salgı balında en az 0.8 mS/cm olmalıdır. Bal içeriğindeki mineral maddeler ile organik asitler balın asitlik derecesini belirler, balın ortalama pH değeri 3.4-6.1 arası olduğu belirtilmiştir (Çiftçi ve Parlat 2018). Diastaz sayısı, balın ısıl işlem görüp görmediğini belirlemek için kullanılan bir parametre olup balın tazeliği hakkında da bilgi verir. TGKBT' ye göre ortalama değeri en az 8 olarak belirlenmiştir. Balın olgunluk ve saflık parametrelerinden biri de serbest aminoasitlerden olan prolin değeridir. Prolin, nektarın bala dönüşürken arılar tarafından eklenen bir maddedir ve sonradan ilave edilmesi mümkün değildir (Karadal ve Yıldırım 2012). TGKBT'ye göre baldaki prolin değeri en az 300 mg/kg olmalıdır. Balın rengi, balın coğrafik ve ekolojik şartlarına, kaynağına ve fenolik bileşen miktarına göre açık sarıdan siyaha kadar değişebilir. Açık renkli bal örneklerinin fenolik bileşen içeriğinin koyu renkli ballara göre genellikle daha düşük olduğu bildirilmektedir (Bogdanov ve ark. 2004; Mutlu ve ark. 2017). Balın rengi, müşteri tercihi olarak pazar fiyatı üzerine de etkili olabilir (Gonzales ve ark. 1999; Özkök ve Bayram 2021).

Bal üretildiği bölge, iklim koşulları ve bitki örtüsüne göre bileşimlerinde farklılıklar gösterir (Sagdiç ve ark. 2013). Zengin bitki örtüsü, coğrafik ve iklim şartları göz önüne alındığında ülkemiz bal üretimi açısından önemli bir yere sahiptir. Farklı bölgelerin bal bileşimleri ile ilgili çeşitli araştırmalar mevcutken (Akbulut ve ark. 2009; Özcan ve Ölmez 2014; Can ve ark. 2015), 4. Uluslararası Muğla Arıcılık ve Çam Balı Kongresiyle eşzamanlı olarak düzenlenen 20. Apislavıa Kongresi Uluslararası Bal Yarışmasın da "Farklı Orjinli Ballar" kategorisinde dünya 2.si olan Çankırı balına ait söz konusu alanda bir veri bulunmamaktadır. Bu kapsamda Çankırı'nın 14 farklı bölgelerinde üretilen balların antibakteriyel, antioksidan özellikleri ve bazı kimyasal parametreleri incelenmiştir.

MATERYAL VE METOT

Bal Örnekleri

Çankırı Karatekin Üniversitesi Sağlık Bilimleri Etik Kurulu'nun 2023-8 sayılı izini alınarak yapılan çalışmada, Çankırı İli Arı Yetiştiricileri Birliği aracılığıyla Şekil 1 ve Tablo 1'de koordinatları verilen Çankırı ilinin 14 farklı bölgesindeki kovanlardan 14 adet bal numunesi metal kapaklı steril cam kavanozlar içerisine alınmıştır. Örnekler etiketlenerek oda sıcaklığında, karanlık bir ortamda analizler yapılıncaya kadar bekletilmiştir. Analizler öncesinde bal örnekleri steril spatül ile karıştırılarak homojenize edilmiştir.



Şekil 1: Bal numunelerinin alındığı Çankırı İli Haritası.

Figure 1: Çankırı Province Map where honey samples were taken.

Antibakteriyel Aktivitenin Belirlenmesi

Bakteri suşları

Antibakteriyel aktivite çalışmalarında geniş spektrumlu beta laktamaz (GSBL) üreten *E. coli* (ATCC 35218) ve metisilin dirençli *S. aureus* (MRSA) (Klinik izolat) kullanılmıştır. Testlerde kullanılacak bakteriler nutrient brothda (Merck, Almanya) 37 ^{o}C'de 24 saat ardından nutrient agara (NA) (Merck, Almanya) ekim yapılarak 37 ^{o}C'de 24 saat inkübe edilmiştir.

Tablo 1: Bal numunelerinin alındığı bölgeler ve koordinatları.

Table 1: Honey sampling locations and coordinates.

İlçe	Köy	Koordinatlar
Çankırı-Merkez	Süleymanlı	40.51754, 33.61850
Eldivan	Gölezkayı	40.51533, 33.54239
	Ekinne Yaylası	40.53429, 33.49834
	İldivan Yaylası	
Kızılırmak	Halaçlı	40.28890, 33.94844
Orta	Kayılar	40.53768, 33.08727
	Merkez-1	40.62759, 33.10532
	Merkez-2	
	Yaylakent	40.59155, 33.09491
Yapraklı	Merkez	40.75655, 33.78546
Ilgaz	Saraycık	40.99981, 33.76787
	Satılar	40.98353, 33.71758
	Kuyupınar	40.85735, 33.60225
Çerkeş	Merkez	40.81710, 32.88524

Disk difüzyon testi

Çankırı'nın 14 farklı bölgesinden elde edilen bal örneklerine ait antibakteriyel aktivitelerin belirlenmesinde ilk olarak disk difüzyon testi (CLSI-2012a) kullanılmıştır. McFarland cihazında (Biosan DEN-1B) 0.5'e (~$1.5x10^8$kob/mL) ayarlanmış bakteri süspansiyonlarından Mueller-Hinton agar (MHA) (Merck, Almanya) yüzeyine yayma ekim yapılmıştır. Takiben steril distile su içerisinde 85 mg/mL olacak şekilde hazırlanan bal örneklerinin her birinden 20 µL (1.7 mg/disk) boş disklere (Bioanalyse, Türkiye) emdirilmiştir. Bu diskler steril pens ile MHA yüzeyine yerleştirilmiştir. Araştırmada referans antibiyotik olarak gentamisin (10µg/disk, Bioanalyse, Türkiye) diskleri kullanılmıştır. Kalite kontrol suşu olarak Avrupa Antimikrobik Duyarlılık Testleri Komitesi-EUCAST'ın (2018) önerdiği *S. aureus* ATCC 29213 kullanılmıştır. Besiyerleri 37 ^oC'de 20-24 saat inkübasyona bırakılmış, süre sonunda disk (7mm) etrafından oluşan inhibisyon zonlarının çapları kaydedilmiştir. Analizler üç paralelli yapılmıştır.

Minimum inhibitor ve bakterisidal konsantrasyon değerinin belirlenmesi

Balların bakteriler üzerine minimum inhibitör konsantrasyonu (MİK) belirlemek için, broth mikrodilüsyon yöntemi (CLSI-2012b) kullanılmıştır. Steril distile su içerisinde 85 mg/mL olacak şekilde hazırlanan bal örnekleri mikropleytlerde 42.5 mg/mL ile 0.33 mg/mL aralığında iki katlı seri sulandırılması yapılmıştır. Her kuyucuğa son konsantrasyonu ~$1x10^5$ kob/mL olacak şekilde bakteri süspansiyonundan kuyucuklara eklenmiştir. Çalışmada gentamisin (Sigma Aldrich, Amerika) (0.1 mg/mL) referans antibiyotik olarak kullanılmıştır. Her bakteri için bal örneği içermeyen bir kuyucuk üreme kontrolü amacıyla, Mueller-Hinton II broth (BBL, Amerika) ve bal örneği içeren birer kuyucuk da sterilite kontrolü amacıyla kullanılmıştır. Mikropleytler 37°C'de 20-24 saat inkübe edilmiştir. İnkübasyon sonunda bulanıklığın olmadığı son dilüsyon kuyucukları minimum inhibitör konsantrasyon (MİK) değeri olarak belirlenmiştir. MİK değeri belirlenen kuyucukların bir alt ve tüm üst dilüsyonlarından NA yüzeyine ekim yapılarak 37°C'de 20-24 saat inkübe edilmiştir. Üreme olup olmamasına göre minimum bakterisidal konsantrasyon (MBK) değerleri saptanmıştır. Analizler üç paralelli yapılmıştır.

Toplam Antioksidan Aktivite Analizleri

Toplam fenolik içeriğin belirlenmesi

Çankırı'nın 14 farklı bölgesinden elde edilen bal örneklerinin toplam fenolik bileşenleri belirlemek için folin–ciocalteu yöntemi bazı modifikasyonlar yapılarak kullanılmıştır (Singleton ve Rossi, 1965). Her bir bal numunesi (1 g) vorteks (Ika, Almanya) karıştırıcı kullanılarak 10 mL metanol içinde ekstrakte edildi. Ekstraksiyon sonrasında solüsyon 0.45 µm filtre kullanılarak filtre edilmiştir. 200 µL seyreltilmiş bal örneği üzerine 2400 µL saf su eklenmiştir. Daha sonra 200 µL 0.2 N folin-ciocalteu (Sigma-Aldrich, Amerika) reaktifi eklenmiş ve vortekslenip 3 dakika bekletilmiştir. Üzerine %7.5 Na_2CO_3 (Merck, Almanya) çözeltisinden 600 µL ilave edilmiştir. Oda sıcaklığında 2 saat inkübasyondan sonra, reaksiyon karışımının absorbansı metanol körüne karşı spektrofotometre (Agilent, Carry 60 UV-Vis, Amerika) ile 765 nm'de ölçülmüştür. Toplam fenolik içerik, standart olarak gallik asit (Sigma Aldrich, Amerika) (0-1 mg/mL) ile kalibrasyon eğrisi kullanılarak belirlenmiştir. Sonuçlar gallik asit eşdeğeri (GAE µg/mL) olarak hesaplanmıştır.

Radikal süpürme aktivitesinin belirlenmesi

Bal örneklerinin radikal süpürme aktivitesi, belirli konsantrasyonlarda hazırlanan 2.2-difenil-1-pikrilhidrazil (DPPH) solüsyonu ile bazı modifikasyonlarla Sánchez-Moreno ve ark. (1998) tarafından önerilen DPPH' nin ağartılması yöntemi izlenerek test edilmiştir. Her bir bal numunesi (1 g) 10 mL metanol içerisinde vorteks karıştırıcı kullanılarak çözdürülmüş ve 0.45 µm filtre kullanılarak filtre edilmiştir. 3 mL 0.1 mM DPPH (Sigma Aldrich, Amerika) çözeltisi üzerine 25 µL örnek eklenmiştir. Karışımlar, karanlıkta oda sıcaklığında 30 dakika bekletilmiş ve absorbanslar 517 nm'de kör olarak metanol kullanılarak spektrofotometre (Agilent, Carry 60 UV-Vis, Amerika) ile ölçülmüştür. Örneklerin antioksidan aktivitesi (%) aşağıdaki formüle göre hesaplanmıştır.

Antioksidan aktivite (%) = (kontrolün absorbansı-numunenin absorbansı) / kontrolün absorbansı x100

Bazı Kimyasal Parametrelerin Belirlenmesi

Bal örneklerinin nem tayini refraktometre (Abbe) ile ölçülmüştür. Bal örneklerinden 20 g alınarak saf su ile 100 mL'ye tamamlanıp homojenize edildikten sonra pH metre cihazı (Hanna, Amerika) ile pH ve elektriksel iletkenliği belirlenmiştir. Balın diastaz aktivitesi ve prolin içeriği spektrofotometre (Rayleigh VIS-7236, Çin) ile tespit edilmiştir (Bogdanov ve ark. 2002). Bal örneklerinin renk analizleri CIE Lab renk sistemine göre; L^*, a^*, b^* değerleri kolorimetrik yöntemle renk ölçümü cihazı-kolorimetre (Konica Minolta CR400, Japonya) kullanılarak belirlenmiştir. Her bir örnek için analizler 2 paralel olacak şekilde gerçekleştirilmiştir.

BULGULAR

Antibakteriyel Aktivite Bulguları

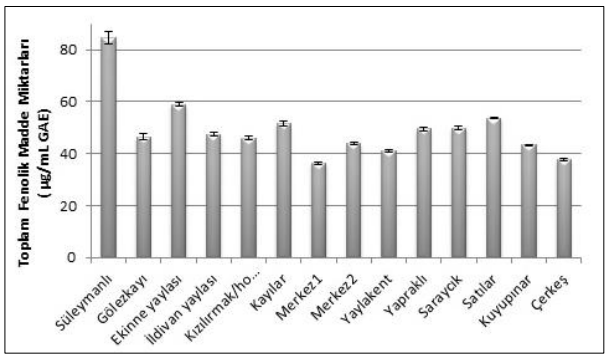
Çankırı'nın 14 farklı bölgesinden elde edilen bal örneklerinin antibakteriyel aktivitesini araştırmak amacıyla yapılan disk difüzyon testi sonucunda kullanılan bal örneklerinin 1.7mg/disk dozunda antibakteriyel aktiviteye sahip olmadığı belirlenmiştir.

Bal örneklerinin bakterilere karşı MİK değerlerini belirlemek ve disk difüzyon test sonuçlarını doğrulamak amacıyla yapılan broth mikrodilüsyon testi bulguları Tablo 2'de verilmiştir. Bu sonuçlara göre, *E.coli*'ye karşı Orta

Merkez-1, Yaylakent ve Satılar bal örneklerinin MİK değeri 42.5 mg/mL, MBK değeri 42.5 mg/mL'den büyük, diğer bal örneklerinde ise MİK değeri 21.5 mg/mL ve MBK değeri 42.5 mg/mL olarak belirlenmiştir. *S.aureus'a* karşı Gölezkayı, Kayılar, Orta merkez-1 ve Satılar bal örneklerinin MİK değeri 42.5 mg/mL, MBK değeri 42.5 mg/mL'den büyük, diğer bal örneklerinde ise MİK değeri 21.5 mg/mL ve MBK değeri 42.5 mg/mL olarak belirlenmiştir. Tüm balların 21.5 mg/mL ile 42.5 mg/mL aralığında farklı MİK değerlerine sahip olduğu saptanmıştır.

Toplam Antioksidan Aktivite Bulguları

Çankırı'nın 14 farklı bölgesinden elde edilen bal örneklerinin toplam fenolik madde miktarları 36-84 µg/mL GAE aralığında bulunmuştur ve Şekil 2'de gösterilmektedir. Süleymanlı bölgesinden alınan numunenin toplam fenolik madde miktarı 84 µg/mL GAE olarak analizi yapılan ballar içerisindeki en yüksek bulunan değerdir. Ekinne Yaylası, Satılar, Saraycık, Yapraklı ve Kayılar bölgelerinden toplanmış bal numunelerine ait fenolik içerikleri 50-58 µg/mL GAE aralığında birbirine yakın değerler bulunmuştur. Diğer lokasyonlardan toplanmış örneklerin fenolik içerikleri ise 36-48 µg/mL GAE aralığında bulunmuştur.



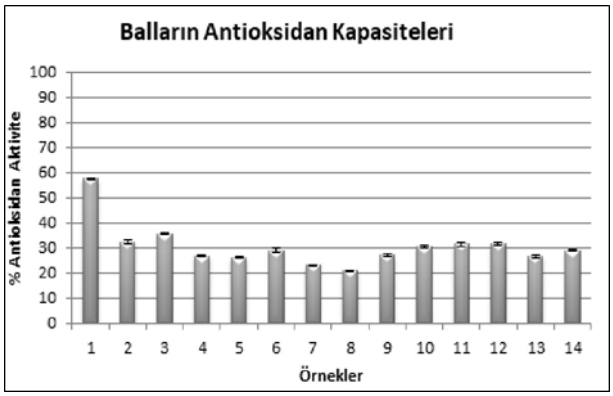
Şekil 2: Çankırı'nın farklı bölgelerinden toplanan balların toplam fenolik içerikleri.

Figure2: Total phenolic contents of honey collected from different regions of Çankırı.

Bal numunelerinden en yüksek antioksidan aktiviteye sahip örnek yine %57.46 değeriyle Süleymanlı bölgesinden alınan örnek olmuştur. Ekinne Yaylası, Satılar ve Saraycık bölgelerinden alınan örneklere ait antioksidan aktivite değerleri sırasıyla %35.6, 31.6 ve 31.5 olarak bulunmuştur. Diğer lokasyonlara ait antioksidan aktivite değerleri ise Şekil 3'te karşılaştırmalı olarak gözlenmektedir.

Kimyasal Parametre Bulguları

Çankırı'nın 14 farklı bölgesinden elde edilen bal örneklerinin kimyasal içeriklerinden nem oranı, elektriksel iletkenliği, asitlik düzeyleri, diastaz sayısı ve prolin analiz sonuçları Tablo 3'te verilmiştir. En yüksek nem oranı 20.80 ile Orta Merkez-2'de belirlenirken en düşük nem oranı 15.40 ile Süleymanlı'da saptanmıştır. En yüksek iletkenlik değeri 0.334 mS/cm ile Saraycık'ta belirlenirken en düşük iletkenlik değeri 0.188 mS/cm ile Çerkeş'te saptanmıştır. En yüksek pH değeri 4.57 Orta merkez-1'de belirlenirken en düşük pH değeri 3.59 ile Yaylakent'te belirlenmiştir. Diastaz sayısı en yüksek 29.40 ile Orta merkez-2'de, en düşük 8.10 ile Yapraklı'da saptanmıştır. En yüksek prolin değeri 1398 mg/kg ile İldivan yaylası, en düşük 515 mg/kg ile Orta merkez-2'de bulunmuştur.



Şekil 3: Çankırı'nın farklı bölgelerinden toplanan balların antioksidan aktivite oranları.

Figure 3: Antioxidant activity rates of honey collected from different regions of Çankırı.

Tablo 2: Çankırı'nın farklı bölgelerinden toplanan balların broth mikrodilüsyon yöntemine göre MİK (Minimum İnhibitör Konsantrasyon) ve MBK (Minimum Bakterisidal Konsantrasyon) değerleri.

Table 2: MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of honey collected from different regions of Çankırı according to broth microdilution method.

Bölgelere göre bal örnekleri (85 mg/mL)	**E.coli*		***S.aureus*	
	MİK (mg/mL)	MBK (mg/mL)	MİK (mg/mL)	MBK (mg/mL)
Süleymanlı	21.5	42.5	21.5	42.5
Gölezkayı	21.5	42.5	42.5	>42.5
Ekinne yaylası	21.5	42.5	21.5	42.5
İldivan yaylası	21.5	42.5	21.5	42.5
Hallaçlı	21.5	42.5	42.5	>42.5
Kayılar	21.5	42.5	42.5	>42.5
Orta Merkez-1	21.5	42.5	42.5	>42.5
Orta Merkez-2	42.5	>42.5	21.5	42.5
Yaylakent	42.5	>42.5	21.5	42.5
Yapraklı	21.5	42.5	21.5	42.5
Saraycık	21.5	42.5	21.5	42.5
Satılar	42.5	>42.5	42.5	>42.5
Kuyupınar	21.5	42.5	21.5	42.5
Çerkeş	21.5	42.5	21.5	42.5
Gentamisin (0.1 mg/mL)	0.0003	0.0006	0.0006	0.0012

* Geniş spektrumlu beta laktamaz üreten, ** Metisilin dirençli.

Tablo3: Çankırı'nın farklı bölgelerinden toplanan balların kimyasal özellikleri.

Table3: Chemical properties of honey collected from different regions of Çankırı.

Bölgelere göre bal örnekleri	*Nem (%)	*İletkenlik (mS/cm)	*Asitlik (pH)	*Diastaz Sayısı (DA)	*Prolin mg/kg	*Renk		
						*L **	*a**	*b**
Süleymanlı	15.40	0.21	4.13	26.30	1086	40.78	3.31	21.40
Gölezkayı	18.60	0.26	3.86	17.60	1260	37.97	4.37	17.80
Ekinne	16.60	0.24	3.81	29.10	1245	45.10	1.75	22.31
İldivan	18.80	0.28	3.90	10.50	1398	46.33	0.90	19.22
Hallaçlı	16.40	0.27	3.83	18.40	603	51.90	-0.11	20.20
Kayılar	19.60	0.27	4.44	9.10	983	46.34	2.32	20.27
Orta Merkez-1	19.80	0.23	4.57	8.60	734	45.55	-0.21	15.34
Orta Merkez-2	20.80	0.17	3.47	29.40	515	45.28	1.57	17.04
Yaylakent	18.80	0.13	3.59	13.60	721	42.14	0.39	15.38
Yapraklı	19.60	0.21	3.79	8.10	825	39.81	2.69	18.42
Saraycık	16.20	0.33	4.07	9.70	1156	43.70	-0.25	16.79
Satılar	17.40	0.27	3.81	28.30	775	39.19	1.54	20.34
Kuyupınar	17.80	0.24	3.72	19.00	1100	46.07	1.25	18.26
Çerkeş	17.00	0.19	3.75	22.90	821	50.30	0.25	22.18
Std sapma	1.61	0.05	0.30	8.15	268.83	4.04	1.40	2.31

*parametrelerin her biri ortalama değer olarak verilmiştir.

Çankırı'nın 14 farklı bölgesinden elde edilen bal örneklerinin ortalama nem değerinin % 18.05±1.61, ortalama iletkenlik (mS/cm) değerinin ise 0.23±0.05 ve ortalama pH değerinin 3.91±0.30 olduğu saptanmıştır. Bal numunelerinin genel ortalama diastaz sayısı ise 17.90±8.15, prolin değeri 944.42±268.83 olarak tespit edilmiştir.

Bal örneklerinin renklerindeki çeşitlilik, L*, a*, b* ve net absorbans değerleri noktasında gerçekleştirilen analiz sonuçlarına göre, L* değerleri 37.97-51.89, a* değerleri -0.25-4.36 ve b* değerleri de 15.33-22.30 aralıklarında ölçülmüştür.

TARTIŞMA VE SONUÇ

Yapılan antibakteriyel aktivite çalışmaları sonucunda, tüm balların *S.aureus* ve *E.coli* bakterileri üzerine 21.5 mg/mL ile 42.5 mg/mL aralığında farklı MİK değerlerine sahip olduğu ve MBK değerlerinin 42.5 mg/mL ve üstünde olduğu belirlenmiştir.

Çakır ve ark. (2022) Bingöl ilinin 4 farklı bölgesinden elde ettikleri bal örneklerinde yaptıkları disk difüzyon testi sonucunda *S.aureus*'a karşı 20 mg/disk dozunda tüm bal örneklerinin 8-9.5 mm aralığında, 10 mg/disk dozunda 6-7 mm aralığında inhibisyon zonu oluşturduğunu, 5 mg/disk dozunda ise etkisiz olduğunu, *E.coli*'ye karşı sadece 20 mg/mL dozunda Genç ve Yedisu bölgesinden alınan bal örneklerinin 6 mm çapında inhibisyon zonu oluşturduğunu bildirmiştir. Osho ve Bello (2010) Nijerya'nın farklı bölgelerinden elde ettikleri iki farklı bal örneğinin antibakteriyel etkinliği ile ilgi yaptıkları çalışma sonucunda 50 µl miktarında 4 farklı konsantrasyonda (%5, %25, %50 ve %100 w/v) kullandıkları ballardan, agar well difüzyon testi sonucunda *S.aureus*'a karşı %50 ve %100 w/v konsantrasyonda sırasıyla 10 mm ve 17 mm, *E.coli*'ye karşı 13 mm ve 20 mm inhibisyon çapı elde ettiklerini bildirmişlerdir. Çakır ve ark. (2022) ile Osho ve Bello (2010) kullandıkları dozlarda elde ettiği sonuçlar bu çalışmada belirlenen dozlarla karşılaştığında benzer olduğu belirlenmiştir.

Maželienė ve ark. (2022) %9'luk bal örneklerinden 0.1 ml kullanarak yaptıkları agar well difüzyon testi sonucunda *S.aureus*'a karşı 19 mm, *E.coli*'ye karşı 11 mm çapında inhibisyon zon alanı belirlediklerini bildirmiştir. Maželienė ve ark. (2022) bu çalışmada belirlenenden farklı olarak daha düşük dozda etkinlik bildirmişledir. Grabek-Lejko ve ark. (2022) mikrodilüsyon yöntemi ile % 6.25, % 12.5 ve % 25 oranlarında hazırlanmış balın antibakteriyel etkinliğinin spektrofotometrik olarak değerlendirdikleri çalışmaları sonucunda kontrol grubuna göre *S.aureus*'a karşı sırasıyla % 30, % 35 ve % 45 oranında, *E.coli*'ye karşı sırasıyla % 20, % 30 ve % 45 oranında bakteriyel üreme inhibisyonu elde ettiklerini bildirmişlerdir. Grabek-Lejko ve ark. (2022) bu çalışmadan farklı olarak daha yüksek dozda etkinlik bildirmişledir. İki çalışma arasındaki farklılıklar, coğrafi özelliklere bağlı olarak balın elde edildiği bitkilerin içeriklerinin çeşitliliğine ve/veya kullanılan test ile ölçüm yöntemlerinden kaynaklanabilir.

Balların fenolik ve antioksidan madde içerikleri ile ilgili olarak literatürde geniş aralıkta sonuçlar elde edilen birçok çalışma mevcuttur. Güzel ve Bahçeci (2019) Çorum yöresi balları ile yaptıkları çalışmada toplam fenolik miktarlarını Folin yöntemi ile 243-546 mg GAE/kg aralığında, antioksidan aktivite değerlerini ise DPPH yöntemi ile 0.17-0.605 mM trolox eşdeğeri antioksidan kapasite (TEAC) olarak rapor etmişleridir. Elde edilen sonuçlar sunulan çalışmadaki Çankırı yöresi balları ile benzerlik göstermektedir. Sagdic ve ark. (2013) tarafından yapılan araştırmada, 5 farklı balın antioksidan aktiviteleri fosfomolibden ve DPPH yöntemleri ile belirlenmiş ve sonuçlar toplam fenolik içeriği 1.50–108.21 mg GAE/100 g bal aralığında belirlenmiş ve DPPH testinde %20.06–58.12'lik yüzde inhibisyon aralığında bulunmuştur. Küçükaydın ve ark. (2023) gevenotunun yaygın olduğu İç Anadolu ve Doğu Anadolu bölgelerinden topladıkları ballarda yaptıkları çalışmada 37 bal numunesinin DPPH aktivitesinin 12.54±0.25 ila 40.00±0.95 mg/mL IC50 arasında değiştiği bulmuşlardır. Shen ve diğerleri (2018), tarafından yapılan çalışmada Çin yöresi ballarında yapılan çalışmada toplam fenolik içeriği, 9.43 ile 26.78 mg GAE/100 g bal arasında değişmektedir. Analiz edilen bal

örneklerinin DPPH radikal süpürme aktivite oranı ise % 7.11 ile % 51.42 arasında değişmektedir. Kara ve ark. (2020), Erzincan-Türkiye ballarının antioksidan kapasitelerini araştırmış ve fosfomolibden yöntemiyle 204±64 µmol askorbik asit/100g ve DPPH yönteminde ise SC50 değeri 146±38 mg/mL bulunmuştur. Balların antioksidan ve fenolik özellikleri balın üretildiği coğrafik koşullara, nektarın toplandığı bitkisel kaynağa, genel floraya, mevsimsel ve çevresel faktörlere bağlı olarak değişkenlik göstermektedir. Tüm incelenen çalışmaların sonuçlarına bakılacak olursa elde edilen değerlerin farklılığı öncelikli olarak balın orijini ve kullanılan yöntemlerin farklılığından kaynaklanabilir.

Baldaki kalite parametrelerinden nem oranının ortalamanın üzerinde olması balın kalitesini olumsuz yönde etkileyebilmektedir. Su içeriğinin yüksek oluşu fermantasyona yatkınlığa sebep olarak raf ömrünü kısalması ile tat ve aroma değişikliğine neden olabilmektedir (Terrab ark. 2004). Çalışmadaki numunelerin ortalama nem değerinin % 18.05 olduğu tespit edilmesi balların uygun dönemlerde hasat edildiğinin göstermektedir. Farklı rakımlarda üretilen balların nem oranlarının karşılaştırıldığı bir çalışmada toplanan bal örneklerinin nem içerikleri % 15.60 ile % 19.70 arasında değişmekte olması (Erdoğan ve Çıvracı 2022) bu çalışmanın bulgularını destekler niteliktedir.

Balın elektriksel iletkenliği, bitkisel kaynağa ve mineral tuzların yoğunluğuna göre değişir. Elektriksel iletkenlik çiçek balını salgı balından ayıran en önemli parametrelerden biri olduğu kabul edilmektedir (Apaydın 2022). Çalışmada ortalama iletkenlik değerinin 0.23 mS/cm olması numunelerin çiçek balı olduğunun göstermektedir.

Balın pH'sı bakteri ve mantar üremesi açısından önemlidir. Bakterilerin çoğu hafif alkali ve nötr ortamlarda çoğalabilir (Conti 2000). Bazı kaynaklar balın pH değerinin 3.2 ile 4.5 arasında olması gerektiğini bildirmiştir (Bogdanov ve ark. 2002). Çalışmada numunelerin ortalama pH değerinin 3.91 olması balların yeterli olgunluğa erişince hasat edildiğini ve raf ömrünün uzun olabileceğini göstermiştir.

Bal bileşiminde bulunan önemli enzimlerden ve kalite parametrelerinden olan diastaz enzimi nektarı bala dönüşmesinde görev alır. TGKBT'ye göre 8'in altında olması uygun görülmemektedir. Diastaz aktivitesi balın uygun muhafaza koşullarının sağlandığının ve yüksek ısıl işlem uygulanıp uygulanmadığını gösteren parametrelerden biridir (Silva ve ark. 2009). Özgüven ve ark. (2020) Türkiye'nin 9 farklı ilinde üretilen çiçek ballarının diastaz sayısı 9-25.4 olarak tespit etmişlerdir. Araştırmacılar Çankırı'dan aldıkları bal örneğinde diastaz sayısı 14.4 olarak bildirmişlerdir. Bu çalışmada ortalama diastaz sayısının 17.90 oluşu bölgede üretilen balların muhafaza koşullarının uygun olduğunu göstermiştir.

Balın kalitesinin belirlenmesinde en önemli parametrelerden biri de prolin değeridir. Prolin arıların tükürük bezlerinden salgıladıkları ve nektarı bala dönüştüren aminoasittir (Kalaycıoğlu ve ark. 2006). Konya Bölgesinde yapılan bir çalışma sonucunda kullanılan bal örneklerinin prolin miktarları 487.8–699.0 mg/kg arasında bulunmuştur (Çiftçi ve Parlat 2018). Özgüven ve ark. (2020) Türkiye'nin 9 farklı ilinde üretilen çiçek ballarının prolin değerlerini 271-928.2 mg/kg aralığında tespit etmişlerdir. Araştırmacılar Çankırı'dan aldıkları bal örneğinde prolin miktarını 629.7 mg/kg belirlemişlerdir. Bu çalışmada ortalama prolin değeri 944.42 mg/kg olarak tespit edilmiş ve diğer çalışmalardan daha yüksek bulunmuştur. Ayrıca elde edilen prolin değerleri TGKB alt sınır olarak belirtilen 300mg/kg değerinin üzerinde saptanmıştır.

Balın rengi en değişken parametrelerden olup bitki kaynağına göre de değişkenlik gösterir. Çalışmada renk analizi sonucu, L* değerleri 37.97-51.89 (ort. 44.31), a* değerleri -0.25-4.36 (ort. 1.42) ve b* değerleri de 15.33-22.30 (ort. 18.92) aralıklarında ölçülmüştür. Çorum yöresinde yapılan çalışma sonucunda, L*, a* ve b* değerleri sırasıyla 32.02-41.48, 0.20-6.82 ve 10.76-20.58 aralığında belirlenmiştir (Güzel ve Bahçeci 2019). Çankırı ballarının Çorum ballarına göre L* değerinin yüksek, a* değerinin yakın, b* değerinin yüksek olduğu tespit edilmiştir.

Sonuç olarak, Çankırı ilinden toplanan 14 farklı bal örneğinin referans antibiyotiğe göre düşük olsa da farklı konsantrasyonlarda antibakteriyel aktiviteye sahip olduğu, yapılan analizler sonucunda fenolik içerik ve antioksidan aktivitenin paralellik gösterdiği ve farklı düzeylerde olduğu, kimyasal parametreler açısından Türk Gıda Kodeksi standartlara uygun olduğu ve yakın bölgelerde yapılan diğer çalışmalara göre prolin içeriğinin yüksek olduğu saptanmıştır. Prolin, bir amino asit türüdür ve balın kalitesini etkileyen önemli bir bileşendir. Çankırı balının antioksidan aktivite, antibakteriyel özellikler, kimyasal uygunluk göstermesi ve prolin başta olmak üzere diğer besin değeri taşıyan bileşenler açısından, beslenme ve sağlık açısından değerlidir.

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YAZAR KATKILARI

Fikir/Kavram: PP, ŞA
Denetleme/Danışmanlık: PP, ŞA, SÖ
Veri Toplama ve/veya İşleme: PP, ŞA, SÖ
Analiz ve/veya Yorum: PP, ŞA, SÖ
Makalenin Yazımı: PP, ŞA, SÖ
Eleştirel İnceleme: PP, ŞA

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Effects of Different Stress Applications on Some Reproductive Hormones, Sperm Parameters, Lipid Profile, Immunohistochemical and Immunofluorescent Markers

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ABSTRACT The current study assessed how 5 stress protocols applied affected sperm parameters, lipid profile, and some reproductive hormones. Live materials of the study consisted of 50 rats. The number of rats in the groups was equal and they were randomly assigned. Each group consisted of 10 rats. No stress application was conducted in the control group. The rats in the psychological stress group were subjected to a cycle of 4-hour light and 20-hour dark per day. The rats in the physical stress group were deprived of feed and water for two hours per day. In the psychological stress + physical stress group, the psychological and physical stress protocol was conducted. In the first 4 groups, all the applications were conducted for 14 days. A different stress application was applied to the rats in the depression group every day. It was determined that the abnormal sperm rate was high in the stress and depression groups, but the highest rate was in the depression group. In addition, sperm motility and sperm concentration were the lowest in the depression group. While the stress and depression groups had significantly lower serum triglyceride and HDL levels and LH and FSH levels, cholesterol and LDL values were significantly higher. Bax expression and 8 OHdG expression were severe in psychological stress+physical stress group and depression group. When the findings are evaluated collectively; it was determined that stress negatively affected sperm parameters, lipid profile, reproductive hormones, immunofluorescence and immunohistochemical parameters.

Keywords: *Bax, LDL, LH, Sperm, Stress, Triglyceride.*

ÖZ

Farklı Stres Uygulamalarının Bazı Üreme Hormonları, Sperm Parametreleri, Lipid Profili, İmmünohistokimyasal ve İmmünofloresan Belirteçler Üzerine Etkileri

Mevcut çalışma, uygulanan 5 stres protokolünün sperm parametrelerini, lipid profilini ve bazı üreme hormonlarını nasıl etkilediğini değerlendirdi. Çalışmanın canlı materyalini 50 sıçan oluşturdu. Gruplardaki sıçan sayısı eşitti ve rastgele dağıtıldılar. Her grup 10 sıçandan oluşuyordu. Kontrol grubuna herhangi bir stres uygulaması yapılmadı. Psikolojik stres grubundaki sıçanlar günde 4 saat aydınlık, 20 saat karanlık döngüsüne tabi tutuldu. Fiziksel stres grubundaki sıçanlar günde 2 saat yem ve sudan mahrum bırakıldı. Psikolojik stres + fiziksel stres grubunda psikolojik ve fiziksel stres protokolü uygulandı. İlk 4 grupta tüm uygulamalar 14 gün süreyle yapılmıştır. Depresyon grubundaki sıçanlara her gün farklı bir stres uygulama uygulandı. Anormal sperm oranının stres ve depresyon gruplarında yüksek olduğu ancak en yüksek oranın depresyon grubunda olduğu belirlendi. Ayrıca sperm motilitesi ve yoğunluğu depresyon grubunda en düşüktü. Stres ve depresyon gruplarında serum trigliserit ve HDL düzeyleri ile LH ve FSH düzeyleri anlamlı olarak düşük bulunurken, kolesterol ve LDL değerleri anlamlı olarak yüksekti. Bax ekspresyonu ve 8 OHdG ekspresyonu psikolojik stress + fiziksel stres grubu ve depresyon grubunda şiddetli düzeydeydi. Bulgular toplu olarak değerlendirildiğinde; stresin sperm parametrelerini, lipid profilini, üreme hormonlarını, immünofloresan ve immünohistokimyasal parametreleri olumsuz etkilediği belirlendi.

Anahtar Kelimeler: *Bax, LDL, LH, Sperm, Stres, Trigliserit.*

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INTRODUCTION

While industrial and technological developments make life easier, they also bring problems. The reproductive system is very sensitive to such environmental factors and is heavily affected by them (Fahim et al. 2019). People are placing more importance on reproductive health than they used to because their lifestyles are changing and they're exposed to more stress, both of which in turn tend to have a negative impact on them and their reproductive health (Awadalla et al. 2011). While short-term (acute) stress affects the process of adaptation to the environment, long-term and excessive (chronic) stress adversely influences health (Lee et al. 2015). It is known that an appropriate micro-environment (appropriate temperature, blood supply and hormonal stimulation) is required for testicular morphology and function (Damber and Janson 1978; Shalet 2009).

During stress, cortisol levels normally elevate, and when the stressful situations end, cortisol levels lower. However, cortisol accumulation increases when the organism is exposed to chronic stress (Stephens and Wand 2012). In this case, cortisol affects muscle, liver and adipose tissues to provide the necessary fuel. Thus, the fat accumulation in the tissues is mobilized and the lipid levels in the blood increase (Bower and Sergerstrom 2001). Hyperlipidemia has a major role in testicular damage (Mehta et al. 2003).

In case of chronic stress, various body activities are inhibited and cellular proliferation and differentiation are negatively affected (Rosmond and Bjorntorp 2000). Chronic stress causes very serious problems in reproduction (decrease in reproductive hormones, impaired sperm quality, erectile dysfunction, decreased sexual desire) (Kennedy et al. 1999; Baldwin 2001). In addition, chronic stress causes atrophy in seminiferous tubules, decreases tubule diameters, increases thickness of basement membrane, and causes necrosis in Leydig cells and interstitial edema (Arun et al. 2016; Fahim et al. 2019).

Numerous studies have emphasized that chronic stress causes very serious problems in male reproduction. However, they generally used a single stress protocol. In the present study, 5 stress protocols were applied. The aim of this study is to determine to what extent different stress protocols affect sperm parameters, reproductive hormones, lipid profile, immunohistochemical and immunofluorescent markers.

MATERIAL AND METHODS

Animals and Experimental Groups

All experimental applications were carried out based on the guidelines of the National Institutes of Health on the care and use of laboratory animals. Before the study, approval was obtained from the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University with the "decision dated 30.0.2022 and numbered 2022/06-02". 50 male Wistar albino rats, aged 3 months (200–250 g) provided by the Experimental Research Unit of Van Yuzuncu Yil University, were used in the study. They were randomly selected and divided into 5 groups including 10 rats in each. In order to make a statistically sound comparison, the number of rats in each group was adjusted equally. Rats were grouped considering their similar physical characteristics (especially their weight). In addition, attention was paid to the same age, species and gender. The number of subjects was kept high in order to increase the reliability, validity and sensitivity of the study and to guarantee the results. Caging was done on a group basis and 10 rats in each group were kept in a single cage. The rats were housed in a ventilated, 50±5% humidity environment under standard conditions during the study period, in laboratory conditions with a 12-hour light-dark cycle. Sterile corn cobs (MBD Feed Mill, Kocaeli, Turkey) were used as litter and changed daily. The rats were fed with standard rat pellet feed (Bayramoglu Feed Factory, Erzurum, Turkey).

In order to minimize the effects of these five protocols applied, a 1-week run-in period was applied. During this 1-week period, the same researcher checked the rats at the same time every day to adapt to the environment, the researcher, and routine procedures.

Control group, (n=10): No application was made on the rats in this group.

Psychological stress group (PS), (n= 10): In this group, rats were deprived of light. For this purpose, it was kept in laboratory conditions with a 4-hour light and 20-hour dark cycle for 14 days (Bulmus 2018).

Physical stress group (FS), (n=10): Rats in this group were placed in a semi-cylindrical acrylic tube with only ventilation holes (4.5 cm wide and 12 cm long). They were not allowed to take feed and water for 2 hours a day (between 10:00 and 12:00 in the morning) for 14 days.

Psychological stress + physical stress group (PS+FS), (n=10): Applications in the psychological and physical stress protocol were performed.

Depression group (DEP), (n=10): Rats in this group were deprived of food and water for 24 hours on the first day. On the second day, they were placed in 50 ml falcon tubes (between 8:00 and 11:00). On the third day, they were left in wet and dirty cages for 24 hours (between 08:00 and 08:00). On the fourth day, they were forced to swim for 5 minutes in a glass aquarium filled with ice water. On the fifth day, they were again placed in wet and dirty cages and deprived of food. On the sixth day, they were exposed to restrictive stress and crowded cages. On the seventh day, they were placed in empty cages and forced to swim in icy water. The same applications were applied to the rats in the depression group in the same order for 14 days (Basar and Ertugrul 2005).

In order to observe whether stress behavior occurred in rats, observations were made using components such as tail hanging and forced swimming (rising on the hind legs, standing still, etc.).

Sample Collection

Cardiac blood samples were taken under general anesthesia by inserting a needle from the lower end of the thorax into the area of the beats. They were centrifuged (5 minutes at 3.000 x g) and their serum was separated. These serums were utilized to assess reproductive hormones and lipid profiles. One testis was resected before the body cooled down following the blood collection process and its spermatological parameters were analyzed. The other testis was left in a 10% solution of formalin and then analyzed for histopathological and immunohistochemical examinations. For euthanasia, the ketamine/xylazine combination was used at four times the anesthetic dose (Belhan et al. 2017).

Spermatological Examination

The cauda epididymis of the testis, which was resected from the body after blood collection procedure, was first used to evaluate motility. This evaluation was made under a light microscope with a heating plate. The semen was

diluted in saline at 37 °C. While assessing the motility, the researchers did their best to avoid cooling down the cauda epididymis part and wasting time. The mixture obtained by slicing this part in 2 ml of saline was used to evaluate the sperm concentration and abnormal sperm ratio (Aksu et al. 2015; Turk et al. 2008).

Evaluation of Reproductive Hormones and Lipid Profile

Follicle stimulating hormone (FSH), Luteinizing hormone (LH), and lipid parameters were measured in the Abbott Architect I6200 SR device, using the chemiluminescence microparticle immunology method (Belhan et al. 2020).

Histopathological Examination

Tissue samples left in formaldehyde (10%) solution were fixed for 48 hours. Afterwars, routine tissue follow-up procedures were performed and embedded in paraffin blocks. Preparations were prepared by taking 4-µm thick sections from each block. These preparations were stained with hematoxylin-eosin (HE) and analyzed using light microscopy (Olympus BX 51, JAPAN). Examples were rated as none (-), mild (+), moderate (++), and severe (+++) in histopathological examination (Belhan et al. 2020).

Immunohistochemical Examination

The tissue sections were first taken on adhesive (poly-L-Lysin) slides to carry out immunoperoxidase examination. They were then deparaffinized and dehydrated. This process was followed by inactivation of endogenous peroxidase by keeping it in 3% H_2O_2 for 10 minutes. In the next step, the tissues boiled in 1% antigen retrieval solution were left to cool down at room temperature. Nonspecific background staining of the tissues was prevented by incubating the sections in protein block for 5 minutes. Tissues were incubated after instillation of primary antibody = Bcl-2-associated X protein (Bax) (Bax Cat No: sc-7480, Dilution Ratio: 1/100, US). 3-3' Diaminobenzidine chromogen was used as chromogen. The stained sections were analyzed by using a light microscope (Zeiss AXIO GERMANY) and based on their immune positivity. They were evaluated as none (-), mild (+), moderate (++), and severe (+++) (Temel et al. 2020).

Immunofluorescence Examination

Immunofluorescence examination was performed by taking the sections on adhesive (poly-L-Lysin) slides and then deparaffinizing and dehydrating them. This process was followed by inactivation of endogenous peroxidase by keeping it in 3% H_2O_2 for 10 minutes. In the next step, the tissues boiled in 1% antigen retrieval solution were left to cool down at room temperature. Their nonspecific background staining was prevented by incubating the prepared sections in protein block for 5 minutes. Then, primary antibody = 8-hydroxy-2-deoxyguanosine (8 OHdG) (8 OHdG Cat No: sc-66036, Dilution Ratio: 1/100, US) was dripped onto the tissues and incubated in accordance with the use instructions. Immunofluorescence secondary antibody (FITC Cat No: ab6717, Diluent Ratio: 1/1000, UK) was used as a secondary marker and incubated in the dark for 45 minutes. Finally, DAPI with mounting medium (Cat no: D1306, Dilution Ratio: 1/200 UK) was dripped onto the sections and incubated in the dark for 5 minutes, and the sections were covered with a coverslip. The stained tissues were analyzed under a fluorescent microscope (Zeiss AXIO GERMANY). The immune positivity of the sections was rated as none (-), mild (+), moderate (++), and severe (+++) (Belhan et al. 2017).

Statistical Analysis

SPSS package software (Version 21) was used for statistical analysis of lipid profile and reproductive hormones. The Shapiro-Wilk test was applied to analyze the compatibility of the data for normal distribution. The groups were normally distributed; therefore, Kruskal Wallis test was run to identify the presence of significant differences between the groups in terms of the same parameter. Post hoc analysis was run to determine which group caused the differences. The *P* value of ≤0.05 was accepted as significant. Descriptive statistics were presented as mean and standard deviation.

Five random areas were selected from each of immunohistochemical and immunofluorescent staining images and analyzed using the ZEISS Zen Imaging Software software to determine the intensity of positive staining. Data were statistically presented as mean and standard deviation (mean±SD) for % value of the area. Mann-Whitney U test was run in comparison of positive immunoreactive cells and immunopositive stained areas with healthy controls. As a result of the test, a p value of <0.05 was accepted as significant.

RESULTS

Sperm Parameters

It was determined that sperm parameters showed significant differences between the groups. Sperm concentration and motility were found to be lowest in the depression group. It was found that the rate of abnormal sperm was higher in the stress groups compared to the control group. However, the highest rate of abnormal sperm was found in the depression group. Findings regarding sperm parameters are presented in Table 1.

Table 1: Sperm parameter findings of the rats in the control group and the groups applied different stress protocols.

Groups	Motility (%)	Sperm Concentration (x106)	Abnormal sperm rate (%)
Control group	76.20±1.22^{a}	111.10±1.28^{a}	10.80±0.88^{e}
Psychological stress group	65.00±0.66^{b}	95.80±1.13^{b}	17.50±0.97^{d}
Physical stress group	60.10±1.37^{c}	84.90±0.99^{c}	21.60±0.96^{c}
Psychological stress + physical stress group	51.60±2.01^{d}	64.00±1.24^{d}	28.70±0.82^{b}
Depression group	38.50±1.58^{e}	57.60±1.50^{e}	32.70±1.33^{a}

$^{a, b, c, d}$ p*: Different letters in the same column indicate the difference between the groups (p<0.001).

Reproductive Hormones and Lipid Profile Findings

Triglyceride values of the stress and depression groups were significantly lower than the control group (p<0.001). However, the most significant decrease in triglyceride values was detected in the depression group (p<0.001). The highest cholesterol level was found in the depression group, and the lowest cholesterol levels were found in the control group (p<0.001).

When high-density lipoprotein (HDL) levels were examined in serum samples, the most significant decrease was detected in the psychological stress group ($p<0.001$). Additionally, HDL values of the depression group were interestingly higher than both the psychological and psychological+physical stress groups ($p<0.001$). Low-density lipoprotein (LDL) levels in the psychological+physical stress and depression groups were significantly higher than the control group ($p<0.001$). However, LDL values in the physical stress group were lower than the other groups ($p<0.001$).

LH and FSH levels were lower in the stress and depression groups than in the control group ($p<0.001$). The decrease in LH levels was even more pronounced, especially in the psychological stress, psychological + physical stress and depression groups. FSH levels in the physical stress, psychological+physical stress and depression groups were lower than the control group ($p<0.001$). No significant difference was detected between FSH levels of the control and psychological stress groups ($p\geq0.05$). Both physical stress and depression groups caused a significant decrease in FSH levels ($p<0.001$). Findings regarding the lipid profile and reproductive hormones of all groups are presented in Table 2 and Figure 1.

Histopathological Findings

Histopathological examination revealed that the testicular tissues in the control group had a normal histological structure (Figure 2). In the testes of the psychological stress group, moderate edema in the intertubular spaces and hyperemia in the vessels, mild degeneration in some spermatocytes and a moderate decrease in the number of sperm in the tubular lumens were detected (Figure 2). In the physical stress group, in addition to the symptoms in the psychological stress group, hyperemia in the vessels was found to be severe and there was moderate degeneration in spermatocytes (Figure 2). In the psychological stress + physical stress group; It was determined that the edema and hypermia observed in the psychological stress and physical stress groups were severe, and there was a significant decrease in the number of sperm in the tubules (Figure 2). There was a statistically significant difference between this group and the control group. In the depression group; In addition to the symptoms seen in the psychological stress and physical stress groups, advanced degeneration of spermatocytes in the tubular wall was detected (Figure 2). It was determined that this group had a statistically significant difference with the control group. Table 3 shows the histopathological findings.

Immunohistochemical Findings

It was determined that cytoplasmic Bax expression in spermatocytes was negative in the control group (Figure 2), moderate in the psychological stress group and physical stress group (Figure 2), and high in the psychological stress + physical stress group and depression group (Figure 2). A statistically significant difference was found in the depression group compared to the control, psychological stress and physical stress groups (Table 4). Table 4 shows the immunohistochemical findings.

Immunofluorescent Findings

In the evaluation of testicular tissues using the immunofluorescence method; It was observed that 8-OHdG expression was negative in the control group (Figure 2), mild-moderate in the psychological stress group (Figure 2), moderate in the physical stress group (Figure 2), and severe in the psychological stress + physical stress group (Figure 2). Psychological stress + physical stress group; showed a statistically significant difference compared to the control, psychological stress and physical stress groups (Table 4). Severe 8-OHdG expression was detected in the testicular tissues of the depression group (Figure 2). Depression group; showed a statistically significant difference according to the control, psychological stress and physical stress groups (Table 4). Immunofluorescence findings are presented in Table 4.

Table 2: Mean and standard deviation values of lipid profile and reproductive hormone levels measured in serum samples.

	Control group	Psychological stress group	Physical stress group	Psychological stress + physical stress group	Depression group	p value
Triglyceride (mg/dL)	77.20±7.51^{a}	66.00±6.93^{b}	69.25±8.14^{b}	69.13±4.89^{b}	51.44±5.99^{c}	0.001
Cholesterol (mg/dL)	37.07±2.83^{c}	52.13±4.64^{b}	53.78±3.15^{b}	54.60±4.45^{b}	62.22±6.22^{a}	0.001
HDL (mg/dL)	50.88±4.12^{a}	32.79±3.26^{d}	46.54±3.19^{b}	34.73±2.91^{d}	39.19±3.15^{c}	0.001
LDL (mg/dL)	4.93±0.42^{c}	5.27±0.52^{bc}	2.86±0.23^{d}	5.31±0.37^{b}	5.68±0.26^{a}	0.001
LH (mlU/mL)	0.57±0.05^{a}	0.39±0.03^{c}	0.44±0.03^{b}	0.35±0.04^{d}	0.4±0.03^{bc}	0.001
FSH (mlU/mL)	0.27±0.01^{a}	0.26±0.02^{ab}	0.24±0.01^{bc}	0.22±0.02^{cd}	0.21±0.03^{d}	0.001

LDL: Low density lipoprotein, HDL: High density lipoprotein, LH: Luteinizing hormone, FSH: Follicle stimulating hormone,

^{a,b,c,d}p: Values with different letters in the same row are significant when compared to each other ($p<0.001$).

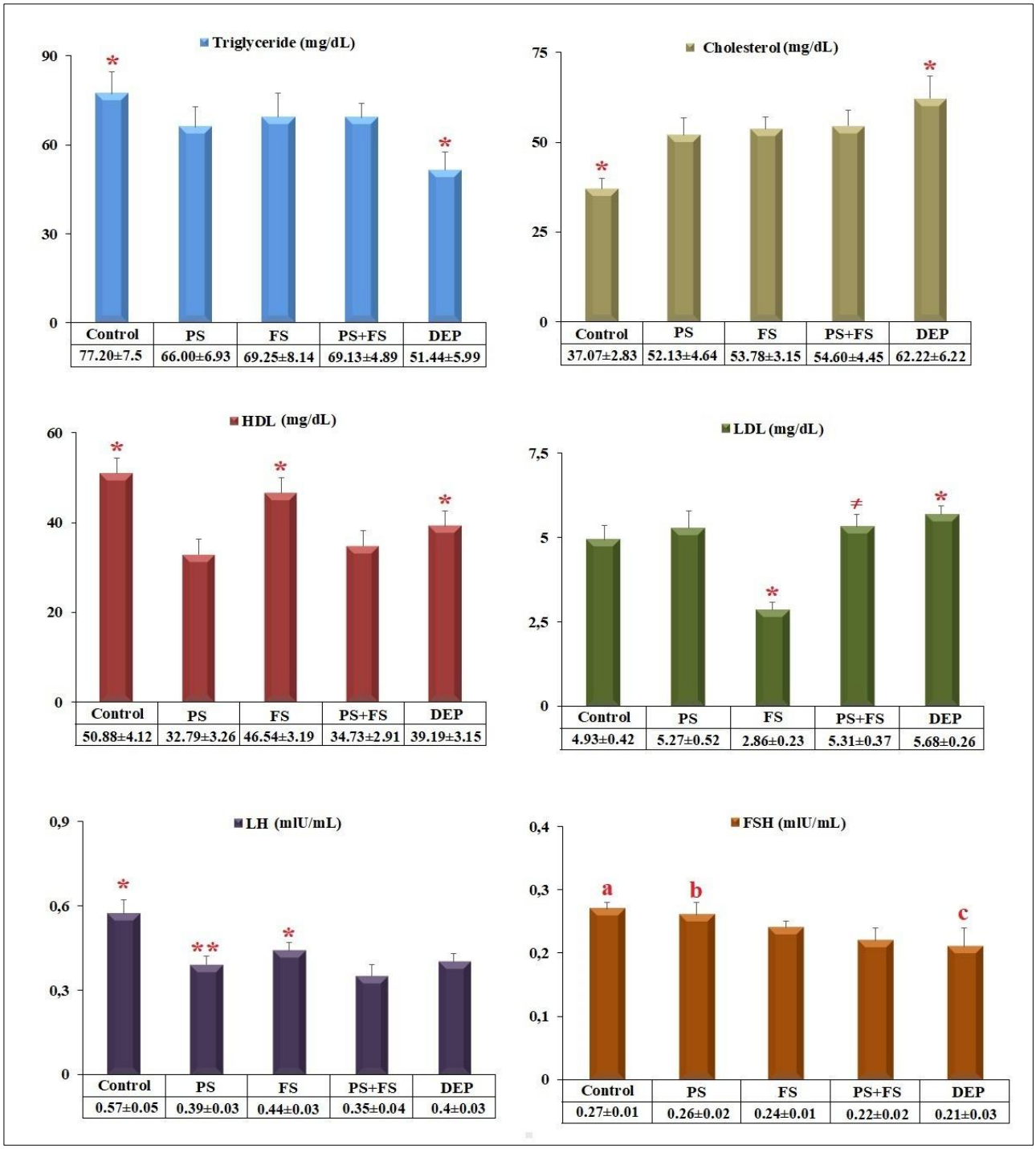

Figure 1: Comparison of the lipid profile and reproductive hormone levels measured in serum samples.

Triglyceride (mg/dL)

LDL; Low density lipoprotein (mg/L)

HDL; High density lipoprotein (mg/dL)

LH; Luteinizing hormone (mlU/dL)

FSH; Follicle stimulating hormone (mlU/dL)

*p: Significant when compared to other groups (p<0.001),

≠p: Significant when compared to psychological stress and psychological+physical stress groups (p<0.001),

[a]p: Significant when compared to psychological stress, psychological+physical stress, and depression groups (p<0.001),

[a]p: Significant when compared to psychological+physical stress and depression groups (p<0.001) Significant when compared to the control group (p<0.001),

[c]p: Significant when compared to the control, psychological stress, and physical stress groups.

Table 3: Scoring the histopathological, immunohistochemical, and immunofluorescence findings observed in testicular tissues.

	Control group	Psychological stress group	Physical stress group	Psychological stress + physical stress group	Depression group
Edema in intertubular spaces	-	++	++	+++	+++
Hyperemia in veins	-	++	+++	+++	+++
Degeneration of spermatocytes	-	+	++	++	+++
Decrease in the sperm count	-	++	++	+++	+++
Bax expression	-	++	++	+++	+++
8 OHdG expression	-	++	++	+++	+++

Table 4: Statistical assessment of immunohistochemical and immunofluorescence findings observed in testicular tissues.

	Control group	**Psychological stress group**	**Physical stress group**	**Psychological stress + physical stress group**	**Depression group**
Bax	86.55±2.78[a]	256.73±14.22[b]	248.22±9.85[b]	330.56±7.65[c]	335.42±6.38[c]
8 OHdG	123.48±2.56[a]	358.22±11.45[b]	362.28±8.79[b]	483.32±6.53[c]	488.73±9.46[c]

The results in the same row were compared with each other. Different letters include significant results.

For the statistical differences among groups ($p<0.05$), the results were expressed as mean±SD.

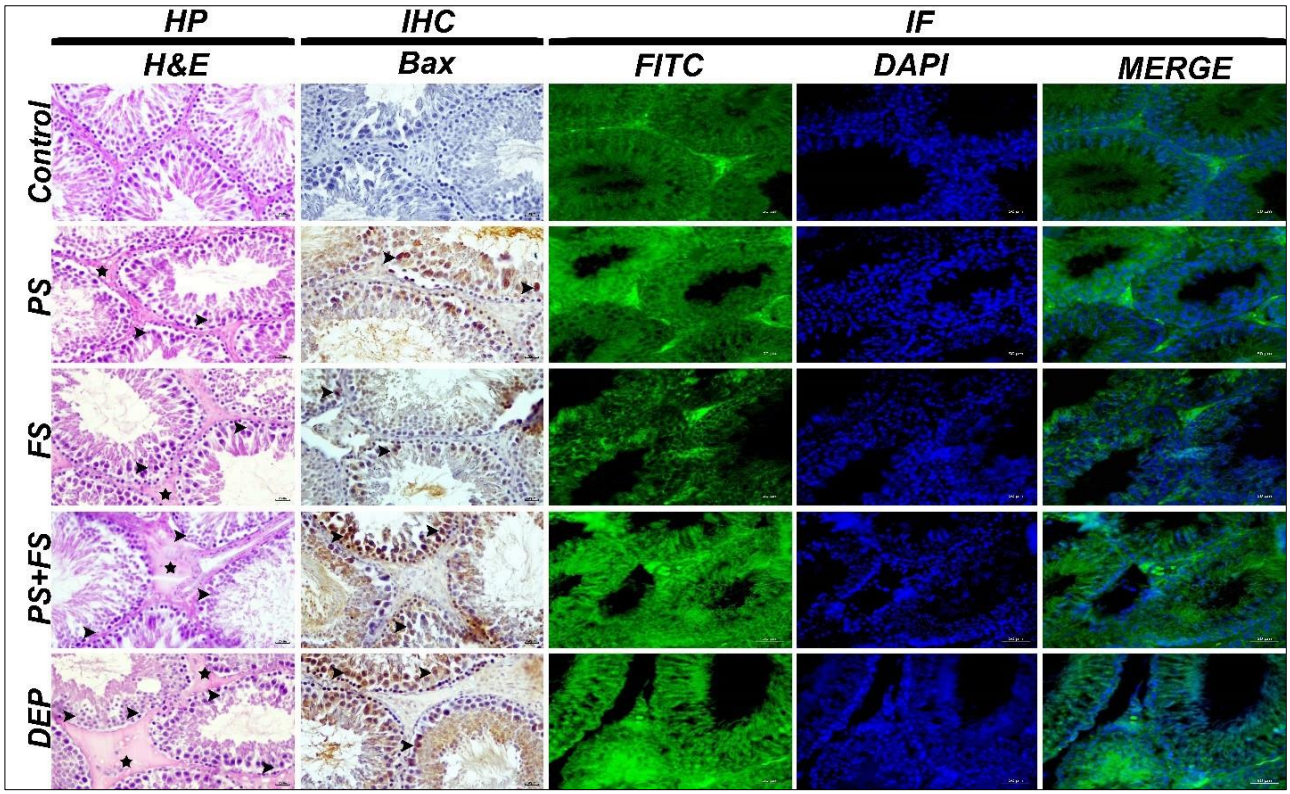

Figure 2: Testicular tissue, H&E-IHC, Bar: 20 µm. IF, Bar: 50 µm.

DISCUSSION AND CONCLUSION

The reaction occurs when the body is exposed to stress and varies depending on the intensity, unpredictability and uncontrollability of the stimulus (Koolhaas et al. 2011). In this case, increased glucocorticoid levels promote gluconeogenesis, mobilizeamino acids, and stimulate fat breakdown to maintain circulating glucose levels (Whirledge and Cidlowski 2010).

It is known that immobilization stress causes both psychological and physiological stress as it creates aggression and subsequent burnout (Yazawa et al. 1999). Animal models used to mimic the development and progression of clinical depression include chronic stress models like chronic unpredictable mild stress, chronic restraint stress, separation from the mother, and social isolation (Stepanichev et al. 2014; Wang et al. 2016). It is known that physical and psychological stress can affect reproductive ability in men. Various stressors activate the hypothalamic-pituitary-adrenal axis, suppress the hypothalamic-pituitary-testicular axis and cause dysfunction in the male reproductive system (Kirby et al. 2009).

The results obtained in the current study regarding sperm parameters are compatible with the depression study performed by applying the forced swimming test and unpredictable mild stress study (Roboon et al. 2016; Salami et al. 2020; Bagheri et al. 2021; Moustafa 2021). It is known that stress increases the formation of reactive oxygen species (ROS) in the male reproductive system. Therefore, ROS resulting from stress may have caused problems in sperm parameters (García-Díaz et al. 2015).

In histopathological evaluation, it can be said that the decrease in spermatogenic cell lines is compatible with the previous study (Fahim et al. 2019). Bax expression, which was detected at a severe level in the psychological stress + physical stress group and depression group, and at a moderate level in the physical stress and psychological stress groups, was compatible with previous stress studies (Fahim et al. 2019; Zou et al. 2019).

Findings obtained after immunofluorescence staining of testicular tissues in the current study; It supports the results of studies in which depression was induced by applying corticosterone and chronic restraint stress (Uchihara et al. 2016; Liu et al. 2019).

The hormonal evaluation revealed that the stress and depression groups had significantly lower FSH and LH values. This result supports the results of previous studies (Bagheri et al. 2021; Moustafa 2021). However, it differs from the result of another study (Mohamadpour et al. 2017). Because Mohamadpour et al (2017) stated in their study that FSH and LH levels elevated in stress groups. This may be due to the difference in the stress protocol and the duration of the application applied by the researchers

The results obtained regarding cholesterol levels in serum samples support the results of previous studies (Neves et al. 2009; Devaki et al. 2013; Zeeni et al. 2013; Kopalli et al. 2019). However, other studies reported that cholesterol and LDL levels decreased in stress groups (Lee et al. 2019; Pan et al. 2019). This may be due to the time difference in the applied stress.

The results of this study regarding triglycerides support previous studies (Lee et al. 2019; Pan et al. 2019). However, other studies reported that triglyceride values increased (Neves et al. 2009; Devaki et al. 2013; Kopalli et al. 2019). The reason for this increase may be the time difference in the applied protocols. In this study, the result showing that HDL values were low in the stress and depression groups is compatible with previous studies (Devaki et al. 2013; Zeeni et al. 2013; Kopalli et al. 2019; Lee et al. 2019; Pan et al. 2019).

In the current study, while the rate of abnormal sperm was found to be high in the stress and depression groups, the highest rate was found in the depression group. In the stress and depression groups, serum triglyceride and HDL levels, as well as LH and FSH levels, were found to be significantly lower, while cholesterol and LDL levels were significantly higher. In addition, cytoplasmic Bax and 8-OHdG expression was observed to be moderate in the psychological stress and physical stress groups, and severe in the psychological stress + physical stress and depression groups. It is thought that the results of this study will contribute to the literature on the relationship between stress and male fertility and may guide clinical studies in this field.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: SB, SA
Supervision / Consultancy: SB
Data Collection and / or Processing: SB
Analysis and / or Interpretation: SB, ZH, SY, SA
Writing the Article: SB
Critical Review: SB, ZH, SY, SA

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Chemical Composition and Physical Properties of Milk in Norduz Sheep

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ABSTRACT The objective of this study was to uncover the milk composition traits of Norduz sheep, which are bred in the Gürpınar district of the Van province, during the mid-term lactation period. The chosen ewes exhibited no signs of clinical or subclinical mastitis. A total of 104 sheep milk samples were meticulously collected to undergo comprehensive milk composition analysis. The chemical and physical attributes of Norduz sheep milk were methodically determined utilizing a milk autoanalyzer. The mean values for various milk constituents were as follows: milk fat (%), 2.48±0.11; solids-not-fat (SNF) (%), 10.76±0.08; milk protein (%), 5.09±0.04; lactose (%), 4.79±0.04; pH, 6.93±0.02; conductivity (mS/cm), 4.41±0.04; freezing point (°C), -0.602±0.80; salt (%), 0.8035±0.66; and density (kg/m^3), 1039.73±0.39. Noteworthy negative and statistically significant correlations were observed between milk fat and protein (r=-0.36, p<0.001), milk fat and SNF (r=-0.32, p<0.001), as well as milk fat and lactose (r=-0.36, p<0.001). Conversely, positive and significant correlations emerged between SNF and milk protein (r=0.90, p<0.001), SNF and salt (r=0.87, p<0.001), and SNF and lactose (r=0.90, p<0.001). In summation, the protein content of Norduz sheep's milk exceeded that of the majority of sheep breeds reared in Türkiye. These findings carry the potential to make a valuable contribution to enhancing the milk composition of Norduz sheep.

Keywords: *Sheep, Lactation, Milk, Milk protein.*

ÖZ

Norduz Koyunlarında Sütün Kimyasal Yapısı ve Fiziksel Özellikleri

Bu çalışmada, Van ili Gürpınar ilçesinde yetiştirilen Norduz koyunlarının orta laktasyon döneminde süt kompozisyon özelliklerinin ortaya konulması amaçlanmıştır. Koyunlar klinik ve subklinik mastitis yönünden değerlendirilmiş ve örnekler yalnızca sağlıklı koyunlardan toplanmıştır. Bu amaçla toplam 104 koyun sütü örneği toplandı. Norduz koyun sütünün kimyasal ve fiziksel özellikleri, süt otoanalizörü kullanılarak belirlenmiştir. Çalışmada Norduz sütünde süt yağı (%), 2.48±0.11; yağsız kuru madde (SNF) (%), 10.76±0,08; süt proteini (%), 5.09±0.04; laktoz (%), 4.79±0.04; pH, 6.93±0.02; iletkenlik (mS/cm), 4.41±0.04; donma noktası (°C),-0.602±0.80; tuz (%), 0.8035±0.66; ve yoğunluk (kg/m^3), 1039.73±0.39 olarak belirlenmiştir. Süt yağı ile protein (r=-0.36, p<0.001), süt yağı ile SNF (r=-0.32, p<0.001) ve süt yağı ile laktoz (r=-0.36, p<0.001) arasında negatif ve istatistiksel olarak anlamlı korelasyonlar gözlenmiştir. SNF ile süt proteini (r=0.90, p<0.001), SNF ile tuz (r=0.87, p<0.001) ve SNF ile laktoz (r=0.90, p<0.001) arasında ise pozitif ve anlamlı ilişkiler belirlenmiştir. Özetle, Norduz koyun sütünün protein içeriği Türkiye'de yetiştirilen koyun ırklarının çoğunluğunun protein içeriğinden yüksek olduğu tespit edildi. Bu bulgular Norduz koyunlarının süt bileşiminin arttırılmasına değerli bir katkı yapma potansiyeli taşımaktadır.

Anahtar Kelimeler: *Koyun, Laktasyon, Süt, Süt proteini.*

INTRODUCTION

Sheep are versatile animals, raised for their meat, milk, skin, and fleece. They are predominantly raised in developing countries across Asia and Africa. Türkiye stands out as a significant country in Asian sheep breeding (Aysondu et al. 2022). Based on current statistics, Türkiye boasts a sheep population of 44.6 million, encompassing various breeds (TUIK 2022). The majority of these breeds comprise indigenous breeds such as Akkaraman, Morkaraman, and Awassi. Due to its large sheep population, Turkey holds the second position in terms of sheep milk production after China (Mohapatra et al. 2019).

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Sheep milk holds significant nutritional value. The content of total solids and other components of sheep milk are higher than cow's milk. This characteristic allows for the utilization of smaller quantities of sheep milk in the production of dairy products (Wendorff and Haenlein 2017). The composition of sheep milk can be influenced by various factors, including genetic factors (breeds and genotypes), management practices, and the lactation stage. Studies indicate that there are variations in milk composition among the native sheep breeds of Türkiye (Yılmaz et al. 2004; Şahan et al. 2005; Ocak et al. 2009; Kiper and Alkan 2016; Koyun et al. 2021; Turgut et al. 2023).

The Norduz sheep breed is predominantly bred within the Gürpınar region of the Van province, primarily by smallholder farms. The population of Norduz sheep is recorded at 13,900 individuals (Dayan and Bingöl 2008). These sheep are recognized as a variant of the Akkaraman sheep breed. As a versatile breed, Norduz sheep are raised for their meat, milk, and wool production, so they are a crucial source of income for local breeders. The anticipated milk yield per lactation for Norduz sheep is approximately 137.2 kg, with a lactation period lasting roughly six months (Ocak et al. 2009). Notably, a substantial portion of Norduz sheep milk is used in the production of Van herbed cheese and butter (Yıldız and Aygün 2021). Nevertheless, limited information is available regarding the chemical composition and physical properties of Norduz sheep milk (Yılmaz et al. 2004; Ocak et al. 2009).

In light of these circumstances, the present study aimed to investigate the chemical composition and certain physical attributes of Norduz sheep milk during the mid-term lactation phase.

MATERIAL AND METHODS

This study was approved by Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (Approval no: 2023/08-02).

Collection of Milk Samples

This study conducted the assessment of a total of 104 Norduz sheep milk samples. Milk samples were taken from sheep that were not found to have clinical or subclinical mastitis. The collection of milk samples was carried out using 50 ml sterile tubes prior to the morning milking routine. These samples were collected from a flock situated within the Gürpınar district of the Van province. Throughout the milk collection process, the sheep were provided with pasture-based feeding. In addition, each animal was fed 0.35 kg of barley and 0.50 kg of hay daily. The collected milk samples were transported to the laboratory at a temperature of +4 °C to facilitate the subsequent analysis.

Analysis of Milk Samples

The milk samples underwent analysis through the utilization of a ultrasonic milk analyzer (Lactoscan SA Milkanalyzer, Nova Zagora, Bulgaria). Device were calibrated for sheep milk. For the analysis, a total of 15 ml of milk sample was used. Each sample was subjected to analysis twice, and the outcomes were recorded. The analysis encompassed the determination of various components including milk fat (%), solids-not-fat (SNF) (%), milk protein (%), lactose (%), pH, conductivity (mS/cm), freezing point (°C), salt (%), and density (kg/ m^3). Deviation of the device for fat, SNF, density, protein, lactose, freezing point and salts were ±0.06 (%), ±0.15 (%), ±0.3 kg/m^3, ±0.20 (%), ±0.005 °C, ±0.05 (%) respectively. The results of milk autoanalyzer were also verified, using methods described by Association of Official Analytical Chemists (AOAC). Total solids in milk assessed by AOAC Official Method 925.23 Solids (Total) in Milk. Protein content of in milk was determined by AOAC official method (Kjeldahl's method) (Barbano et al. 1990). Fat in raw milk was assessed by Gerber method (Kleyn et al. 2001). Following verification, the results of milk autoanalyzer were used for statistical analysis.

Statistical Analysis

Descriptive statistics were computed to summarize the distribution for each parameter of milk composition separately. To report the central tendency and variation of the collected milk samples, mean±SE values were used with median (Q1-Q3). Minimum and maximum values of each measurement were also reported for better interpretation of results. Pearson's correlation analysis was performed to examine the possible correlations between milk composition traits. A critical value of $p<0.05$ was considered as a criterion of significance and all analyses were conducted by using The Statistical Package for the Social Sciences (SPSS 26.0, IBM) software package.

RESULTS

The mean values for the various milk components were as follows: milk fat (%), 2.48±0.11; solids-not-fat (SNF) (%), 10.76±0.08; milk protein (%), 5.09±0.04; lactose (%), 4.79±0.04 (Table 1); pH, 6.93±0.02; conductivity (mS/cm), 4.41±0.04; freezing point (°C), -0.602±0; salt (%), 0.8035±0.66; and density (kg/m^3), 1039.73±0.39 (Table 2).

Table 1: Descriptive statistics showing milk components of Norduz sheep.

	N	Mean±SE	Median (Q1-Q3)	Min.	Max.
Fat (%)	104	2.48±0.11	2.4 (1.8-2.9)	0.53	8.60
SNF (%)	104	10.76±0.08	10.8 (10.4-11.2)	7.92	12.60
Protein (%)	104	5.09±0.04	5.1 (4.9-5.3)	3.49	5.96
Lactose (%)	104	4.79±0.04	4.9 (4.6-5)	3.30	5.64
Salt (%)	104	0.8035±0.66	0.81 (0.78-0.84.3)	0.54	0.95

Table 2: Descriptive statistics showing physical properties of Norduz sheep.

	N	Mean±SE	Median (Q1-Q3)	Min.	Max.
Density (kg/m^3)	104	1039.73± 0.39	40.3 (38.6-42.3)	21.77	48.24
Conductivity (mS/cm)	104	4.41±0.04	4.4 (4.1-4.7)	3.54	5.97
pH	104	6.93±0.02	6.9 (6.8-7)	5.95	7.80
Freezing point (°C)	104	-0.602±0	-0.6 (-0.6--0.6)	-0.71	-0.40

Significant and negative correlations were observed between milk fat and protein ($r=-0.36$, $p<0.001$), milk fat and SNF ($r=-0.32$, $p<0.001$), as well as milk fat and lactose ($r=-0.36$, $p<0.001$). Conversely, positive and significant correlations were identified between SNF and milk protein ($r=0.90$, $p<0.001$), SNF and salt ($r=0.87$, $p<0.001$), and SNF and lactose ($r=0.90$, $p<0.001$). The correlation coefficients between the various milk characteristics are presented in Table 3 and Figure 1.

Table 3: Correlations between milk characteristics.

		Fat (%)	SNF (%)	Density (kg/m³)	Protein (%)	Conductivity (mS/cm)	Lactose (%)	pH
SNF (%)	Pearson's r	-0.32***	—					
Density (kg/m³)	Pearson's r	-0.522***	0.894***	—				
Protein (%)	Pearson's r	-0.36***	0.902***	0.936***	—			
Conductivity (mS/cm)	Pearson's r	-0.026	-0.161	-0.26**	-0.267**	—		
Lactose (%)	Pearson's r	-0.366***	0.907***	0.94***	0.96***	-0.281**	—	
pH	Pearson's r	0.04	0.014	-0.052	-0.096	-0.191	-0.077	—
Salt (%)	Pearson's r	-0.436***	0.87***	0.947***	0.928***	-0.213*	0.928***	-0.075

Note: * p <0.05, ** p <0.01, *** p < 0.001.

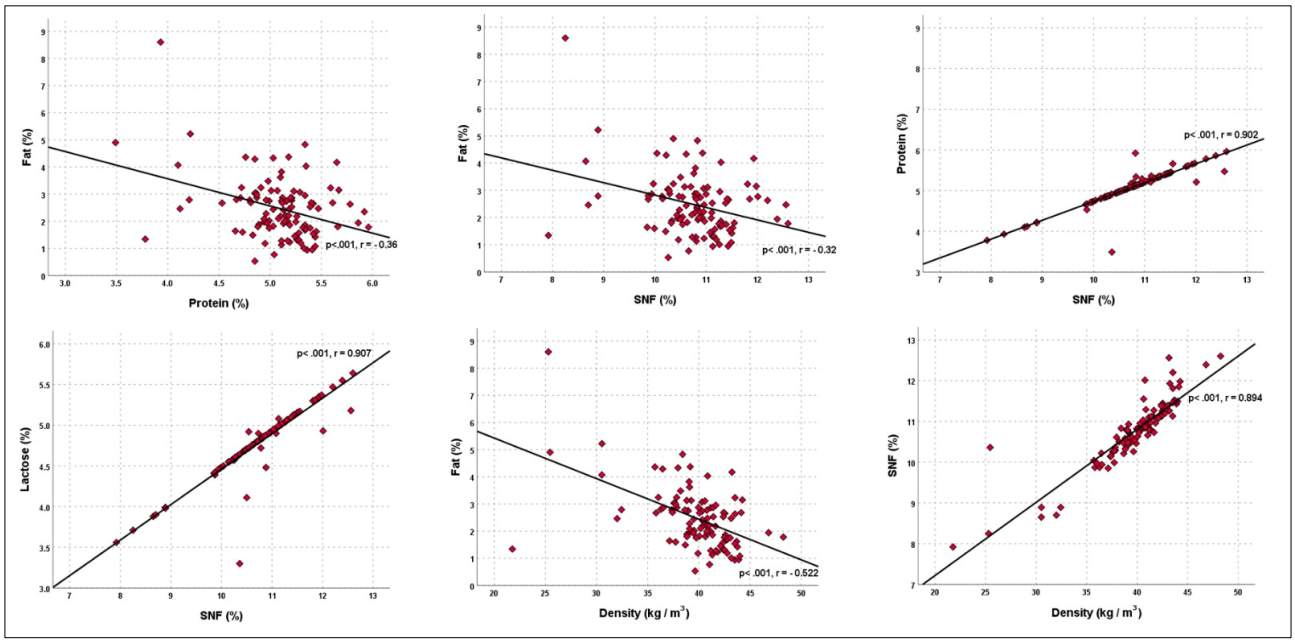

Figure 1: A scatter plot depicting the correlation between various milk characteristics.

DISCUSSION AND CONCLUSION

The current study revealed the milk composition of Norduz sheep during mid-term lactation. The milk composition of Norduz sheep has been investigated in a variety of studies. Previous research by Ocak et al. (2009) documented percentages of milk fat, SNF, and protein as 4.00±1.00, 10.56±1.50, and 7.4±0.69 during the early stage of lactation. In another study, percentages of milk fat, protein, and lactose were reported as 6.49±0.07, 6.11±0.08, and 5.07±0.17 (Yılmaz et al. 2004). This study, however, found notably lower milk fat percentages in Norduz sheep when compared to these other investigations. It's important to note that the stage of lactation significantly influences milk composition in sheep. Typically, a negative correlation exists between daily milk yield and milk composition (Pulina et al. 2005). Koncagül et al. (2012) elucidated that Norduz sheep exhibit a distinctive lactation curve, differing from other sheep breeds (Komprej et al. 2012; Kahraman et al. 2020). According to their findings, the daily milk yield of Norduz sheep peaks during the mid-term of lactation and subsequently decreases. Considering that the milk samples for this study were collected during the mid-term of lactation, it's plausible that the lower percentages of milk fat and protein in Norduz sheep could be attributed to their peak lactation phase. This distinctive timing may account for the observed disparities in milk composition when compared to previous studies in Norduz sheep.

Various studies have delved into the milk composition of native sheep breeds in Turkey, yielding diverse findings. For instance, in Awassi sheep, Şahan et al. (2005) documented percentages of milk fat, SNF, protein, and lactose as 6.61±1.33, 10.93±0.44, 5.68±0.47, and 4.34±0.27, respectively. Meanwhile, in Akkaraman sheep, the reported percentages of milk fat, protein, and lactose were 4.69±0.2, 4.76±0.07, and 5.58±0.08, respectively. In the case of Morkaraman sheep, Türkyılmaz et al. (2018) identified percentages of milk fat, SNF, protein, and lactose as 7.19±0.35, 9.67±0.26, 3.18±0.09, and 5.55±0.15, respectively. Moving to crossbreed Hamdani sheep, Turgut et al. (2023) observed percentages of milk fat, SNF, protein, and lactose as 7.49±0.15, 8.69±0.08, 4.13±0.04, and 3.89±0.04, respectively. Notably, comparisons between Norduz sheep and other breeds reveal higher milk fat percentages in Karayaka (Kiper and Alkan, 2016) and Tuj (Türkyılmaz et al. 2018) sheep. On the other hand, Slovenian Bovec and Istrian Pramenka dairy sheep breeds exhibited milk fat and protein percentages of 6.59±1.60, 5.53±1.14, and 7.20±1.62, 5.63±0.90, respectively (Komprej et al. 2012). Meanwhile, in Sarda sheep, the percentages of milk fat and protein were 6.70 and 6.09. Comparable values were reported in Chios sheep, with milk fat and protein percentages of 6.60 and 6.05 (Pulina et al. 2016).

The composition of sheep milk is significantly influenced by nutritional factors. Generally, milk composition tends to be more favorable in dairy sheep breeds subjected to intensive management practices (Pulina et al. 2005). However, in Turkey, sheep primarily graze on pastures,

with only a small quantity of hay and grains added to their diet, a practice observed in Norduz sheep as well. Within the Gürpınar district of the Van province, sheep predominantly rely on pasture feeding, particularly during the plant-rich spring season. It has been proposed that the elevated protein percentage in Norduz sheep milk could be attributed to the plant composition prevalent in the region (Ocak et al. 2009). This study further supports the findings of Ocak et al. (2009), revealing that during the mid-term lactation phase, the milk protein percentage of Norduz sheep surpasses that of other native Turkish sheep breeds. Thus, it can be inferred that the plant composition of the region significantly impacts the milk composition of Norduz sheep. Additionally, these outcomes underscore the suitability of Norduz sheep milk for cheese production due to its elevated protein content.

This study revealed noteworthy correlations among milk components. A significant negative correlation ($r=-0.36$, $p<0.001$) was identified between milk fat and protein percentages, and a similar negative correlation ($r=-0.366$, $p<0.001$) observed between milk fat and lactose percentages. This observation aligns with findings by Turgut et al. (2023) in crossbreed Hamdani sheep, where a negative and significant correlation between milk fat and protein as well as milk fat and lactose content was noted, akin to the current study. However, Pavić et al. (2002) reported a contrasting outcome, revealing a positive and significant correlation between milk fat and protein percentages. Additionally, this study unveiled positive correlations between solids-not-fat (SNF) and milk protein and lactose content. This can be attributed to the fact that protein, lactose, and minerals constitute the primary constituents of SNF, rendering the observed positive correlations between SNF and protein and lactose content plausible.

Across diverse native sheep breeds of Türkiye, including Norduz sheep, the pH range of milk typically falls between 6.5 and 6.9 (Şahan et al. 2005; Akdağ et al. 2018; Koyun et al. 2021; Turgut et al. 2023). In accordance with these previous findings, the present study established a milk pH of 6.93, corroborating similar reports. The density of sheep milk serves as a significant indicator for assessing milk components. Prior research indicated sheep milk density values of 1.030 kg/m^3 in Morkaraman, Tuj, and Awassi sheep (Türkyılmaz et al. 2018). In alignment with these results, the current study's findings revealed a sheep milk density of 1.039, which closely mirrors the reports in Norduz sheep by Ocak et al. (2009).

Indeed, the density of sheep milk tends to decrease with higher fat content due to the lower density of milk fat molecules. This phenomenon has been highlighted by Turgut et al. (2023), who suggested that such a relationship could result in a negative correlation between milk fat and density in crossbred Hamdani sheep. Similarly, in the present study, a negative correlation ($r=-0.522$) that holds statistical significance ($p<0.001$) was observed between milk fat and density. This aligns with the anticipated trend stemming from the milk composition.

It's important to emphasize that milk composition is notably influenced by genetic factors. Particularly, genetic variations within genes associated with milk traits can exert a significant impact on milk composition (Koyun et al. 2021). Given this consideration, the exploration of genetic variations tied to milk composition through molecular techniques could potentially offer a means to enhance the milk composition of Norduz sheep. This avenue of research holds the promise of fostering improvements in the quality and attributes of Norduz sheep milk.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: DK
Supervision / Consultancy: DK, NÇ
Data Collection and / or Processing: DK, NÇ, AOT, BK
Analysis and / or Interpretation: SÜ, EG
Writing the Article: DK, AOT
Critical Review: DK, AOT, NÇ

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Aydın İlinde Yetiştirilen Atlarda Tespit Edilen Gastrointestinal Sistem Parazitlerinin Dağılımı

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ÖZ

Çalışma, Aydın ilinde meralarda beslenen atlarda dışkı muayenesi yapılarak sindirim sistemi parazitlerinin çeşitliliğinin ve yaygınlığının belirlenmesi amacıyla yapılmıştır. Bu amaçla farklı yaş, cinsiyet ve ırktan toplam 242 attan taze dışkı örnekleri alınmış ve Fülleborn'un doymuş tuzlu su flotasyon ve Benedect sedimentasyon yöntemleriyle muayene edilmiştir. Çalışmada farklı ırklara ait toplam 242 at dışkı örneği incelenmiş ve 182 hayvan (%75.20) sindirim sistemi parazitleri yönünden pozitif olarak değerlendirilmiştir. Elde edilen bulgular sonucunda örneklerin 150'sinde tekli, 32'sinde ikili miks enfeksiyonlar saptanmıştır. Örneklerin, 180'inde (%74.38) Strongylid tip yumurta, 7'sinde (%2.89) *Anaplocephalidae spp*, 4'ünde (%1.65*) Parascaris equorum*, 7'sinde (%2.89) *Dicrocoelium dendriticum*, 6'sında (%2.47) *Fasciola spp*. yumurtaları tespit edilirken, 10'unda (%4.13) ise *Eimeria spp*. ookistleri saptanmıştır. Aydın iline ait ilçelerde yetiştirilen atlarda mera kaynaklı gastrointestinal sistem enfeksiyonlarının oldukça yaygın olduğu görülmüş ve bu parazitlere karşı yetiştiricilerin bilgilendirilerek etkili mücadele programlarının oluşturulması gerektiği belirlenmiştir.

Anahtar Kelimeler: *At, Dışkı, Parazitik intestinal hastalıklar, Prevalans.*

ABSTRACT

The Distribution of Gastrointestinal System Parasites Detected in Horses in Aydın

In the present study aimed to determine the diversity and prevalence of gastrointestinal parasites in horses grazing on pastures in Aydın province through fecal examination. Fresh fecal samples were collected from a total of 242 horses of different ages, genders, and breeds. The samples were examined using Fülleborn's saturated salt flotation and Benedect sedimentation methods. According to the fecal examinations, 182 animals (75.20%) were assessed as positive for digestive system parasites and based on the obtained findings, singular and mixed infections were detected in 150 and 32 samples, respectively. Among the findings, Strongylid-type eggs were detected in 180 samples (74.38%), *Anaplocephalidae spp*. in 7 samples (2.89%), *Parascaris equorum* in 4 samples (1.65%), *Dicrocoelium dendriticum* in 7 samples (2.89%), *Fasciola spp*. eggs in 6 samples (2.47%), and *Eimeria spp*. oocysts in 10 samples (4.13%). The study revealed a high prevalence of pasture-related gastrointestinal infections in horses raised in various districts of Aydın province. Consequently, it is recommended that horse breeders be educated and effective control programs be established to combat these parasites.

Keywords: *Feces, Horse, Parasitic intestinal diseases, Prevalence.*

GİRİŞ

Motorlu taşıtların yaygın olarak kullanıma girmesinin ardından, tek tırnaklı hayvanlar ulaşımda eski önemini yitirmekle beraber, taşıt kullanımının zor olduğu durumlarda, kırsal kesimlerde, tarım işlerinde, sportif faaliyetlerde askeri ve turizm amaçlı olarak günümüzde hala kendisine kullanım alanı bulmaktadır. Türkiye İstatistik Kurumu (TÜİK)'nun 2022 verilerine göre Türkiye'de tek tırnaklı hayvan sayısı son on yılda %50'ye yakın azalma gösterse de hala 83.718 baş at, 95.809 baş eşek, 22.164 baş katır bulunmaktadır.

Atlarda görülen bakteriyel, viral ve paraziter hastalıklar hayvanların sağlığını etkilemekte ve ciddi performans düşüklüğüne, hatta ölümlere neden olmaktadır. Gelişmekte olan ülkelerde at ve eşekler için en önemli problem gastrointestinal sisteme yerleşen parazitlerdir (Pereira ve ark. 2006). Özellikle sindirim sistemine yerleşen parazitler otlayan atlarda oldukça sık görülmekte olup, başta nematodlar olmak üzere sestod, trematod ve daha az yoğun olarak da protozoon ve artropodlara da rastlanmaktadır. Bu parazitler atlarda gelişme geriliği, yemden yararlanmada azalma, ağırlık kaybı ile kolik ve diyare gibi çeşitli klinik bulgulara neden olmaktadır (Öge 2003; Teixeira ve ark. 2014; Buzatu ve ark. 2016).

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Ayrıca gastrointestinal sistem parazitleri konaklara sadece doğrudan zarar vermekle kalmaz, aynı zamanda enfekte hayvanların bağışıklığını düşürerek çeşitli hastalıklara yatkın hale gelmelerine ve hatta ölümlerine neden olabilmektedir (Buzatu ve ark. 2016).

Çırak ve Girişgin (2021) yaptıkları derlemede Türkiye'de atlarda 62, eşeklerde 52, katırlarda 21 farklı helmintin tespit edildiğini bildirmişlerdir. Bu parazitler arasında küçük Strongylidae'ler (*Cyathostomum spp*., *Cylicostephanus spp.*), büyük Strongylidae'ler (*Strongylus vulgaris*, *S. equinus*, *S. edentatus*), *Parascaris equorum*, *Oxyuris equi*, *Strongyloides westeri*, *Trichostrongylus axei*, *Habronema spp*., *Dictyocaulus arnfieldi* ve Anoplocephala türleri yer almaktadır. Ayrıca trematodlardan zoonoz etkenler olan *Dicrocoelium dendriticum* ve Fasciola gibi türlere de rastlanılmaktadır. Daha az yoğun tespit edilmekle beraber şimdiye dek atlardan 13, eşeklerden 13 ve katırlardan 3 protozoon türü; yine atlardan 40, eşeklerden 23 ve katırlardan 6 tür artropod bildirilmiştir (Clairton ve ark. 2017; Çırak ve Girişgin 2021).

Helmintlerin teşhisinde; uygulaması kolay ve özellikle moleküler yöntemlere göre oldukça ucuz olan flotasyon, sedimantasyon ve Baerman-Wetzel gibi konvansiyonel yöntemler sıklıkla tercih edilmektedir. Türkiye'nin farklı yörelerinde yaşayan atlarda dışkı bakısında bahsedilen bu yöntemleri kullanarak yapılan sindirim sistemi parazitlerinin yaygınlığını inceleyen pek çok çalışma bulunmaktadır. Çırak ve Girişgin (2021) yaptıkları derlemede bu çalışmalarda Türkiye'de at, eşek ve katırlarda kaydedilen paraziter enfeksiyonları detaylı şekilde göstermiştir. Deniz ve ark. (2008)'nın *Babesia* yönünden yaptıkları çalışma haricinde Aydın ilinde tek tırnaklıların paraziter enfeksiyonlarını belirlemeye yönelik herhangi bir bilimsel veriye rastlanamamıştır. Bu doğrultuda bu çalışma ile Aydın ilinde bulunan atlarda dışkı muayenesi yapılarak sindirim sistemi parazitlerinin çeşitliliğinin ve yaygınlığının belirlenmesi hedeflenmiştir.

MATERYAL VE METOT

Bu çalışma, Aydın Adnan Menderes Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan 26.08.2021 tarihinde 64583101/2021/118 sayılı izin alınarak yapılmıştır.

Materyal

Çalışmanın materyalini; Aydın ilinin farklı ilçelerinden (Kuşadası, Söke, Didim, Germencik, İncirliova, Koçarlı, Çine, Efeler, Köşk, Sultanhisar, Bozdoğan, Nazilli, Kuyucak, Karacasu ve Buharkent) (Şekil 1)

Şekil 1: Çalışmada kullanılan materyalin coğrafi dağılımı.

Figure 1: Geographic distribution of the material used in the study.

özellikle otlaklarda otlatılarak beslenen 129'u dişi (%53.3) 113'ü (%46.7) erkek olmak üzere toplam 242 attan toplanan dışkı örneği oluşturmaktadır. Toplanan örneklerin ilçelere göre dağılımı Tablo1'de verilmiştir. Çalışmaya dahil edilen atların %50'si 7 yaş ve üzeri, %32.2'si 4-6 yaş aralığında ve %17.8'i ise 1-3 yaş aralığındadır. Bununla beraber çalışmada kullanılan atların ırklara göre dağılımı ise; Melez (%54.1), Arap (%33.1) ve Rahvan (%12.8) şeklinde gerçekleşmiştir.

Tablo 1: İlçelere göre toplanan örnek sayıları.

Table 1: Number of collected samples according to districts.

İlçe adı	n*	%
İncirliova	17	7.0
Germencik	18	7.4
Bozdoğan	12	5.0
Kuyucak	34	14.0
Buharkent	10	4.1
Karacasu	16	6.6
Merkez	16	6.6
Nazilli	16	6.6
Köşk	8	3.3
Sultanhisar	15	6.2
Çine	12	5.0
Didim	10	4.1
Kuşadası	16	6.6
Söke	27	11.2
Koçarlı	15	6.2
	242	100

*n= Hayvan sayısı.

Dışkı Örneklerinin Toplanması

Özellikle serbest olarak otlaklarda beslenen, tedavi veya koruyucu amaçlı antiparaziter uygulama yapılmamış hayvanların rektumundan, yaklaşık 200-300 gram dışkı örneği alınarak örnekler poşetler içerisine konulmuş, her bir örneğin kodlaması yapılarak hayvana ait ırk, yaş, cinsiyet gibi bilgileri not edilmiştir. Örnekleri rektumdan almanın mümkün olmadığı durumlarda taze dışkı örnekleri dışkının yere temas etmeyen yüzeyinden alınmıştır. Alınan dışkı örnekleri aynı gün içerisinde Parazitoloji Anabilim Dalı Laboratuvarına getirilerek incelenmiştir. Hemen incelemesi yapılamayacak örnekler +4°C de muhafaza edilerek maksimum 24 saat içerisinde incelemeleri yapılmıştır.

Mikroskobik Bakı

Laboratuvara getirilen dışkı örnekleri mikroskobik incelemeye alınmadan önce sestod halkaları yönünden makroskobik olarak incelenmiştir. Makroskobik inceleme sonrasında helmint yumurtaları ve protozoon ookistleri yönünden önce nativ muayene yapılmıştır. Daha sonra Fülleborn'un (Thienpoint ve ark. 1986) doymuş tuzlu su yöntemi kullanılarak flotasyon yöntemi uygulanmış, hemen ardından Benedect (Thienpoint ve ark. 1986) sedimantasyon yöntemi ile örneklerin incelenmesi tamamlanmıştır.

İstatiksel Analiz

Çalışmaya alınan değişkenlere ait tanımlayıcı istatistikler hesaplanmış ve "Frekans-Yüzde" şeklinde gösterilmiştir. Flotasyon ve sedimentasyon sonucu belirlenen parazit varlığı ile bölge, yaş, ırk ve cinsiyet özellikleri arasındaki ilişkileri değerlendirmek için ki-kare testi kullanılmış, $p<0.05$ olan farklılıklar istatistiksel olarak anlamlı kabul edilmiştir. İstatistiksel analizler Stata 15.1 programı kullanılarak gerçekleştirilmiştir.

BULGULAR

Çalışmada 80 arap, 131 melez ve 31 rahvan, olmak üzere toplam 242 at dışkısı incelenmiş ve 180 hayvanda (%75.20) çeşitli paraziter enfeksiyonlar saptanmıştır (Tablo 2). Enfekte at ırkları arasında paraziter enfeksiyon dağılımı incelendiğinde; Arap atlarında toplam enfeksiyon oranı %80, melez ırklarda %77.86 ve rahvan atlarında %45.16 olarak saptanmıştır (Tablo 2).

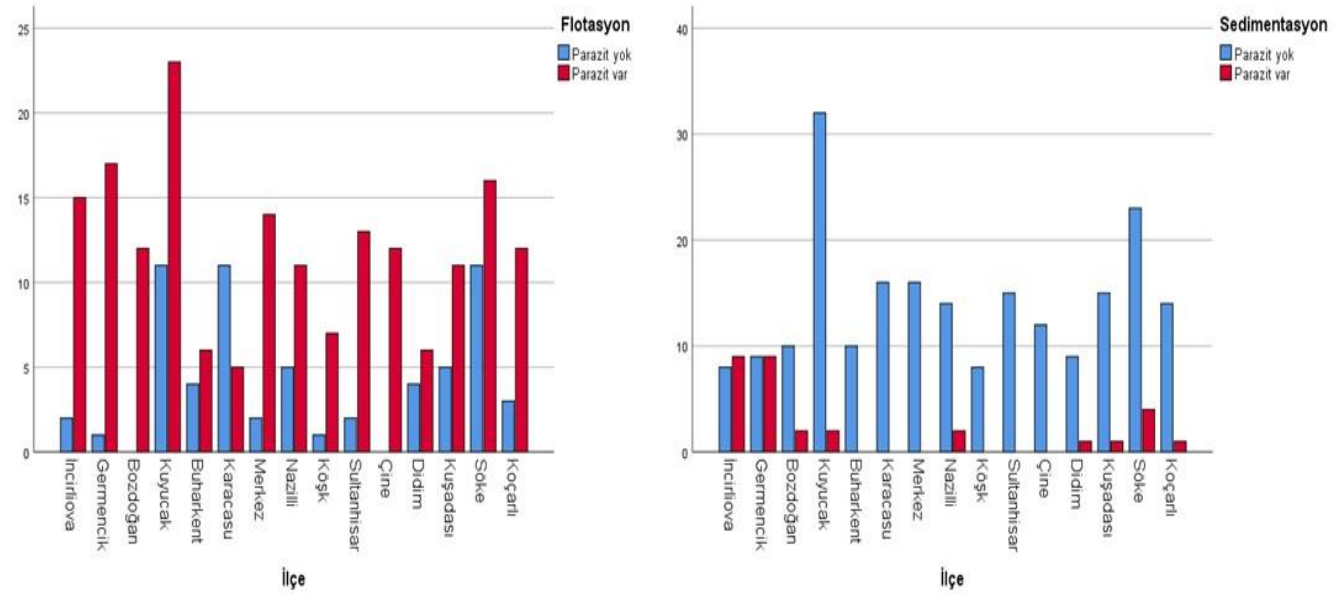
Enfekte bulunan atlarda en fazla Stronglidae ailesine ait Strongylid tip yumurta saptanırken (%74.38), Eimeria spp. %4.13, *Anaplocephalidae spp* ve *Dicrocoelium dentriticum* %2.89, *Fasciola hepatica* %2.47, *Parsascaris equorum* %1.65 oranında belirlenmiştir (Tablo 2). Enfekte atlardan 150'sinin (%61.98)tek türle, 32 atın ise farklı iki tür ile (%13.22) enfekte olduğu belirlenmiştir.

Tablo 2: Dışkı muayenesi yapılan örneklerin ve saptanan paraziter enfeksiyonların at ırklarına göre dağılımı.

Table 2: Distribution of examined stool samples and detected parasitic infections according to breeds.

Parazit türü	Arap	Melez	Rahvan
Strongylidae spp	63	102	14
Parascaris equorum	_	4	_
Anaplocephalidae spp	2	5	_
Dicrocoelium dentriticum	1	6	_
Fasciola hepatica	3	3	_
Eimeria spp.	2	8	_
Enfekte hayvan sayısı	**64**	**102**	**14**
Toplam hayvan sayısı	**80**	**131**	**31**
Enfeksiyon oranı (%)	**80**	**73.2**	**45.16**

* Irklar arasında istatistiksel olarak anlamlı fark belirlenmiştir ($p<0.001$).



Şekil 2: İlçelere ve yöntemlere göre parazit varlığının grafik dağılımı.

Figure 2: Graphic distribution of parasite presence according to districts and methods.

Flotasyon yöntemi ile incelenen at örneklerinde hayvanlar yaş ve cinsiyet gruplarına göre değerlendirildiğinde paraziter enfeksiyonlar açısından istatistiksel olarak anlamlı bir fark görülmemiştir, buna karşın örnek toplanan ilçe ve ırklara ($p<0.001$) göre $p<0.05$ olarak tespit edildiğinden fark anlamlı olarak değerlendirilmiştir. Sedimantasyon yöntemi ile incelenen örneklerde ise ırk ($p<0.001$), yaş ($p=0.003$) ve bölgelere ($p<0.001$) göre istatistiki olarak anlamlı fark saptanmıştır, fakat cinsiyet gruplarına göre anlamlı bir fark belirlenememiştir.

Enfeksiyon oranları bir- üç yaş grubunda %79.06, dört-altı yaş grubunda %66.66 ve yedi yaş üzeri hayvanlarda ise %79.33 olarak belirlenmiştir (Tablo 3). Tüm yaş gruplarında en sık olarak Strongylid tip yumurtalara rastlanılmış, bunu Eimeria ookistleri takip etmiştir. Çalışmada kullanılan örneklerde iki cinsiyet arasındaki enfeksiyon oranları incelendiğinde her iki teşhis yönteminde de istatistiki olarak anlamlı bir fark belirlenememiştir.

Tablo 3: Yaş gruplarına göre tespit edilen parazit türleri.

Table 3: Detected parasite species according to age groups.

	Enfekte at yaşı						
	1-3 yaş n (%) (n=43)		4-6 yaş n (%) (n=78)		7 ve üzeri yaşlı n (%) (n=121)		Toplam n (%) (n=242)
Parazit türü	**Dişi**	**Erkek**	**Dişi**	**Erkek**	**Dişi**	**Erkek**	
Strongylidae sp	18 (41.86)	16 (37.20)	29 (37.17)	23 (29.48)	52(42.97)	42 (34.71)	180 (74.38)
P. equorum	3 (6.97)	_	_	1 (1.28)	_	_	4 (1.65)
Anaplocephalidae sp	_	1 (2.32)	_	2 (2.56)	4 (3.30)	_	7 (2.89)
D. dendriticum	_	1 (2.32)	2 (2.56)	_	_	4 (3.30)	7 (2.89)
F. hepatica	2 (4.65)	2 (4.65)	1 (1.28)	1 (1.28)	_	_	6 (2.47)
Eimeria spp.	_	_	2 (2.56)	_	6 (4.95)	2 (1.65)	10 (4.13)
Enfekte hayvan sayısı (% enfeksiyon oranı)	34 (79.06)		52 (66.66)		96 (79.33)		

n= Hayvan sayısı

TARTIŞMA VE SONUÇ

Merada beslenen atlarda helmint enfeksiyonları başta olmak üzere paraziter enfeksiyonlar daha sık olarak görülmektedir. Bu çalışmada mera ile iletişimi olan ve bu alanlarda beslenen atlar bölgedeki parazit yaygınlığın ve çeşitliliğin ortaya konulabilmesi amacıyla özellikle seçilmiştir. Çalışmada incelenen at dışkılarında daha önceki çalışmalarda da sıklıkla tespit edilen *Strongylidae spp*, *P. equorum*, *Anaplocephalidae spp*, *D. dendriticum*, *F. hepatica, Eimeria spp.* türleri saptanmıştır. Gastrointestinal parazitler gelişmekte olan ülkelerde atların önemli problemleri arasında gelmektedir. Bu durum hayvanlarda sağlığı olumsuz yönde etkilemekte aynı zamanda performansı da düşürmektedir (Pereira ve ark. 2006). Dünyada atlarda dışkı ve otopsi bulgularına bakılarak yapılan çalışmalarda sindirim sistemi paraziter enfeksiyonlarının yaygınlığı %27.6-100 (Dunsmore ve Jue 1985; Pereira ve Vianna 2006; Mezgebu ve ark. 2013) arasında belirlenmiştir. Türkiye'nin farklı yörelerinde yapılan çeşitli araştırmalarda ise paraziter enfeksiyon yayılışının %16.2-100 arasında olduğu bildirilmiştir (Öge 1991, Arslan ve Umur 1998, Bakırcı ve ark. 2004, Uslu ve Güçlü 2007, Toktamış ve Yaman 2012, Aypak 2013, Kozan ve Güzel 2015, Ceylan ve ark. 2020). Tüm dünyada yapılan çalışmaların sonuçlarına benzer şekilde bu çalışmada dışkı bakısı ile atlarda saptanan sindirim sistemi parazitlerinin oranı %74.38 olarak saptanmıştır.

Atların helmintleriyle ilgili dünyada ve Türkiye'de yapılan çalışmalarda en sık rastlanan tür Stronglidae ailesine ait türler olmuştur. Tüm dünyada dışkı ve otopsi bakılarına göre parazitin atlarda yayılışı %62.7-100 (Öge 1991, Uslu ve Güçlü 2007, Mezgebu ve ark. 2013, Asefa ve Dulo 2017, Mathewos ve ark. 2021) olarak belirlenmiştir. Türkiye'de ise bu oranın %1-100 arasında değiştiği bildirilmektedir (Çırak ve Girişgin 2021). Özellikle meralarda beslenen hayvanlarda Strongyloides enfeksiyonlarının ahırda yetiştirilen atlara oranla daha fazla görüldüğü bildirilmektedir (Toktamış ve Yaman 2012). Bu çalışmada merada beslenen atların dışkılarından yapılan incelemelerde literatür bilgiye uyumlu olarak Stronglidae ailesine ait türlerin yaygınlığı yüksek bulunmuş ve %74.38 olarak tespit edilmiştir.

Literatür bilgiye göre Parascaris enfeksiyonlarına, bağışıklığın paraziter etkenin alınmasından sonra gelişmesi nedeniyle yaşlı hayvanlarda daha az oranda rastlanılırken genellikle taylarda veya yaşça genç olan atlarda görülmekte ve asemptomatik olarak seyretmektedir (Suderman ve ark. 1997, Cribb ve ark. 2006, Upjohn ve ark. 2010, Von Samson-Himmelstjerna 2012). Dünyada P. equorum'un dağılımı %1.7-22.4 (Dunsmore ve Jue 1985; Lyons ve Tolliver 2004; Eslami ve ark. 2005; Pereira ve Vianna 2006), benzer olarak Türkiye'de bu oran %1-37 arasındadır (Demir ve ark. 1995; Pişkin ve ark. 1999; Aydenizöz 2004; Gül ve ark. 2003; Bakırcı ve ark. 2004; Altaş ve ark. 2005; Karaca ve ark. 2005; Uslu ve Güçlü 2007; Umur ve Açıcı 2009; Esatgil ve Efil 2012; Kozan ve Güzel 2015). Bu çalışmada P. equorum %1.65 oranında saptanmış olup, genç atlarda parazit saptanırken 7 yaş ve üzeri hayvanlarda belirlenememiştir.

Atların sestodlarından Anoplocephalidae ailesine ait *A. perfoliata* hayvanların sindirim sistemlerinde önemli bir hastalık tablosu oluşturmakta ve en sık rastlanan sestod olarak bilinmekte, bunu A. magna izlemektedir (Pişkin ve ark. 1999; Çırak ve ark. 2004, Gasser 2005). Anoplocephalidae enfeksiyonları dünyanın çeşitli bölgelerinde yapılan çalışmalar sonucunda %1-66.6 (Hinney et al. 2011, Tomczuk et al. 2015, Slivinska et al. 2016, Hedberg-Alm ve ark. 2020, Ilić ve ark. 2022), Türkiye'de ise %1-15.8 (Öge 2002; Aydenizöz 2004; Altaş ve ark. 2005; Umur ve Açıcı 2009; Toktamış ve Yaman 2012) arasında tespit edilmiştir. Bu çalışmada Anoplacephalidae ailesine ait parazitlerin yaygınlığı %2.89 olarak saptanmış ve elde edilen sonuç Türkiye'de yapılan çalışmalarla uyumlu bulunmuştur. Yapılan çalışmalarda post mortem olarak incelenen hayvanlarda parazitemi oranlarının dışkı bakısı ile incelenenlere oranla daha yüksek olduğu saptanmıştır. Ayrıca parazite karşı şekillenen antikorların kan serumu ve tükürükteki oranları incelenmiş ve parazit oranı dışkı bakısıyla kıyaslandığında serolojik yöntemler ile daha yüksek sonuçlar elde edildiği görülmüştür (Jürgenschellert 2020). Bu çalışmada klasik flotasyon yönteminin kullanıldığı düşünüldüğünde sahadaki hayvanların Anoplacephalidae ailesine ait parazitlerle daha yüksek oranlarda enfekte olduğu kanısına varılmıştır.

Atların trematodlardan olan *F. hepatica* ve *D. dentriticum*' un yaygınlığı Türkiye'deki çalışmalarda sırasıyla %0.9- 3.6 ve %1.1-3 oranlarında (Demir ve ark. 1995, Gül ve ark. 2003, Uslu ve Güçlü 2007; Umur ve Açıcı 2009; Avcıoğlu ve ark. 2016) saptanmış olup, bu çalışmada söz konusu

helmintlerin görülme sıklığı sırasıyla %2.47 ve %2.89 olarak belirlenmiştir.

Sindirim kanalına yerleşen protozoonlardan *Eimeria leuckarti*'nin Türkiye'de tek tırnaklılarda yapılan çalışmalarla %0.6 ile %100 (Oğuz 1971; Tınar ve ark. 1994; Arslan ve ark. 1998; Gülegen ve ark. 2016) arasında yayılış gösterdiği ortaya konulmuştur. Bu çalışmada belirtilen parazit türüne rastlanmazken, incelenen on atın (%4.13) dışkısında farklı Eimeria ookistleri saptanmış fakat tür tayini için sporlandırma yapılamamıştır.

Bu çalışmada elde edilen sonuçlar istatiksel olarak incelenmiştir. Elde edilen bulgular ırk ve ilçeler arasında anlamlı farklılıklar ortaya koymuştur. İlçelere göre parazit var/yok incelemesi yapıldığında flotasyon yönteminde Karacasu ilçesi hariç tüm ilçelerde incelenen atlarda parazit varlığı daha fazla iken, sedimantasyon yönteminde İncirliova ilçesi hariç diğer tüm ilçelerde parazit yokluğu daha fazla görülmektedir (Şekil 2).

Bölgeler arasında belirlenen bu farklılık kullanılan yöntemden ziyade bu bölgelerde yetiştirilen at ırklarıyla ilgili olarak bulunmuştur. Karacasu ilçesinde daha çok yarışlarda kullanılan rahvan atları yetiştirilmektedir ve materyal genellikle bu atlardan toplanmıştır. Yetiştirici bu özellikteki atlara daha özenle bakım-besleme yapmakta, bu nedenle antiparaziter uygulamaların daha düzenli yapıldığı bilinmektedir. Uygulanan daha iyi bakım-besleme koşullarının etkisiyle istatistiki olarak anlamlı bir fark belirlenmiş ve bakım koşullarının geliştirilmesinin atlardaki enfeksiyon oranı üzerine olumlu etkileri gözlemlenmiştir.

Sonuç olarak bu çalışmanın yapıldığı Aydın iline ait ilçelerde yetiştirilen atlarda mera kaynaklı helmint enfeksiyonlarının oldukça yaygın olduğu görülmüş, atlarda sırasıyla en fazla Strongylidae, *Dicrocoelium dentriticum*, Anoplocephalidae*, Fasciola hepatica* ve *Parascaris equorum* enfeksiyonlarına rastlanmıştır. Ayrıca protozooer etkenlerden Eimeria türleri %3.65 oranında tespit edilmiştir. Paraziter enfeksiyonlarda yaygın olarak görülen subklinik hastalık tablosuna rağmen özellikle ko-infeksiyon durumlarında bu etkenlerin konaklar için öldürücü olabileceği unutulmamalıdır. Elde edilen bu veriler kontamine meraların hayvanlar için enfeksiyon kaynağı olduğunu, tek tırnaklılarda görülen bu parazitlere karşı hayvan sahiplerinin bilgilendirilerek etkili mücadele programlarının oluşturulması gerektiğini ortaya koymaktadır.

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YAZAR KATKILARI

Fikir/Kavram: SH, MP
Denetleme/Danışmanlık: SH, MP
Veri Toplama ve/veya İşleme: SH, MP
Analiz ve/veya Yorum: SH, MP
Makalenin Yazımı: SH, MP
Eleştirel İnceleme: SH, MP

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