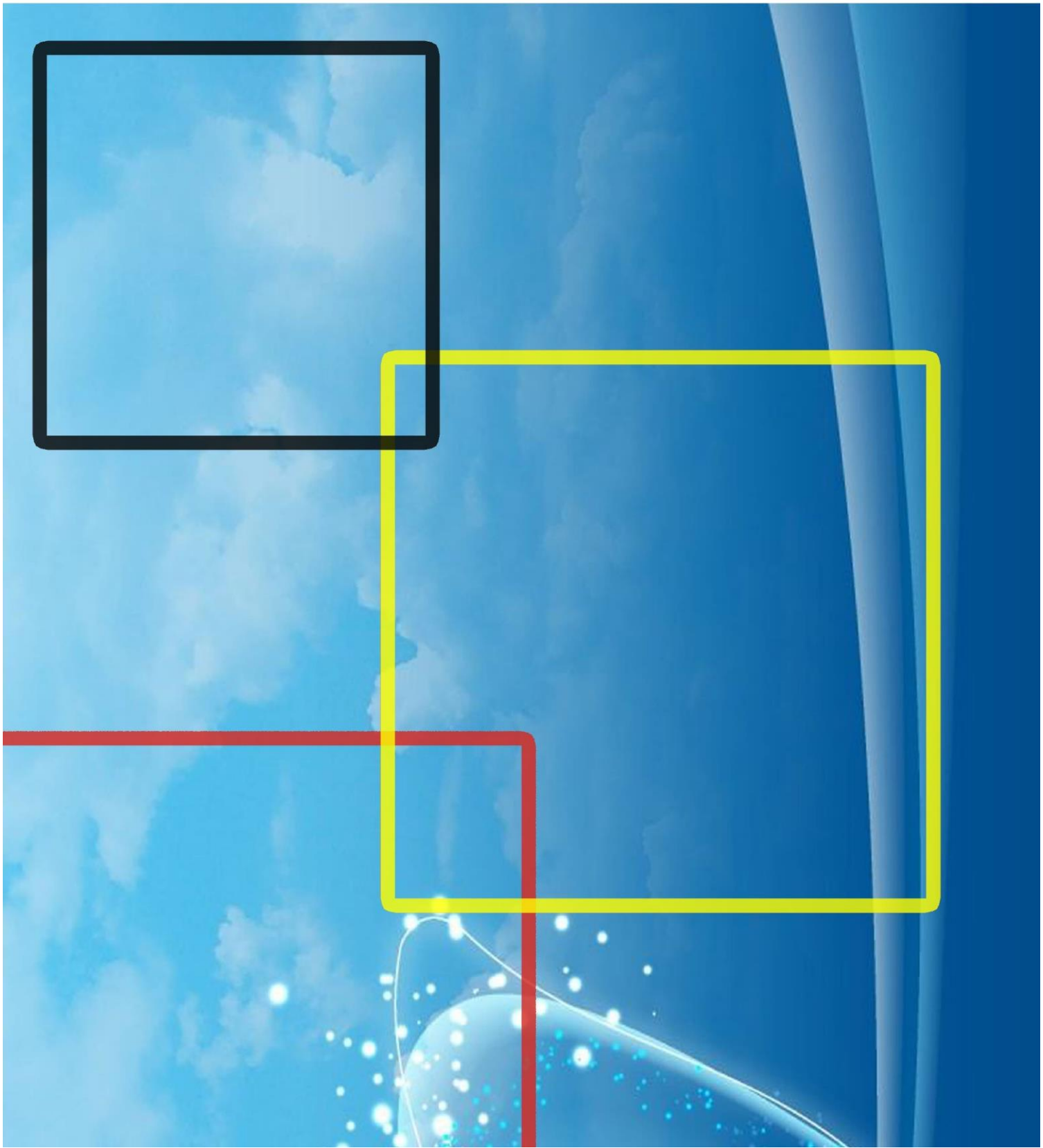
VAN VETERINARY JOURNAL

Year: 2021 / Volume: 32 / Issue: 3

ISSN : 2149-3359
e-ISSN : 2149-8644



VAN VETERİNARY JOURNAL

CONTENTS

Page

Original Articles

Review

November - 2021

VAN VETERINARY JOURNAL

This journal previously published as: **Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi**

ISSN: 2149-3359 E-ISSN: 2149-8644

This journal is published three times a year. Journal Tittle Abbreviation: Van Vet J

All articles in this journal are available free of charge from https://dergipark.org.tr/tr/pub/vanvetj

Year	Volume	Issue
2021	32	3

This journal indexed / abstracted in Asos Index, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Sobiad and Turkiye Atif Dizini

November - 2021

VAN VETERINARY JOURNAL

This journal previously published as: **Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi**

This journal is published three times a year. Journal Tittle Abbreviation: Van Vet J

All articles in this journal are available free of charge from https://dergipark.org.tr/tr/pub/vanvetj

Year	Volume	Issue
2021	**32**	**3**

This journal indexed / abstracted in Asos Index, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Sobiad and Turkiye Atif Dizini

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Hassan Ahmed N, Gradaščević N, Katica M (2021).** Influence of Calcium Chloride on Osteoblast Like Cells of Both Sexes in Rats in In Vitro Conditions. *Van Vet J*, 32(3), 92-97. DOI: https://doi.org/10.36483/vanvetj.942284

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Influence of Calcium Chloride on Osteoblast Like Cells of Both Sexes in Rats in *In Vitro* Conditions

Nasreldin HASSAN AHMED[1] Nedžad GRADAŠČEVIĆ[2] Muhamed KATICA[3,*]

[1] *University of Sarajevo, University Clinical Center Sarajevo, Emergency Medicine Clinic, Sarajevo, Bosnia and Herzegovina*
[2] *University of Sarajevo, Veterinary Faculty, Department of Food Safety and Environmental Protection, Sarajevo, Bosnia and Herzegovina*
[3] *University of Sarajevo, Veterinary Faculty, Department of Clinical Sciences of Veterinary Medicine, Sarajevo, Bosnia and Herzegovina*

Received: 24.05.2021 Accepted: 11.11.2021

ABSTRACT The aim of the study was to determine whether calcium chloride affects the proliferation of osteoblast like cells in a sex-dependent manner, as well as to determine the most effective concentration on proliferation of osteoblast like cells, in in vitro conditions. Bone marrow was used as biological material from young adult rats, both sexes, aged 90-95 days. Six different concentrations of calcium chloride were tested, determining the numerical representation of osteoblast like cells after 24 and 48 hours. Test results of mean values between males and females after 24 hours, indicate significant differences with a probability of $P<0.05$ at calcium chloride concentrations of: 0.25 mM and 1.8 mM. Results after 48 hours showed that there were no significant differences at most $CaCl_2$ concentrations. It was found that male osteoblast like cells show a higher affinity for different calcium chloride concentrations when compared to the female osteoblast like cells. The calcium chloride concentration of 0.25 mM affected the proliferation of osteoblast like cells most favorably, which is 26.6% higher than the control values.

Keywords: *Bone marrow, Calcium chloride, Cell proliferation, Osteoblast.*

ÖZ

In Vitro Ortamda Kalsiyum Klorürün Osteoblast Benzeri Hücreler Üzerine Etkisi

Bu çalışmada in vitro ortamda kalsiyum klorürün osteoblast benzeri hücrelerin proliferasyonu üzerine cinsiyet bağımlı olarak etkisi olup olmadığının belirlenmesi ve osteoblast benzeri hücrelerin proliferasyonlarını etkileyen en uygun konsantrasyonun belirlenmesi amaçlanmıştır. Her iki cinsiyetten 90-95 günlük ratların kemik ilikleri çalışmanın biyolojik materyalini oluşturmuştur. Altı farklı kalsiyum klorür konsantrasyonu test edildi ve 24 ve 48 saat sonra osteoblast benzeri hücrelerin sayısal değerleri tespit edildi. 24 saat sonunda erkekler ve dişiler arasındaki ortalama değerlerin test sonuçları, 0.25 mM ve 1.8 mM kalsiyum klorür konsantrasyonları istatistiki olarak anlamlı derecede farklılık göstermiştir($P<0.05$). 48 saat sonraki sonuçlar, çoğu $CaCl_2$ konsantrasyonunda önemli farklılıklar olmadığını gösterdi. Erkek osteoblast benzeri hücrelerin, dişi osteoblast benzeri hücrelere kıyasla farklı kalsiyum klorür konsantrasyonları için daha yüksek bir afinite gösterdiği belirlendi. 0.25 mM'lik kalsiyum klorür konsantrasyonu osteoblast benzeri hücrenin proliferasyonunu en olumlu şekilde etkileyen konsantrasyon olarak belirlenmiştir. Bu konsantrasyonda proliferasyon kontrol değerine oranla %26.6 daha fazla olduğu belirlenmiştir.

Anahtar Kelimeler: *Hücre proliferasyonu, Kemik iliği, Kalsiyum klorür, Osteoblast.*

INTRODUCTION

During one year in the human and animal skeleton, about 10% of bone tissue is replaced by new one, and this takes place by a balanced action of osteoblasts and osteoclasts through three phases – decomposition, construction and rest (Clarke 2008). Osteoblasts are defined as young types of bone cells that are responsible for building bone, by producing an organic extracellular matrix and partially controlling bone mineralization (Heino et al. 2004; Katica 2007; Çiçek and Tumer 2018). Osteoblast proliferation and differentiation are important events during bone processing and are controlled by both local growth factors and hormonal regulation. In their final phase of differentiation, osteoblasts become deeply embedded in the mineralized bone matrix during bone formation and become osteocytes (Heino et al. 2004).

A key stimulus for bone remodeling is the occurrence of focal bone micro-damage caused by mechanical stress, and

✉ *Corresponding author: muhamed.katica@vfs.unsa.ba

in addition to its role in repairing such damage, this process plays an irreplaceable role in calcium homeostasis (Clarke 2008).

Bone tissue is a kind of calcium depot, in order to balance, if necessary, the concentrations of calcium in the muscles and serum (Brini et al. 2013; Modi et al. 2019). Calcium is, therefore, the most abundant mineral in the body with different physiological roles. Extracellular calcium is extremely important for the formation of skeletons and teeth, as well as for the normal course of hemostasis, and the regulation of signal transduction pathways, neurotransmission, etc. (Živančević-Simonović 2006; Brini et al. 2014; Modi et al. 2019).

Calcium deficiency in the body can be regulated by replacement with calcium salts. The most commonly used salts are: calcium carbonate, calcium citrate malate, calcium chloride, calcium gluconate, calcium gluceptate, calcium glucoheptonate and calcium acetate. They differ in calcium content, solubility, bioavailability and taste (Trailokya et al. 2017; Modi et al. 2019).

The effect of some calcium salts and/or calcium ions on the proliferation and/or expression of osteoblast like cells salts has already been described (Lei et al. 2018; Modi et al. 2019; Nakamura et al. 2020).

However, the effect of calcium chloride salt on osteoblast like cells in in vitro conditions is not completely clear. For this reason, we conducted an in vitro experimental study using rat bone tissue, to establish the beneficial or possibly undesirable effect of different concentrations of calcium chloride salt on osteoblast like cells proliferation.

MATERIAL AND METHODS

Ethical Declaration

This research was approved by the Ethics Committee of the University of Sarajevo, Faculty of Veterinary Medicine, under registration number 01-02-421-2/21, Sarajevo, Bosnia and Herzegovina.

Rats

Fourteen young adult Wistar rats, aged 90 to 95 days, weighing 170-210 grams, both sexes were used in the study.

Rats were housed in cages and acclimatized to standard laboratory conditions (temperature 20°C to 24°C; 12 hours light, 12 hours dark), with humidity 60% ± 70%. All experimental animals consumed pelleted food that contained a mixture of different dietary components such as protein, fiber and minerals (Katica and Gradascevic 2017).

Femur preparation

The proximal parts of the femur were dissected for bone marrow extraction. The procedure was performed in – α minimal essential medium (α MEM), which contained the usual antibiotic (Katica and Tepeköy 2020).

Isolation and primary cell culture of bone marrow cells

Bone marrow suspension was dispersed by pipetting, filtered through 70-μm mesh nylon filter, and centrifuged at 200 × *g* for 10 min. After centrifugation, the supernatant containing erythrocytes was decanted and the bone marrow cells were resuspended with 10 ml α- MEM, where the common antibiotics were previously placed: 100 μg/ml Penicillin G, 50 μg/ml Gentamicin sulphate. The cells in the medium supplemented with 10% FBS were transferred to a plastic culture flask. The prepared vials with a half-closed stopper were immediately transferred to a special incubator with a temperature ambient of 37^0C, with a presence of 95% air and 5% CO_2. The cells were approximately seeded in the density of $1X10^3$ cell/vial, after the first preliminary microscopic examination. We constantly monitored the maturation of OB-like cells under a light microscope, with the magnification of 10X or 40 X. On the third day the medium was changed by adding 0,7 ml 10% of FBS. On day six, the medium was enriched with 10 mM Na-β-glycerophosphate (β-GP), 10^{-8} M Dexamethasone (D_x) and 50 μg/mL ascorbic acid (Maniatopoulos et al. 1988; Katica and Tepeköy 2020). After the sixth day, the medium was being changed every second day.

Subculture of bone marrow cells

We approached day 12 by subculturing the bone marrow cells of the examined rats (I passage), applying the conventional methods of porcine trypsin use obtained from the exocrine portion of the pancreas (Maniatopoulos et al. 1988; Katica and Tepeköy 2020). We emptied the medium from small plastic vials and injected 1ml of pork trypsin, which was previously heated in a water bath at 37^0C, for 3-5 minutes. 18 ml α MEM was added into the same plastic culture flasks and then using a sterile pipette, after one minute of "shaking" the cells in suspension, they were transferred to a prepared larger pot in which the medium was previously placed. Finally, 1.8 ml FBS was added. The subcultured cells were placed back into an incubator at the temperature of 37^0C, 100% relative humidity, and 5% of CO_2 and 95% of pure air presence. The medium was being changed every other day, and the cultured cells constantly monitored using a light microscope. Over the next six days, the cells gradually progressed and the preconditions were made for the passage II, that is, for their preparation in suspension for the test.

Application of Calcium Chloride ($CaCl_2$)

Prepared cells in suspension after treatment with trypsin were transferred to a sterile plastic plate with appropriate dents (24 pieces). 1 ml of cells in suspension were placed into every dent using an automatic pipette and two plates were filled, 48 dents. A third, separate plate with 24 dents was a control one. $CaCl_2$ was applied, fresh magistral preparation, prepared immediately before the test at six (6) different concentrations (mM): 0.25, 1.0, 1.3, 1.8, 2.3 and 2.5. Each concentration was tested four times, counting the cells after 24 and 48 hours.

Counting cells

The cells were counted manually, using Thoma's hemocytometer, under the binocular light microscope Motic Type 102 M, with magnification 40X, according to the below-mentioned formula: Counted cell number X 1000 X dilution factor, to obtain the number of cells in $1mm^3$ in the suspension (1×10^{-3}/ml) (Davis 1996; Katica and Tepeköy 2020). Most representative fields were electronically recorded using Motic Images Plus 2.0 software.

Statistical Analysis

All data obtained in the study were processed and analyzed using IBM SPSS Statistics for Windows, Version 24 for statistical data processing: Standard Deviation, Statistical Significance (T-Test), T-Test (difference) for small dependent samples, where a value of $P<0.05$ was considered statistically significant.

RESULTS

In Table 1, significant differences from control osteoblast like cells were observed when $CaCl_2$ was administered to males after 24 hours, with a probability of P<0.01 at $CaCl_2$ concentrations of 0.25 mM, 1,3 mM, 2.3 mM and 2.5 mM. Significance of differences with a probability of P<0.001 was recorded at a concentration of 1mM (Table 1). It was noticed that osteoblast like cells treated with a concentration of 1 mM were more numerous than osteoblast like cells from the control group by 5.4%.

Results after 48 hours in males after administration with calcium chloride (Table 2) indicate a tendency of a slight decrease of about 5% of the mean values of the number of treated osteoblast like cells compared to the mean values in males of 24 hours (Table 1).

In Table 2, significant differences were found in mean values compared to control values after $CaCl_2$ treatment after 48 hours and differ from the results of those of 24 hours. Significant differences with a probability of P<0.001 were found at concentrations of: 1.8 mM, 2.3 mM and 2.5 mM. Significant differences with a probability of P<0.01 were found at concentrations of: 0.25 mM and 1.3 mM, and at concentrations of 1.0 mM there were no significant differences (Table 2).

When examining the effect of $CaCl_2$ on the proliferation of female osteoblast like cells after 24 hours (Table 1), significant differences were recorded compared to control osteoblast like cells at the following concentrations: 0.25 mM, 1.3 mM, 1.3 mM and 2.3 mM with a probability of P<0.01. At a concentration of 2.5 mM, no cells were found (mean value was 0) (Figure 1C) and the significance of the differences in this case was expected, P<0.001. No significant difference was recorded at a concentration of 1.0 mM, and these osteoblast-like cells were more represented than the controls by 2.9%.

Mean values after application of $CaCl_2$ of 2.3 mM after 48 hours decreased when compared to the same 24 hours by 66.6%, and the mentioned mean values ($CaCl_2$ of 2.3 mM) of 24 and 48 hours were lower than the control values by 63% or 87%. Only $CaCl_2$ concentration of 0.25 mM had a favorable effect on proliferation and the mean value was 26.6% higher than 24 and 48 hours compared to the control values.

The test results of mean values between males and females by t-test, and after 24 hours, indicate significant differences with a probability of P<0.05 at $CaCl_2$ concentrations of: 0.25 mM and 1.8 mM (Table 3). Testing of the same parameters, but after 48 hours showed that there were no significant differences at most $CaCl_2$ concentrations. Exceptions were concentrations of 1.0 mM and 2.3 mM, where significant differences were found (Table 3).

The results of the study of the significance of the differences between the findings after 48 and 24 hours by t-test of differentiation in males for $CaCl_2$ concentration of 0.25 mM, the average difference was -0-3 which corresponds to a significant difference with probability P<0.05 (Table 4). Other t-test results showed no significant differences. The same analysis in females found the presence of a significant difference in $CaCl_2$ concentration of 2.3 mM. It had an average difference value of -0.8, and the t-test was 6.92, which is a significant difference with a probability of P<0.01 (Table 4).

Table 1. Results of application of $CaCl_2$ to osteoblasts like cells in males and females after 24 hours.

Ca Cl_2 (mM)	Males (n=7)		Females (n=7)		t -test Significance difference compared to the control group	
after 24 hours	Mean value (10^3)	SD	Mean value (10^3)	SD	Males	Females
2.5	0.2	0.14	0.0	0.0	t=23.33 P<0.01	t=33.00 P<0.001
2.3	1.6	0.21	1.2	0.15	t=11.70 P<0.01	t=16.47 P<0.01
1.8	1.4	0.12	1.2	0.17	t=34.29 P<0.001	t=15.87 P<0.01
1.3	1.9	0.31	2.2	0.15	t=7.69 P<0.01	t=13.91 P<0.01
1.0	3.7	0.24	3.4	0.30	t=1.15 NS	t=0.55 NS
0.25	4.8	0.07	4.5	0.12	t=10.17 P<0.01	t=10.23 P<0.01
Control	3.5	0.24	3.3	0.20	-	-

NS: Not significant, SD: Standard deviation, n: Number of rats used in the experiment.

Table 2. Results of application of $CaCl_2$ to osteoblasts like cells in males and females after 48 hours.

Ca Cl_2 (mM)	Males (n=7)		Females (n=7)		t -test Significance difference compared to the control group	
after 48 hours	Mean value (10^3)	SD	Mean value (10^3)	SD	Males	Females
2.5	0.1	0.00	0.0	0.0	t=33.00 P<0.001	t=31.50 P<0.001
2.3	1.4	1.10	0.4	0.07	t=18.85 P<0.001	t=58.00 P<0.001
1.8	1.3	0.20	1.0	0.12	t=15.33 P<0.001	t=37.55 P<0.001
1.3	2.0	0.21	2.0	0.28	t=9.89 P<0.01	t=9.19 p <0.01
1.0	3.7	0.18	3.1	0.18	t=2.26 P<0.001	t=2.00 P<0.001
0.25	4.5	0.18	4.4	0.07	t=8.31 P<0.01	t=22.00 P<0.001
Control	3.4	0.28	3.3	0.43	-	-

SD: Standard deviation, n: Number of rats used in the experiment.

Table 3. Significance of differences in mean values between males and females after $CaCl_2$ application after 24 and 48 hours.

$CaCl_2$ (mM)	Testing significance of differences in average values between male and female by t-test	
	After 24 hours	After 48 hours
2.5	t=2.82 NS	t=2.82 NS
2.3	t=3.02 NS	t=20.00 P<0.001
1.8	t=3.25 P<0.05	t=3.00 NS
1.3	t=1.44 NS	t=0.00 NS
1.0	t=0.75 NS	t=4.53 P<0.05
0.25	t=4.24 P<0.05	t=1.06 NS
Control	t=1.26 NS	t=1.06 NS

NS: Not significant.

Table 4. Results of testing the significance of differences after application of $CaCl_2$ 48 hours and 24 hours by t-test of differences between females and males.

$CaCl_2$ (mM)	Average difference (d)		t-test of difference	
	Males	Females	Males	Females
2.5	-0.1	0.0	t=0.471 NS	t=0.462
2.3	-0.2	-0.8	t=1.188 NS	t=6.92 P<0.01
1.8	-0.1	-0.2	t=0.926 NS	t=2.19 NS
1.3	0.1	-0.2	t=0.451 NS	t=1.18 NS
1.0	0.0	-0.3	t=1.18 NS	t=1.12 NS
0.25	-0.3	-0.1	t=3.0 P<0.05	t=1.73 NS
Control	-0.1	0.0	t=0.594 NS	t=0.597 NS

NS: Not significant.

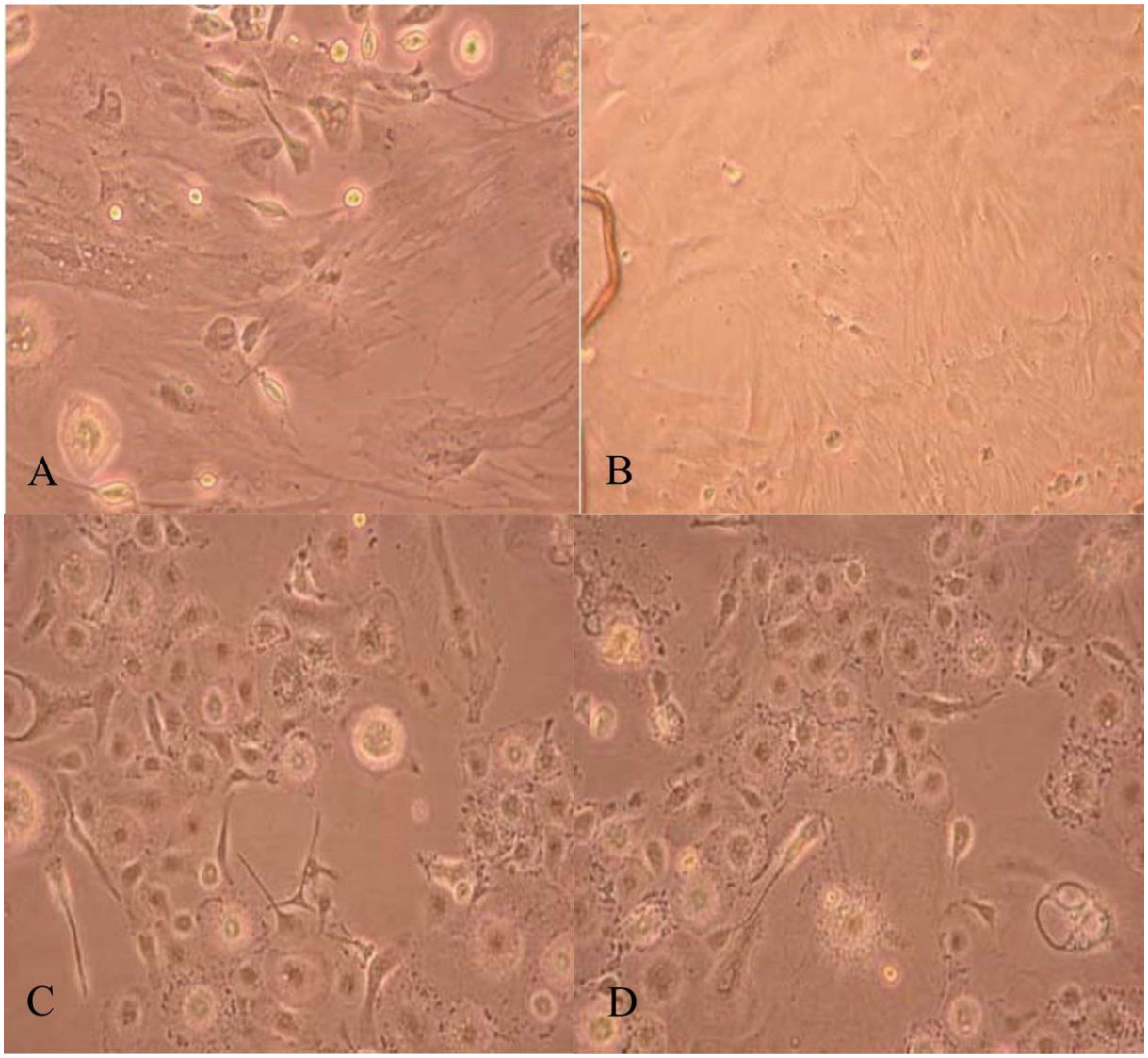


Figure 1. Osteoblast like cells after application of different concentration of $CaCl_2$.

DISCUSSION AND CONCLUSION

In cell culture, osteoblasts are difficult to distinguish from fibroblasts. They most commonly take on spindle shape (Figure 1A, 1B, 1C, 1D), although there are not all spindle cells in in vitro fibroblast cell culture (Davis 1996). All genes found in fibroblasts are also expressed in osteoblasts and vice versa, thus osteoblasts can be viewed as a sophisticated version of fibroblasts, and as such are called osteoblast like cells (Wlodarski 1990; Katica 2007). The exceptions are two gene products identified in osteoblasts, one is Cbfa1 transcription factor and the other is osteocalcin, as well as the presence of a mineralized extracellular matrix (Katica 2007).

The numerical representation of the cultured osteoblast like cells was not large in our study (Figures 1A, 1B, 1C, 1D and Table 1, 2). The reason for the low numerical representation can be related to the age of rats of 90-95 days. The experimental rats were in the young adult stage, which is equivalent to a 20-year-old man (Ćupić et al. 1999). In animals of this age, the presence of osteoblasts in bone tissue is not pronounced, as is the case in young animals in the period of early puerperium. Therefore, osteoblasts in young adult rats, in our case, are in a certain physiological balance in opposition to antagonists, osteoclasts, and their number is not evident for these reasons.

In vivo studies have found that mineral supplements, primarily calcium, in the diet, support osteoblast proliferation, which helps during bone remodeling in osteoporosis (Marie and Kassem 2011).

Results of study, Modi et al. (2019), indicate that calcium glucoheptonate has an increased effect on the cell proliferation of osteoblasts of similar MG-63 cells. Our study unequivocally found that calcium chloride induced osteoblast like cells proliferation in a dose-dependent manner. Lower concentrations of $CaCl_2$ (0.25 mM and 1.0 mM) in males and females, after 24 and 48 hours, favorably affect the proliferative processes of osteoblast like cells (Figure 1A). Obtained results correspond to a similar study by Modi et al. (2019), where identical calcium glucoheptonate concentrations of 0.25 and 1.0 mM, significantly increased alkaline phosphatase activity, suggesting increased osteoblast like cells activity. Higher concentrations of $CaCl_2$: 1.3, 1.8, 2.4 mM, adversely affect the population of osteoblast like cells in cell culture (Figures 1C, 1D), where the destructive influence of $CaCl_2$ is visible with the appearance of degenerated osteoblast like cells. A concentration of 2.5 mM $CaCl_2$ proved to be very toxic (Figure 1B).

The answer to why calcium salts in sufficient concentration yield good results in terms of supporting proliferation and maturation of osteoblast like cells, lies in the accumulation of calcium in the extracellular space. It is inevitable binding to hydroxyapatite crystals, in fact is a key factor in supporting and maturing osteoblast like cells (Bermudez-Reyes et al. 2018).

The results of our study show that the proliferation of osteoblast like cells in males, after treatment with different concentrations of $CaCl_2$ is more pronounced when compared to females. Our results are consistent with a similar study by Katica and Tepeköy (2020), where it was found that different concentrations of calcitriol also have a more favorable effect on the proliferation of the male population of osteoblast like cells compared to the population of females. It is indicative that the control results of male osteoblast like cells, within our study, are slightly higher than the control results in females. Osteoporosis, various forms of osteoarthritis and pathophysiological disorders of the spine are generally more prevalent in the female population than in males (Tosi et al. 2005). The female population has higher levels of estrogen, compared to testosterone in males (Jochems et al. 2010). So, it is to be assumed that this hormonal factor, as well as a higher susceptibility to osteopathies, may to some extent affect lower osteoblast like cells in female population. According to D'Amelio et al. (2008) evident differences in bone properties, including morphological and genetic aspects in male and female cells contribute to sexual dimorphism between men and women.

However, we can conclude that the results of this study favor a 0.25 mM concentration of calcium chloride, which most effectively acts on cell proliferation similar to osteoblasts. Also, male osteoblast like cells were found to show higher affinity for different $CaCl_2$ concentrations when compared to the osteoblast like cells of the female population. This finding provides guidance that calcium chloride treatment could also affect the proliferation of osteoblast like cells in a sex-dependent manner. A better understanding of osteoblast biology, as the most representative bone tissue cell, is necessary as a prerequisite for more effective curative treatment of various osteopathies.

CONFLICTS OF INTEREST

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

AUTHOR CONTRIBUTIONS

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

REFERENCES

Bermudez-Reyes B, Del Refugio Lara-Banda M et al. (2018). Effect on growth and osteoblast mineralization of hydroxyapatite-zirconia (HAZrO2) obtained by a new low temperature system. *Biomed Mater,* 13,1-9.

Brini M, Ottolini D, Cali T et al. (2013). Calcium in health and disease. *Met Ions Life Sci,* 13, 81–137.

Clarke B (2008). Normal bone anatomy and physiology. *Clin J Am Soc Nephrol,* 3, 131-139.

Clinckspoor I, Verlinden L, Mathieu C et al. (2013). Vitamin D in thyroid tumorigenesis and development. *Prog Histochem Cytochem*, 48 (2), 65–98.

Ćupić V, Kataranovski D, Popović N et al. (1999). Osnovi biologije, kliničke patologije i terapije nedomestifikovanih kućnih ljubimaca. GIK „Ljubostina, Trstenik; Beograd, Srbija.

Çiçek M, Tumer MK (2018). Yeni Bir Hemostatik Ajan Olan Mecsina Hemostopper® 'ın Farklı Testlerle Sitotoksisitesinin Değerlendirilip, Kalvarial Osteoblast Proliferasyonuna Etkisinin Araştırılması. *Dicle Med J*, 45 (3), 291-296.

D'Amelio P, Rossi P, Isaia G et al. (2008). Bone mineral density and singh index predict bone mechanical properties of human femur. *Connect Tissue Res*, 49, 99-104.

Davis JM (1996). Basic cell culture – a practical approach. I Edition, Oxford University Press; England.

Gila A, Plaza-Diaza J, Mesaa DM (2018). Vitamin D: Classic and Novel Actions. *Ann Nutr Metab*, 72 (2), 87–95.

Heino TJ, Hentunen TA, Väänänen HK (2004). Conditioned medium from osteocytes stimulates the proliferation of bone marrow mesenchymal stem cells and their differentiation into osteoblasts. *Exp Cell Res,* 294 (2), 458-468.

Jochems C, Islander U, Erlandsson M et al. (2010). Role of endogenous and exogenous female sex hormones in arthritis and osteoporosis development in b10.Q-ncf1*/* mice with collagen-induced chronic arthritis. *BMC Musculoskelet Disord,* 11, 284.

Katica M (2007). Effects of vitamin D3 and calcium on the establishment of primary osteoblast culture. Master's thesis. University of Sarajevo, Faculty of Natural Sciences and Mathematics, Sarajevo/Bosnia and Herzegovina.

Katica M, Gradascevic N (2017). Hematologic profile of laboratory rats fed with bakery products. *I J Res –Granthaalaya,* 5 (5), 221-231.

Katica M, Tepeköy F (2020). The effect of Calcitriol 1,25 (OH)2 - D3 on osteoblast-like cell proliferation during in vitro cultivation. *MAE Vet Fak Derg,* 5 (1), 11-17.

Lei Q, Lin D, Huang WX et al. (2018). Effects of calcium ion on the migration and osteogenic differentiation of human osteoblasts. *Hua Xi Kou Qiang Yi Xue Za Zhi*, 36 (6), 602-608.

Maniatopoulos C, Sodek J, Melcher AH (1988). Bone formation in vitro by stromal cells obtained from bone marrow of young adult rats. *Cell Tissue Res,* 254 (2), 317-330.

Marie PJ, Kassem M (2011). Osteoblasts in osteoporosis: past, emerging, and future anabolic targets. *Eur J Endocrinol,* 165 (1), 1–10.

Modi PK, Prabhu A, Bhandary YP (2019). Effect of calcium glucoheptonate on proliferation and osteogenesis of osteoblast-like cells in vitro. *PLoS One*, 14 (9), 1-15.

Nakamura S, Matsumoto TT, Sasaki JI et al. (2010). Effect of Calcium Ion Concentrations on Osteogenic Differentiation and Hematopoietic Stem Cell Niche-Related Protein Expression in Osteoblasts. *Tissue Eng Part A,* 16 (8), 2467-2473.

Tosi LL, Boyan BD, Boskey AL (2005). Does sex matter in musculoskeletal health? The influence of sex and gender on musculoskeletal health. *J Bone Joint Surg Am,* 87, 1631-1647.

Trailokya A, Srivastava A, Bhole M, Zalte N (2017). Calcium and Calcium Salts. *J Assoc Physicians India,* 65 (2), 100–103.

Wlodarski K (1990). Propertietis and Origin of Osteoblasts. *Clin Orthopaed Related Res,* 252, 276-293.

Živančević–Simonović S (2006). Opšta patološka fiziologija. Univerzitet u Kragujevcu, Medicinski fakulet Press; Srbija.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Öner AC, Şahin A (2021).** Effects of Marbofloxacin, Diclofenac Sodium and Methylprednisolone on Serum Biochemical Values in Endotoxemia-Shaped Rats. *Van Vet J*, 32(3), 98-103. DOI: https://doi.org/10.36483/vanvetj.948037

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Endotoksemi Şekillendirilmiş Ratlarda Marbofloksasin, Diklofenak Sodyum ve Metilprednizolonun Serum Biyokimyasal Değerler Üzerine Etkisi

Ahmet Cihat ÖNER[1,*] Ali ŞAHİN[2]

[1] *Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, 65080, Van, Turkey*
[2] *Karamanoğlu Mehmet Bey University, Faculty of Health Sciences, Department of Nursing, 70100, Karaman, Turkey*

Received: 04.06.2021 Accepted: 15.10.2021

ÖZ

Escherichia coli'den türetilen lipopolisakkarit (LPS), sepsis ve septik şok için bir model olarak yaygın olarak kullanılmıştır. Çalışmamızda LPS ile deneysel endotoksemi oluşturulan ratlarda, marbofloksasin, diklofenak sodyum, metilprednizolon kullanılarak, bu ilaçların organ yetmezliğinin indirekt belirteçleri olan alkalen fosfotaz (ALP), alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), gama glutamil transferaz (GGT), kan üre azot (BUN), kreatinin değerleri üzerine olan etkilerinin değerlendirilmesi amaçlanmıştır. Çalışma için gerekli 186 adet rat, 5 gruba ayrıldı. Kontrol grubundan 0. saatte kan örnekleri alındı. Ratlarda endotoksemi oluşturmak amacı ile intraperitoneal (IP) yolla LPS (4mg/rat) uygulandı. Gelişen endotoksemiyi tedavi etmek için marbofloksasin IP yolla 100 mg/kg, diklofenak sodyum IP yolla 10 mg/kg, metilprednizolon IP yolla 10 mg/kg dozunda uygulandı. Daha sonra 1, 2, 4, 8, 12 ve 24. saatlerde tiyopental anestezisi altında kan örnekleri alınarak biyokimyasal değerler ölçüldü. Çalışmada serum ALP, ALT, AST, GGT, BUN ve kreatinin düzeylerinin LPS uygulaması ile arttığı ($P<0.05$) ve sepsiste beklenilen etkinin şekillendiği tespit edildi. Sepsis tedavisinde, metilprednizolon dışında diğer ilaçların tek başlarına kullanılamayacağı ancak kombine uygulamanın tercih edilebileceği sonucuna ulaşıldı.

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

ABSTRACT

Effects of Marbofloxacin, Diclofenac Sodium and Methylprednisolone on Serum Biochemical Values in Endotoxemia-Shaped Rats

Escherichia coli-derived lipopolysaccharide (LPS) has been used extensively as a model for septic shock and sepsis. In our study, it was aimed to induce experimental endotoxemia in rats by using LPS and the effects of marbofloxacin, diclofenac sodium and methylprednisolone on alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), blood urea nitrogen (BUN), creatinine, which are indirect indicators of organ insufficiency, were assessed. 186 rats were divided into 5 groups. Blood samples were obtained from control group at 0 h. To shape endotoxemia, LPS was administered at a dose of 4 mg/rat via intraperitonally (IP). Marbofloxacin (100 mg/kg, IP), diclofenac sodium (10 mg/kg, IP) and metylprednisolone (10 mg/kg, IP) were administered at dosage for the treatment of developing endotoxemia. Blood samples were collected under thiopental anesthesia at 1, 2, 4, 8, 12 and 24 h. They were analyzed biochemical levels. In the study, it was determined that serum ALP, ALT, AST, GGT, BUN and creatinine levels increased with LPS application ($P<0.05$) and the expected effect in sepsis was formed. As a result, LPS administration caused sepsis and it had several effects observed in the kidney and liver. It was concluded that in the treatment of sepsis, drugs other than methylprednisolone cannot be used alone, but combined application can be preferred.

Keywords: *Diclofenac sodium, Endotoxemia, Methylprednisolone.*

GİRİŞ

Dolaşımda endotoksin bulunmasına endotoksemi ismi verilir. Bu endotoksinler gram negatif bakterilerin veya gram negatif enterik bakterilerin hücre duvarı bileşeni olan lipopolisakkaritlerin (LPS) intestinal mukozal bariyeri aşması sonucu açığa çıkmaktadır (Hart ve MacKay 2015). Meydana gelen olgu öncelikle sistemik inflamatuvar yanıt sendromu (SIRS), sepsis ve septik şok olarak gelişir. Yeni doğan çiftlik hayvanlarında septisemi genellikle önemli morbitite ve mortalite nedenleri olan *Escherichia coli* ve *Salmonella spp*. ile ilişkilidir. *E. coli*, septisemik buzağılarda en sık kan dolaşımından izole edilen bir bakteridir, ancak septisemik buzağıların%10'unda gram pozitif bakteri

✉ *Corresponding author: afatihdemirel@yyu.edu.tr

ve%28'inde polimikrobiyal enfeksiyon saptanmıştır (Constable ve ark. 2017, Bonelli ve ark. 2018; Pardon ve Perez 2018).

Bakterilerin toksinlerine karşı gelişen hücresel immun cevap, normal koşullarda konağı enfeksiyondan koruyabilirken hücresel cevabın aşırı tepkisi konak için olumsuz sonuçlar doğurabilmektedir. Bu nedenle sepsisin patogenezi, proinflamatuvar ve antiinflamatuvar eşitsizlik sendromu olarak ta tanımlanabilmektedir. Proinflamatuvar ve antiinflamatuvar dengenin bozulmasının konağa zarar verdiği düşünülmektedir (Camcıoğlu ve Aytaç 2007; Boscolo ve ark. 2008).

Başarılı sepsis tedavisi, intravenöz olarak uygulanan geniş spektrumlu, bakterisidal bir ilaçla tedavinin hızlı bir şekilde başlatılmasını gerektirir (Pardon ve Perez 2018). Sepsis/endotoksemi ve bunlara bağlı şok olgularının tedavisinde, çeşitli ilaçlar/ajanlar denenmiştir. Son 20 yıl içerisinde de sepsisin fizyopatolojisi üzerinde araştırmalar yoğunlaşmış, buna bağlı olarak çeşitli ilaç kombinasyonları denenmiştir. Son yıllarda yapılan deneysel araştırmalarda antibiyotik, glukokortikoid, nonsteroid antiinflamatuvar ilaç (NSAID) veya kombinasyon (antibiyotik + glukokortikoid + NSAID) uygulamalarının olumlu sonuçları belirlenmiştir (Elmas ve ark. 2008, Yazar ve ark. 2009; Yazar ve ark. 2010).

Sepsis çalışmalarında en sık denenen ve sahada bu olgularda en çok kullanılan antibiyotiklerden olan florokinolonlar bakterilerde DNA-jirazın etkinliğini engelleyerek etkinlik gösteren geniş etki spektrumlu antibiyotiklerdir. Florokinolonlar, özellikle gram (-) bakteriler ve *Staphylococcus* türlerine karşı etki etmektedir (Traş ve ark. 2007; Yazar ve ark. 2009).

Non-steroid antiinflamatuar ilaçlar (NSAID) NF-KB'nin etkinliğini engelleyerek, proinflamatuar sitokinler, iNOS ve COX oluşumunu engeller (Meduri 1999; Yazar ve ark. 2009). Bu bilinen etkileri düşünülerek çalışmalar gerçekleştirilse de sepsiste kullanılması hekimler arasında tartışmalı bir konu olarak karşımıza çıkmaktadır.

Deneysel septik şok modellerinde glukokortikoid uygulamalarının her zaman olumlu etkileri bildirilmiştir (Traş ve ark. 2007; Yazar ve ark. 2009). Sepsis tedavisi için glukokortikoidlerin kullanımında doz ve sağkalım oranının lineer doğrultuda olduğu saptanmıştır. Bu da glukokortikoidlerin kullanımının sepsis süresince doza bağımlı olduğunu göstermektedir (Minneci ve ark. 2004; Yarema ve Yost 2011).

Bu çalışmada LPS ile deneysel endotoksemi oluşturulan ratlarda, florokinolon grubu antibiyotiklerden marbofloksasin (MAR), NASID grubundan diklofenak sodyum (DS) ve glukokortikoid grubu ilaçlardan metilprednizolonun (MPRED) tek veya kombine kullanımlarının biyokimmyasal parametreler üzerine olası etkilerinin değerlendirilmesi amaçlanmıştır.

MATERYAL VE METOT

Araştırmada Sprague - Dawley ırkı ratlardan 93 erkek, 93 dişi, her bir grupta 6 denek olmak üzere, toplam 186 adet rat kullanıldı. Deney hayvanları Van Yüzüncü Yıl Üniversitesi Tıp Fakültesi Deney Hayvanları Ünitesi'nden temin edildi. Çalışma Selçuk Üniversitesi Veteriner Fakültesi Etik Kurulu tarafından alınan karar ile 11.04.2012 tarih ve 2012/35 numaralı izinle yürütüldü.

Biyokimyasal ölçümler için, AST kiti (IL Test AST, Milano, İtalya), ALT kiti (IL Test ALT, Milano, İtalya), ALP kiti (IL Test ALP, Milano, İtalya), BUN kiti (IL Test BUN, Milano, İtalya), kreatinin kiti (IL Test Creatinine, Milano, İtalya) kullanıldı.

Çalışmada 0.saat olarak ayrılan hayvanlar hiçbir ilaç uygulaması yapılmadan kontrol grubu olarak kullanıldı. Çalışma planı doğrultusunda hiçbir 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.

Diğer gruplar ise LPS, LPS+Marbofloksasin (MAR), LPS+ Diklofenak Sodyum (DS), LPS + MetilPrednizolon (MPRED) ve LPS + Komibnasyon (KOMBİN) olmak üzere adlandırıldı. İlaç uygulamaları sonrası 1, 2, 4, 8, 12 ve 24. saatlerde kalpten anestezi altında kanları alındı ve servikal dislokasyon yöntemi ile ötenazi uygulandı.

Deney hayvanlarına sepsis oluşturmak amaçlandığı için, deney LPS verilmesi ardından ilaç uygulaması şeklinde yapıldı. LPS 4 mg/rat dozunda serum fizyolojik (SF) ile sulandırılarak uygulandı. Marbofloksasin 100 mg/kg, diklofenak sodyum 10 mg/kg ve metilprednizolon 10 mg/kg dozda kullanıldı (Yazar ve ark. 2009).

İstatistik Analiz

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

BULGULAR

ALP düzeyinin 4 ve 12. saatleri dışında sadece LPS uygulanan grup ile LPS + ilaç uygulanan gruplar arasında farkın istatistiki açıdan önemli olmadığı ($P>0.05$) belirlendi. Ayrıca 4. saatte metilprednizolon, 12. saatte ise metilprednizolon ve kombinasyon uygulamasının LPS uygulamasından farklı olduğu ve yükselen ALP seviyesini önemli ölçüde ($P<0.05$) düşürdüğü belirlendi. Her bir grubun saatler arasındaki farkına bakıldığı zaman sadece kombinasyon uygulanan grupta saatler arasında istatistiki bir fark olmadığı ($P>0.05$), diğer gruplarda ise farklılık olduğu ($P<0.05$) gözlendi (Tablo 1).

Serum AST seviyesi üzerinde, gruplar arasında 4, 12 ve 24. saatler dışında istatistiksel olarak fark ($P<0.05$) olmasına rağmen marbofloksasin, metilprednizolon ve kombinasyon uygulamasının LPS ile yükselen AST seviyesini düşüremediği ve istatistiksel olarak benzer olduğu ($P>0.05$) fakat diklofenak sodyum uygulamasının 8. saatte LPS'den farklı ($P<0.05$) olduğu ve AST düzeyini düşürdüğü belirlendi. Her bir grubun saatler arasındaki farkına bakıldığı zaman sadece diklofenak uygulanan grupta saatler arasında istatistiki bir fark olmadığı ($P>0.05$), diğer gruplarda ise farklılık olduğu ($P<0.05$) gözlendi (Tablo 2).

Serum ALT düzeyi üzerinde LPS uygulaması sonrası 8. saatte pik gözlendiği, yükselmenin 4. saatten itibaren 24 saat boyunca devam ettiği, 4. saatte diklofenak sodyum, 8. saatte ise marbofloksasin ve diklofenak sodyum uygulamasının LPS'den farklı ($P<0.05$) olduğu ve serum ALT düzeyini düşürdüğü, diğerlerinin LPS'den farklı olmadığı ($P>0.05$) belirlendi. Saatler arasında grupların karşılaştırılmasında ise sadece 4 ve 8. saatler arasında gruplar arasında fark olduğu ($P<0.05$) diğer saatlerde ise gruplar arasında istatistiksel bir fark olmadığı belirlendi. Her bir grubun saatler arasındaki farkına bakıldığı zaman ise tüm gruplarda istatistiki olarak farklılık olduğu ($P<0.05$) gözlendi (Tablo 3).

Serum GGT düzeyinin 1. ve 2. saatte tüm gruplar arasında istatistiksel açıdan bir fark (P>0.05) bulunamamıştır. Metilprednizolon ile LPS uygulamaları arasında 4. saatte fark olduğu (P<0.05) yükselen GGT düzeyinin düştüğü, 8. saatte marbofloksasin, metilprednizolon ve kombinasyon uygulamalarının LPS'den farklı olduğu (P<0.05) GGT düzeyinin düşürdüğü, 12 ve 24. saatte ise bütün uygulamaların LPS'den farklı olduğu (P<0.05) ve serum GGT düzeyini düşürdükleri tespit edildi. Her bir grubun saatleri arasındaki farkına bakıldığı zaman sadece diklofenak ve kombinasyon uygulanan gruplarda saatler arasında istatistiki bir fark olmadığı (P>0.05), diğer gruplarda ise farklılık olduğu (P<0.05) gözlendi (Tablo 4).

Böbrek hasarı belirteçlerinden BUN düzeyinin LPS uygulaması sonrasında kademeli şekilde yükseldiği (P<0.05), 12 ve 24. saatlerde en yüksek değerine ulaştığı belirlendi. Diklofenak sodyumun LPS düzeyini diğer ilaç uygulamalarına göre 1 ve 2. saatte düşürdüğü fakat istatistiksel olarak farklı olmadığı (P>0.05) gözlendi. Dördüncü saatte metilprednizolon uygulamasının, 8. saatte kombinasyon uygulaması hariç diğer uygulamaların, 12. saatte metilprednizolon ve kombinasyon uygulamasının ve 24. saatte marbofloksasin ile metilprednizolon uygulamalarının LPS'den farklı olduğu (P<0.05) ve BUN düzeyini düşürdüğü tespit edildi. Her bir grubun saatler arasındaki farkına bakıldığı zaman sadece kombinasyon uygulanan grupta saatler arasında istatistiki bir fark olmadığı (P>0.05), diğer gruplarda ise farklılık olduğu (P<0.05) gözlendi (Tablo 5).

Kreatinin düzeyi incelendiğinde, diklofenak sodyum ile kombinasyon uygulamasının 1 ve 2. saatte, 4. saatte yalnız kombinasyon uygulamasının, 12. saatte metilprednizolon ve kombinasyon uygulamasının, 24. saatte ise bütün uygulamaların LPS'den farklı olduğu (P<0.05) ve yükselen kreatinin değerini düşürdüğü belirlendi. 8. saatte ise uygulamalar arası fark olmadığı (P>0.05) ve LPS uygulaması ile benzer değerlere sahip olduğu belirlendi (Tablo 6).

Tablo 1. Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında ALP ortalamaları ve çoklu karşılaştırma testi sonuçları (IU/L).

Table 1. Means of ALP and multiple comparison test results (IU/L) 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	132±12.5^{w}	169±26.5^{wx}	277±24.1^{xy}	315±25.3^{by}	259±42.5^{wxy}	502±67.4^{bz}	308±97.4^{y}	**
LPS+MARBO	132±12.5^{w}	121±19.9 ^{w}	230±13.0 ^{wx}	231±43.8 ^{abwx}	173±40.7 ^{w}	405±150 ^{bx}	179±19.8 ^{w}	*
LPS+DS	132±12.5^{w}	173±11.6 ^{w}	216±25.7 ^{w}	273±84.5 ^{abwx}	202±60.0 ^{w}	413±81.5 ^{bx}	325±211 ^{wx}	*
LPS+MPRED	132±12.5^{wx}	163±12.3 ^{wx}	312±149 ^{x}	169±17.2 ^{awx}	196±21.1 ^{wx}	113±11.9 ^{aw}	134±32.2 ^{wx}	*
LPS+KOMBİN	132±12.5	148±18.8	157±15.2	184±18.6 ^{ab}	142±17.7	146±25.8 ^{a}	163±24.1	-
P	-	-	-	*	-	*	-	

-; P>0.05, *; P<0.05, **; P<0.01.

^{a,b}; Aynı sütun da gösterilen ortalamalar arasında farklılıklar önemlidir.

^{x,w,y,z}; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir.

Tablo 2. Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında AST ortalamaları ve çoklu karşılaştırma testi sonuçları (IU/L).

Table 2. Means of AST and multiple comparison test results (IU/L) 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	148±34.1 ^{wx}	126±6.00 ^{abw}	167±23.7 $^{ab\ wx}$	366±70.7 ^{x}	358±107 ^{bx}	92.8±92.8 ^{w}	173±106^{wx}	*
LPS+MARBO	148±34.1 ^{wx}	132±18.7 ^{abwx}	108±11.4 ^{awx}	223±66.9 ^{x}	143±29.6 ^{abwx}	72.0±35.5 ^{w}	363±67.3 ^{y}	**
LPS+DS	148±34.1	93.8±7.60 ^{a}	159±32.1 ^{ab}	205±32.3	55.8±45.8 ^{a}	252±157	217±174	-
LPS+MPRED	148±34.1 ^{w}	138±12.6 ^{bw}	191±23.4 ^{bw}	242±37.5 ^{wx}	365±84.8 ^{bx}	215±25.9 ^{w}	173±50.4 ^{w}	*
LPS+KOMBİN	148±34.1 ^{wx}	130±16.3 ^{abw}	147±12.1 ^{abwx}	206±35.6 ^{wx}	272±82.4 ^{abwx}	307±83.3 ^{x}	246±24.9 ^{wx}	*
P	-	*	*	-	*	-	-	

-; P>0.05, *; P<0.05, **; P<0.01.

a,b; Aynı sütun da gösterilen ortalamalar arasında farklılıklar önemlidir.

x,w,y; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir.

Tablo 3. Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında ALT ortalamaları ve çoklu karşılaştırma testi sonuçları (IU/L).

Table 3. Means of ALT and multiple comparison test results (IU/L) 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	43.3±9.30 ^{w}	40.6±5.26 ^{w}	81.8±13.9 ^{w}	227±48.7 ^{bwx}	380±135 ^{bx}	108±101 ^{w}	168±109 ^{wx}	*
LPS+MARBO	43.3±9.30 ^{w}	38.1±4.08 ^{w}	59.8±5.07 ^{w}	135±41.8 ^{abwx}	70.5±17.2 ^{aw}	57.8±20.6 ^{w}	218±73.0 ^{x}	**
LPS+DS	43.3±9.30 ^{w}	42.3±2.72 ^{w}	48.6±10.2 ^{w}	92.0±16.5 ^{awx}	28.0±16.2 ^{aw}	300±184^{x}	102±70.5 ^{wx}	**
LPS+MPRED	43.3±9.30 ^{w}	46.1±4.46 ^{w}	78.0±17.1 ^{w}	135±18.6 ^{abw}	259±81.1 ^{abx}	112±19.7 ^{w}	61.3±24.8 ^{w}	*
LPS+KOMBİN	43.3±9.30 ^{w}	43.1±4.82 ^{w}	56.3±3.58 ^{wx}	75.17±9.6 ^{awx}	164±82.5 ^{abx}	132±43.5 ^{wx}	137±14.6 ^{wx}	*
P	-	-	-	*	*	-	-	

-; P>0.05, *; P<0.05, **; P<0.01.

a,b; Aynı sütun da gösterilen ortalamalar arasında farklılıklar önemlidir.

x,w; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir.

Tablo 4. Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında GGT ortalamaları ve çoklu karşılaştırma testi sonuçları (IU/L).

Table 4. Means of GGT and multiple comparison test results (IU/L) 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	2.00±0.52^{w}	3.00±0.52 ^{wx}	7.00±1.07 ^{wx}	6.83±0.87 ^{bwx}	9.83±1.30 ^{bx}	30.2±4.29 ^{cz}	19.0±6.68 ^{by}	***
LPS+MARBO	2.00±0.52^{w}	3.83±0.17 ^{w}	3.60±1.03 ^{w}	6.00±1.63 ^{bw}	4.33±1.05 ^{aw}	15.3±4.90 ^{bx}	3.83±0.40 ^{aw}	**
LPS+DS	2.00±0.52	23.6±21.2	6.00±1.00	6.20±1.02 ^{b}	9.17±2.50 ^{b}	13.6±2.56 ^{b}	9.00±9.00 ^{a}	-
LPS+MPRED	2.00±0.52^{w}	2.50±0.22 ^{w}	6.83±1.92 ^{x}	2.83±0.60 ^{aw}	3.50±0.99 ^{aw}	3.00±0.45 ^{aw}	3.50±1.23 ^{aw}	*
LPS+KOMBİN	2.00±0.52	3.00±0.26	4.17±0.83	3.67±0.42 ^{ab}	2.00±0.37^{a}	3.00±1.29 ^{a}	2.83±0.54 ^{a}	-
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,z}; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir.

Tablo 5. Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında BUN ortalamaları ve çoklu karşılaştırma testi sonuçları (IU/L).

Table 5. BUN averages and multiple comparison test results (IU/L) 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	56.6±5.21 ^{w}	57.6±3.97 ^{abw}	64.1±7.25 ^{abw}	86.3±6.97 ^{bw}	102±12.6 ^{bw}	190±12.7 ^{bx}	190±60.2 ^{bx}	***
LPS+MARBO	56.6±5.21 ^{w}	71.1±3.57 ^{bw}	61.6±10.8 ^{abw}	81.0±12.7 ^{abw}	62.6±14.6 ^{aw}	140±42.5 ^{abx}	75.8±12.9 ^{aw}	*
LPS+DS	56.6±5.21 ^{w}	54.3±5.30 ^{aw}	50.3±4.63 ^{aw}	73.4±12.1 ^{abw}	57.0±9.93 ^{aw}	137±23.1 ^{abx}	151±125 ^{abx}	**
LPS+MPRED	56.6±5.21 ^{w}	67.5±1.84 ^{abwx}	80.0±6.60 ^{bx}	55.1±2.73 ^{aw}	54.5±3.68 ^{aw}	67.1±5.13 ^{awx}	50.6±8.94 ^{aw}	**
LPS+KOMBİN	56.6±5.21	72.5±7.43 ^{b}	67.8±4.69 ^{ab}	63.5±3.68 ^{ab}	73.5±5.66 ^{ab}	86.3±27.2 ^{a}	99.8±21.5 ^{ab}	-
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; Aynı satırda gösterilen ortalamalar arasında farklılıklar önemlidir.

Tablo 6. Endotoksemik ratlarda LPS, LPS+MARBO, LPS+DS, LPS+MPRED ve LPS+KOMBİN gruplarında kreatinin ortalamaları ve çoklu karşılaştırma testi sonuçları (IU/L).

Table 6. Creatinine mean and multiple comparison test results (IU/L) in LPS, LPS+MARBO, LPS+DS, LPS+MPRED and LPS+COMBIN groups in endotoxemic rats.

GRUPLAR	$\bar{X} \pm S_{\bar{X}}$							P
	0. saat	1. saat	2. saat	4. saat	8. saat	12. saat	24. saat	
LPS	0.36±0.04^{w}	0.71±0.05^{bw}	0.63±0.07^{cw}	0.63±0.08^{bw}	0.72±0.13^{w}	1.24±0.19^{bx}	1.36±0.49^{bx}	**
LPS+MARBO	0.36±0.04^{w}	0.71±0.05^{bwx}	0.47±0.05^{abcwx}	0.48±0.09^{abwx}	0.49±0.09^{wx}	0.84±0.29^{abx}	0.59±0.05^{awx}	*
LPS+DS	0.36±0.04^{w}	0.45±0.03 ^{aw}	0.41±0.07 ^{abw}	0.45±0.03 ^{abw}	0.55±0.14 ^{w}	0.98±0.19 ^{abx}	0.43±0.37 ^{aw}	**
LPS+MPRED	0.36±0.04^{w}	0.67±0.01 ^{by}	0.56±0.06 ^{bcxy}	0.45±0.02 ^{abwx}	0.61±0.03 ^{xy}	0.51±0.06 ^{awxy}	0.45±0.10 ^{awx}	**
LPS+KOMBİN	0.36±0.04^{w}	0.52±0.02 ^{ax}	0.35±0.01 ^{aw}	0.36±0.01 ^{aw}	0.54±0.06 ^{x}	0.55±0.09 ^{ax}	0.66±0.05 ^{ax}	***
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.

TARTIŞMA VE SONUÇ

Sepsis ve septik şok olguları hem veteriner sahada hem de beşerî hekimlik alanında önde gelen mortalite nedeni olmaya devam etmektedir. Sepsis; patogenezi gram-negatif ve gram-pozitif bakteriler ve mantarlar ya da her ikisinin de şiddetlenen inflamatuvar yanıtı ile enfeksiyonun bir arada olduğu durumdur. Sepsis ile yapılan büyük mücadelelere rağmen, dünya çapında yılda 8 milyon ölüm gözlenmektedir (Dawulieti ve ark. 2020; Prauchner 2020).

Organizmanın immünolojik savunma mekanizmalarında çok önemli rol oynadığı için sepsis durumunda en çok etkilenen organın karaciğer olduğu düşünülmektedir (Pastor ve ark. 1995; James ve ark. 2002). AST tüm hayvanlarda yumuşak doku nekrozunun nonspesifik indikatörüdür. Serum ALT'nin en spesifik aktivitesi karaciğerdedir. Bu nedenle karaciğer hasarı belirteci olarak kullanılır. Köpek ve kedilerde karaciğer hasarının teşhisinde ALT spesifik bir değerdir. ALT karaciğer hasarında AST'den daha fazla yükselir. Özellikle akut hepatit ve steroid hepatopatisinde miktarı artar. Karaciğer hasarı meydana geldiğinde artan diğer bir enzim ise GGT'dir. İnsanlarda hepatik siroz, hepatik karsinomda GGT miktarı yükselir. ALP testi genelde safra salgısı bozukluklarında, safra yolları tıkanıklıklarında, karaciğer hastalıklarında kullanılan bir parametredir. ALP, vücutta en fazla bulunan enzimlerden biridir. Serum ALP aktivitesi septisemi ve endotoksemide, karaciğer hasarı nedeni ile normal üst sınırın birkaç katı yükselebilir (Turgut 2000; Kerr 2002; Swarnalatha ve ark. 2020).

Deneysel sepsis/septik şok modellerinde ve gerçek hastalarda yapılan araştırmalarda, genellikle bir ilaç için, bir veya birkaç doz örneklemesi yapılmaktadır. Gerçek hastalarda yapılan incelemelerde ise kontrol gruplarının farklılığı bir diğer problemi oluşturmaktadır. Ayrıca septik şokun ilk ve son dönemi ile ölen veya yaşayan hastalarda kan parametrelerinde çok farklı sonuçlar elde edildiği bildirilmektedir (Yazar ve ark. 2009). Bu araştırmada 24 saat süresince aralıklı kan alınarak (1 - 2 - 4 – 8 - 12 ve 24. saatlerde) organ hasarı belirteçleri incelenmiştir.

Karaciğer yetmezliğinin sepsiste erken dönemde oluştuğu ve LPS uygulaması sonucu serum aminotrasferazlarda artışlar ile karaciğer hasarına neden olduğu bildirilmektedir (Berger ve Chiolero 2007). Yazar ve ark. (2009; 2010)'nın yaptıkları çalışmalarda LPS uygulaması sonrası karaciğer enzimlerinde belirgin düzeyde artış bildirilmiştir. LPS uygulamasının tavşan (Elmas ve ark. 2006; Elmas ve ark. 2008) ve ratlarda (Yazar ve ark. 2004) serum AST, ALT ve GGT düzeyini artırdığı bildirilmiştir. Yapılan çalışmada da karaciğer hasarı belirteçleri olan serum AST, ALT, GGT ve safra kanalı hasarı belirteci olan serum ALP düzeyleri değerlendirildiğinde, adı geçen parametrelerin LPS uygulaması ile arttığı (P<0.05) ve sepsiste beklenilen etkinin şekillendiği tespit edildi. Bu sonuçlar yapılan diğer çalışmalar (Yazar ve ark. 2004; Elmas ve ark. 2006) ile paralellik göstermektedir. Sonuçlar aynı zamanda LPS uygulaması ile karaciğer ve safra kanalı hasarının oluştuğunu da göstermektedir.

Tavşanlarda LPS uygulaması sonrasında yükselen ALT düzeyinin, prednizolon uygulanması ile engellendiği belirlenmiştir (Yazar ve ark. 2004). Mevcut çalışmada gelişen karaciğer ve safra kanalı hasarını önlemede, incelenen tüm karaciğer enzimleri ve serum ALP düzeyi dikkate alındığında, metilprednizolon ve kombinasyon uygulamasının başarılı olduğu ve sağlıklı bireyler kadar olmasa da olumlu sonuçlar verdiği tespit edildi. ALT düzeyi ölçümünde metilprednizolon uygulaması ile 4, 8 ve 12. saatlerde yükselme tespit edildi. Bu yükselmenin metilprednizolon uygulamasına bağlı olarak şekillenebilecek steroid hepatopatiden kaynaklabileneceği düşünülmektedir. Metilprednizolon ve kombinasyon uygulamasının yükselen karaciğer enzimlerini düşürmede etkili olmasındaki nedenin karaciğerde Serbest Oksijen Radikalleri (SOR) ve sitokin üretimine engel olabilmesinden kaynaklandığı düşünülmektedir.

Sepsis vb durumlarda serum üre (veya kan üre azotu) ve kreatinin artışları yaygındır ve kreatinin konsantrasyonunda küçük bir artış bile kritik hastalığı olan hastalarda daha kötü sonuçlarla ilişkilidir (Lelubre ve Vincent 2018). Böbrek hasarı belirteçleri serum BUN ve kreatinin düzeyinin LPS uygulanan laboratuvar hayvanlarında yükseldiği bildirilmiştir (Wang ve ark. 2004; Yazar ve ark. 2004; Wedn ve ark. 2020). Üre, nitrojen amonyak metabolizmasının son ürünü olarak, kreatinin ise kreatin fosfatın nonenzimatik parçalanması sonrası son ürün olarak kana geçer. BUN düzeyinin kedilerde ve bazı atlarda 15 mmol/L'ye kadar yükseldiği, BUN için üst sınırın 30 mg/dL, kreatinin için ise üst sınırın 2 mg/dL olabileceği bildirilmiştir (Turgut 2000; Kerr

2002). Yazar ve ark. (2009)'nın yaptığı çalışmada fluniksin meglumin uygulamasının LPS uygulanan ratlarda yükselen BUN seviyesini değiştiremediği, dekzametazonun ise tek başına ve kombine uygulamada etkili olarak BUN düzeyini yükseltmeye engel olduğu bildirilmektedir.

Mevcut çalışmada LPS uygulanmasının ardından dereceli bir BUN yükselmesi gözlendi. Serum BUN düzeyi 12 ve 24. saatlerde maksimum seviyesine ulaştı. 12. saatte yükselen BUN seviyesini metilprednizolon uygulamasının düşürdüğü ($P<0.05$), 24. saatte ise metilprednizolon ve marbofloksasin uygulamasının etkili olduğu ve BUN seviyesini düşürdüğü ($P<0.05$) belirlendi. Çalışmada kreatinin düzeyi LPS uygulamasının 24. saatinde sağlıklı bireylere oranla 4 kat ve üzerinde yükseldi. LPS uygulaması ile yükselen kreatinin düzeyini 24. saatte bütün uygulamaların düşürdüğü ($P<0.05$) belirlendi. Ancak kreatinin ilk saatleri göz önüne alındığında LPS'den kombinasyon uygulaması dışında diğer uygulamaların farkının olmadığı ($P>0.05$) gözlendi. Metilprednizolon'un böbrek hasarı üzerindeki etkisinin yine karaciğer üzerindeki etkisinde olduğu gibi SOR ve sitokin üretimine engel olmasına bağlı olduğu düşünüldü.

Sonuç olarak, LPS ile deneysel endotoksemi şekillendirilen bu araştırmada doğal olarak şekillenen sepsise benzer biyokimyasal bulgular elde edildi. Bu sonuçlara bağlı olarak 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.

ÇIKAR ÇATIŞMASI

Yazarlar bu çalışma için herhangi bir çıkar çatışması olmadığını beyan ederler.

TEŞEKKÜR VE BİLGİLENDİRME

Bu araştırma Van Yüzüncü Yıl Üniversitesi Bilimsel Araştırma Projeleri Başkanlığı tarafından 2010-SBE-D174 no'lu proje ile desteklenmiştir. Bu çalışma Ahmet Cihat ÖNER isimli yazarın doktora tezinden elde edilen veriler ile gerçekleştirilmiş olup "IV. Ulusal Veteriner Farmakoloji ve Toksikoloji Kongresi" isimli kongrede poster sunu olarak sunulmuş, kongre kitabına özet metin olarak basılmıştır.

YAZAR KATKILARI

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

KAYNAKLAR

Berger MM, Chiolero RL (2007). Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. *Crit Care Med,* 35, 584-590.

Bonelli F, Meucci V, Divers TJ, Boccardo A, Pravettoni D ve ark. (2018). Plasma procalcitonin concentration in healthy calves and those with septic systemic inflammatory response syndrome. *Vet J*, 234, 61-65.

Boscolo P, Youinou P, Theoharides TC, Cerulli G, Conti P (2008). Environmental and occupational stress and autoimmunity. *Autoimmun Rev*, 7, 340-343.

Camcıoğlu Y, Aytaç E (2007). Sepsisin İmmunopatogenezi. *Türk Yoğun Bakım Dergisi,* 5, 81-85.

Constable PD, Kenneth W, Hinchcliff KW, Done SH, Grünberg W (2017). Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 11th ed., Saunders, Missouri.

Dawulieti J, Sun M, Zhao Y, Shao D, Yan H, et all (2020). Treatment of severe sepsis with nanoparticulate cell-free DNA scavengers, *Sci Adv*, 6, 7148.

Elmas M, Yazar E, Uney K, Er Karabacak A (2006). Influence of Escherichia coli endotoxin induced endotoxaemia on the pharmacokinetics on enrofloxacin after intravenous administration in rabbits. *J Vet Med A*, 53, 410-414.

Elmas M, Yazar E, Uney K, Er Karabacak A, Traş B (2008). Pharmacokinetics of enrofloxacin and flunixin meglumine and interactions between both drugs after intravenous co-administration in healthy and endotoxaemic rabbits. *Vet J*, 177, 418-424.

Hart KA, MacKay RJ (2015). Endotoxemia and sepsis. In: Smith BP (Editor). Large Animal Internal Medicine (pp.682-695). 5th Edition, St.Louis, Missouri: Elsevier.

James PE, Madhani M, Roebuck W, Jackson SK, Swartz HM (2002). Endotoxin-induced liver hypoxia: Defective oxygen delivery versus oxygen consumption. *Nitric Oxide*, 6, 18-28.

Kerr MG (2002). İndividual enzyme interpretation (pp.101-147). Veterinary Laboratuary Medicine, Blackwell Science, 2th edit.

Lelubre C, Vincent JL (2018). Mechanisms and treatment of organ failure in sepsis. *Nat Rev Nephrol*, 14 (7), 417-27.

Lukashenko PV, Lukashenko TM, Savanovich II, Sandakov DB, Gerein V (2004). Granulocyte macrophage colony-stimulating factor regulates activity of the nervous system. *Neuroimmunomodulation,* 11, 36-40.

Meduri GU (1999). An historical review of glucocorticoid treatment in sepsis. Disease pathophysiology and the design of treatment investigation. *Sepsis,* 3, 21-38.

Minneci PC, Deans KJ, Banks SM, Eichacker PQ, Natanson C (2004). Meta-Analysis: The effect of steroids on survival and shock during sepsis depends on the dose. *Ann Intern Med*, 141 (1), 47-58.

Pardon B, Deprez P (2018). Rational antimicrobial therapy for sepsis in cattle in face of the new legislation on critically important antimicrobials. *Vlaams Diergen Tijds*, 87, 37-46.

Pastor CM, Billiar TR, Losser MR, Payen DM (1995). Liver injury during sepsis. *J Crit Care Med,* 10, 183-197.

Prauchner CA (2017). Oxidative stress in sepsis: Pathophysiologicalimplications justifying antioxidant co-therapy, *Burns*, 43, 471-485.

Swarnalatha Y, Sivakkumar SU, Siddharthan S (2020). Protective role of heptamethoxyflavone on LPS-induced hepatotoxicity. *Toxin Reviews.*

Traş B, Yazar E, Elmas M (2007). Veteriner Hekimliğinde İlaç Kullanımına Pratik ve Akılcı Yaklaşım. Olgun Çelik Ofset Matbaası, Konya.

Turgut K (2000). Veteriner Klinik Laboratuvar Teşhis. Bahçıvanlar A.Ş, Konya.

Wedn AM, El-Gowilly SM, El-Mas MM (2020). Time and sex dependency of hemodynamic, renal, and survivability effects of endotoxemia in rats. *Saudi Pharmaceutical Journal,* 28, 127-135.

Wang CC, Lee YM, Wei HP, Chu CC, Yen MH (2004). Dextromethorphan prevents circulatory failure in rats with endotoxemia. *J Biomed Sci,* 11, 739-747.

Yarema TC, Yost S (2011). Low-Dose Corticosteroids to Treat Septic Shock: A Critical Literature Review. *Crit Care Nurse*, 31, 16-26.

Yazar E, Çöl R, Konyalıoğlu S ve ark. (2004). Effects of vitamin E and prednisolone on biochemical and haematological parameters in endotoxemic New Zealand white rabbits. *B Vet I Pulawy*, 48, 105-108.

Yazar E, Elmas M, Traş B, Üney K, Er A (2009). Septik şok tedavisinde kullanılan ilaçların şokun patofizyolojisine etkileri. Tübitak Projesi, Proje No: 107O042.

Yazar E, Er A, Uney K, Bulbul A, Avcı GB ve ark. (2010). Effects of drugs used in endotoxic shock on oxidative stress and organ damage markers. *Free Rad Res*, 44, 397-402.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Aşılıoğlu B, Gökpınar S (2021).** Prevalence of lungworms according to fecal examination in cats and dogs. *Van Vet J*, 32(3), 104-108. DOI: https://doi.org/10.36483/vanvetj.952844

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Dışkı Bakısına Göre Kedi ve Köpeklerde Akciğer Kıl Kurtlarının Prevalansı

Barış AŞILIOĞLU[1] Sami GÖKPINAR[2*]

[1] *Kırıkkale University, Health Sciences Institute, Department of Veterinary Parasitology, 71450, Kırıkkale, Turkey*
[2] *Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, 71450, Kırıkkale, Turkey*

Received: 16.06.2021 Accepted: 22.11.2021

ÖZ

Bu çalışmanın amacı Kırıkkale ve Ankara illerinde bulunan kedi ve köpeklerden toplanan dışkı örneklerinin akciğer kılkurdu larvalarının varlığı yönünden araştırılmasıdır. Bu amaçla 100 adet kedi ve 100 adet köpek dışkısı ayrı dışkı kaplarına alınmış ve soğuk zincir kurallarına uyularak aynı gün içinde laboratuvara ulaştırılmıştır. Bu örnekler Baermann-Wetzel yöntemi kullanılarak incelenmiştir. İncelenen kedi dışkılarından 4 tanesinde (%4) *Aelurostrongylus abstrusus* birinci dönem larvalarına rastlanırken, köpeklerin hiçbirinde akciğer nematodu larvalarına rastlanmamıştır. Kedilerde *A. abstrusus* larvaları ≤1 yaş kedilerde, 1 yaşından büyük kedilere göre, erkeklerde dişilere, melez ırklarda saf ırklara ve antiparaziter tedavi alan kedilerde almayanlara göre daha yüksek oranda bulunmuştur. Ancak bu etkenin kedilerdeki varlığı yönünden ırk, yaş, cinsiyet ve tedavi durumuna göre istatistiksel olarak anlamlı bir fark bulunmamıştır. Sonuç olarak Türkiye'de kedilerde akciğer kılkurdu olarak baskın türün *A. abstrusus* olduğu, solunum sistemi belirtisi olan kedilerde bu etkenin enfeksiyon nedeni olarak göz önüne alınması gerektiği kanısına varılmıştır. Köpeklerde akciğer nematodlarının teşhisinde dışkı muayenesinin yanısıra salyalarında akciğer kıl kurdu larvaları yönünden incelemesinin kesin teşhis için daha yararlı olacağı düşünülmektedir.

Anahtar Kelimeler: *Akciğer, Kediler, Köpekler, Nematod.*

ABSTRACT

Prevalence of Lungworms According to Fecal Examination in Cats and Dogs

The aim of this study is to investigate the presence of lungworm larvae by examining fecal samples collected from cats and dogs in Kırıkkale and Ankara. For this purpose, feacal saples 100 cats and 100 dogs were taken into separate stool containers and delivered to the laboratory within the same day, following the cold chain rules. These samples were analyzed using the Baermann-Wetzel method. While *A. abstrusus* first instar larvae were found in 4 (4%) of the cat feces examined, lung nematode larvae were not found in any of the dogs. In cats, *A. abstrusus* larvae were found at a higher rate in cats 1 year old and younger than cats older than 1 year, males compared to females, cross breeds compared to pure breeds, and cats that received antiparasitic treatment compared to those that did not. However, there was no statistically significant difference in terms of the presence of this parasite in cats according to breed, age, sex, ownership and treatment status. As a result; It has been concluded that the dominant species as lungworm in cats in Turkey is *A. abstrusus* and that this parasite should be considered as the cause of infection in cats with respiratory system symptoms. In the diagnosis of lung nematodes in dogs, it is thought that the examination of the saliva together with the stool examination will be more useful for the definitive diagnosis.

Keywords: *Cat, Dog, Lung, Nematoda.*

GİRİŞ

Kedi ve köpeklerin akciğer kıl kurtları Metastrongyloidea üst ailesinde bulunur. Türlere göre enfektif dönem birinci veya üçüncü dönem larvalardır. Bazı türler direkt olarak gelişirken bazı türlerde indirekt olarak gelişirler (Doğanay ve ark. 2018). Karnivorların solunum yolu nematodları ile ilgili bildirimler Avrupa'da giderek yaygınlaşmaktadır (Traversa ve ark. 2010; Giannelli ve ark. 2017).

Şiddetli ve bazen ölümcül enfeksiyonlara sebep olan bu nematodlardan köpeklerde *Angiostrongylus vasorum* (Morgan ve Shaw 2010) en yaygın tür olarak görülmektedir. *Oslerus osleri*, *Crenosoma vulpis*, *Filaroides hirthi* ise daha sınırlı bir yayılışa sahiptir (Traversa ve ark. 2010; Latrofa ve ark. 2015; Cervone ve ark. 2018). Evcil kedilerde solunum sistemini etkileyen en önemli tür ise *Aelurostrongylus abstrusus*'tur (Colella ve ark. 2019). Bunun yanında esas olarak yabani kedilerde bulunan *Troglostrongylus subcreanatus*, *Oslerus rostratus* ve *Gurltia paralysans* daha az yaygındır (Bowman ve ark. 2002).

✉ *Corresponding author: samigokpinar@kku.edu.tr

A. abstrusus erişkinleri evcil kedilerin terminal bronşiyolleri, alveolleri ve alveol kanallarında bulunan bir nematoddur (Carruth ve ark. 2019). *A. abstrusus*'un son konaklara bulaşması ara konak olan gastropodların alınması ile gerçekleşebilir. Ancak bulaşmanın daha çok paratenik konak olarak görev yapan omurgalıların yenmesine bağlı olarak gerçekleştiği bildirilmektedir (Anderson 2000). Gastropodlar evcil kedilerin beslenmek için tercih ettikleri avlar değildir (Woods ve ark. 2013). Yumuşakçaların da kediler tarafından alındığında kedilerde kusma etkisi meydana getirdikleri öne sürülmektedir (Brianti ve ark. 2014). Bu nedenle parazitin yaşam çemberinin sürdürülmesinde ve parazitin son konaklara bulaşmasında kedilerin paratenik konak görevi yapan kemirgenler, kuşlar, sürüngenler ve kurbağa gibi canlıları avlama davranışı önemli bir yer tutmaktadır (Anderson 2000; Jezewski ve ark. 2013; Falsone ve ark. 2017).

Türkiye'de kedi ve köpeklerde akciğer kıl kurtlarının belirlenmesine yönelik sınırlı sayıda çalışma mevcuttur. Bu çalışmalarda kedilerde *A. abstrusus* (Tüzer ve ark. 2002; Burgu ve Sarımehmetoğlu 2004; Atasever ve Yazar 2009; Gökpınar ve Yıldız 2010; Yıldız ve Gökpınar 2011) ve *Troglostrongylus brevior* (Umur ve ark. 2020), köpeklerde ise *O. osleri* (Pamukçu ve Ertürk 1961), *F. hirthi* (Doğanay 1983) ve *A. vasorum* (Tiğin 1972; Doğanay 1983) türleri bildirilmiş olup, bu çalışmaların büyük bir kısmı vaka bildirimi şeklindedir. Türkiye'de kedilerde akciğer kıl kurtlarının yaygınlığının belirlenmesine yönelik çalışma bulunmamaktadır. Bu çalışmada Kırıkkale ve Ankara illerinde bulunan kedi ve köpeklerden toplanan dışkı örneklerinin Baermann-Wetzel yöntemi ile incelenerek, akciğer kılkurdu larvalarının varlığı yönünden araştırılması amaçlanmıştır.

MATERYAL VE METOT

Bu çalışma, Kırıkkale Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan 10.09.2020 tarihinde E.5559 sayılı izin alınarak yapılmıştır. Çalışmada 100 kedi ve 100 köpek dışkı örnekleri kullanılmış olup, örneklenen hayvanlara ait veriler sırasıyla Tablo 1 ve Tablo 2'de verilmiştir. Köpek dışkı örnekleri kliniklerde veya barınaklarda bireysel olarak bağlı köpeklerin kulübelerinden, kedilerde ise kliniklerde veya evde bireysel olarak bakılan kedilerin dışkılarını yaptıkları kumlardan veya kafeslerden altlıkla birlikte alınmıştır. Alınan dışkı örnekleri uygun koşullarda laboratuvara ulaştırıldıktan sonra Baermann-Wetzel yöntemi ile (Zajac ve Comboy 2012) ışık mikroskobunda akciğer kıl kurdu larvalarının varlığı yönünden incelenmiştir. Tespit edilen larvalar ilgili literatürler kullanılarak teşhis edilmiştir (Zajac ve Comboy 2012).

İstatistiksel Analiz

Veriler Ki-kare yöntemi kullanılarak analiz edilmiştir. Hesaplamalarda istatistik anlamlılık düzeyi %5 olarak alınmış ve analiz için SPSS (IBM SPSS for Windows ver. 22) istatistik paket programından yararlanılmıştır.

Tablo 1. Kedi dışkı örneklerinin ırk, yaş, cinsiyet ve tedavi durumuna göre dağılımı.

Table 1. Distribution of cat fecal samples by breed, age, gender and treatment status.

IRKLAR	Sayısı(n)	≤1 yaş (n)	>1 yaş (n)	Dişi (n)	Erkek (n)	Tedavi var	Tedavi yok
Melez	71	44	27	41	30	28	43
Chinchilla	1	-	1	1	-	1	-
Bombay	1	1	-	1	-	1	-
Van kedisi	1	1	-	-	1	1	-
Ankara kedisi	18	9	9	9	9	9	9
İran kedisi	2	-	2	2	-	1	1
Scottish	2	1	1	1	1	1	1
British	4	2	2	4	-	2	2
Toplam	**100**	**58**	**42**	**59**	**41**	**44**	**56**

Tablo 2. Köpek dışkı örneklerinin ırk, yaş, cinsiyet ve tedavi durumuna göre dağılımı.

Table 2. Distribution of dog fecal samples by breed, age, gender and treatment status.

IRKLAR	Sayısı (n)	≤1 yaş (n)	>1 yaş (n)	Dişi (n)	Erkek (n)	Tedavi var	Tedavi yok
Melez	49	34	15	31	18	3	45
Samoyed	1	1	-	-	1	-	1
Pomerian	1	-	1	-	1	1	-
Pointer	2	1	1	1	1	1	1
Doberman	3	2	1	2	1	2	1
Kangal	7	2	5	3	4	1	5
Coccier	3	-	3	2	1	3	-
Rotweiler	4	2	2	3	1	2	2
French Bulldog	1	1	-	1	-	-	1
Golden	7	-	7	4	3	2	4
Pitbull	7	2	5	2	5	1	5
Malaklı	2	2	-	-	2	-	1
Labrador	3	2	1	-	3	3	-
Boxer	2	1	1	-	2	1	1
King Charles	1	-	1	-	1	1	-
Border Collie	1	-	1	1	-	1	-
Terrier	6	2	4	4	2	2	4
Toplam	**100**	**52**	**48**	**54**	**46**	**24**	**71**

BULGULAR

Çalışmada dışkı örnekleri alınan kedilerin 4 (%4) tanesinde akciğer kıl kurdu birinci dönem larvalarına rastlanmıştır. Bu larvaların tümü *A. abstrusus* olarak tanımlanmıştır (Şekil 1). Kedi dışkılarının Baermann-Wetzel yöntemi ile muayenesinde *A. abstrusus* larvalarına ≤1 yaş kedilerde, 1 yaş üzerindeki kedilere göre daha yüksek oranda rastlanmıştır. Ancak yaş ile *A. abstrusus* pozitifliği arasında istatistik olarak anlamlı bir ilişki bulunamamıştır (P>0.05).

Irk düzeyinde yapılan karşılaştırmalarda kediler saf ırk ve melez ırklar olarak değerlendirilmiştir. Buna göre melez ırklarda *A. abstrusus* larvalarına, saf ırklardan daha fazla rastlanmıştır. Ancak ırklar arasında anlamlı bir fark bulunamamıştır (P>0.05).

Cinsiyete göre *A. abstrusus*'un yaygınlığı karşılaştırıldığında erkeklerde dişilere oranla daha yüksek bir oran bulunmasına rağmen, yine istatistiki olarak cinsiyet ile *A. abstrusus* pozitifliği arasında anlamlı bir ilişki bulunamamıştır (P>0.05).

Akciğer kıl kurdu larvalarına antiparaziter tedavi uygulanan ve uygulanmayan kedilerde de rastlanmıştır. Tedavi durumu ile *A. abstrusus* pozitifliği arasında istatistik olarak anlamlı bir ilişki bulunmamasına rağmen, antiparaziter tedavi alan kedilerde daha yüksek oranda parazite rastlanmıştır (P>0.05) (Tablo 3).

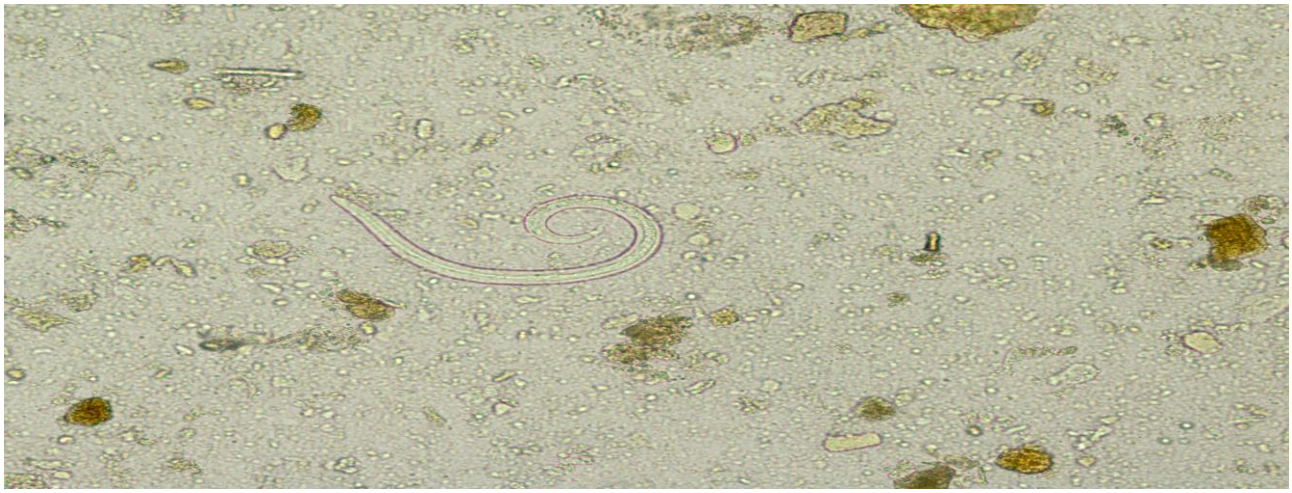
Köpeklerde ise dışkı bakısında akciğer nematodlarının gelişim dönemlerine rastlanmamış, tümü negatif olarak değerlendirilmiştir.

Tablo 3. Örneklenen kedilere ait epidemiyolojik veriler.

Table 3. Epidemiological data of sampled cats.

	İncelenen kedi sayısı (n)	Pozitif kedi sayısı (n)	%
Yaş*			
≤1	58	3	5.2
>1	42	1	2.4
Irk*			
Melez	71	3	4.2
Saf ırk	29	1	3.4
Cinsiyet*			
Dişi	59	2	3.4
Erkek	41	2	4.9
Tedavi durumu*			
Var	44	2	4.5
Yok	56	2	3.6

*: P>0.05 (Ki-kare testi)



Şekil 1. *Aelurostrongylus abstrusus* birinci dönem larva.

Figure 1. First instar larva of *Aelurostrongylus abstrusus.*

TARTIŞMA VE SONUÇ

Dünya'da çeşitli ülkelerde kedi ve köpeklerde akciğer kıl kurtlarının belirlenmesine yönelik çok sayıda çalışma bulunmasına rağmen, Türkiye'de bu etkenlerin belirlenmesine yönelik sınırlı sayıda çalışma bulunmakta ve bu çalışmalar genellikle vaka takdimleri şeklindedir. Bununla birlikte son yıllarda Türkiye'de özellikle kedilerde bu bildirimlerin gittikçe arttığı dikkati çekmektedir (Tüzer ve ark. 2002; Burgu ve Sarımehmetoğlu 2004; Atasever ve Yazar 2009; Gökpınar ve Yıldız 2010; Yıldız ve Gökpınar 2011; Umur ve ark. 2020).

Dünya'nın farklı bölgelerinde evcil kedilerde yapılan çalışmalarda *A. abstrusus* ve *T. brevior* türlerine rastlanmıştır. Son yıllarda Dünya'da çeşitli ülkelerde *A. abstrusus*'un yaygınlığını belirlemeye yönelik çok sayıda çalışma yapılmıştır. Bu çalışmalarda *A. abstrusus* oranı Kolombiya'da %0.4 (Lopez-Osorio ve ark. 2021), Belçika'da %0.92 (Giannelli ve ark. 2017), Fransa'da %4.34 (Giannelli ve ark. 2017), İsviçre'de %0.80 (Giannelli ve ark. 2017), Hollanda'da %2.6 (Robben ve ark. 2004), Yunanistan'da %8 (Diakou ve ark. 2015), İtalya'da %5-17.8 (Traversa ve ark. 2008; Di Cesare ve ark. 2015; Giannelli ve ark. 2015; Giannelli ve ark. 2017) Portekiz'de %0.83-17.4 (Payo-Puente ve ark. 2008; Nabais ve ark. 2014; Waap ve ark. 2014; Giannelli ve ark. 2017),

İspanya'da %1-5 (Miro ve ark. 2004; Giannelli ve ark. 2017), Danimarka'da %8.86-13.6 (Olsen ve ark. 2015; Hansen ve ark. 2017), İngiltere'de %1.7 (Elsheikha ve ark. 2019), Bulgaristan'da %33.3-35.8 (Stoichev ve ark. 1982; Giannelli ve ark. 2017), Macaristan'da %19.8-22.5 (Kiszely ve ark. 2019), Romanya'da %6.10 (Ciopaşiu ve ark. 2018), ABD'de %2.07 (Carruth ve ark. 2019) oranında bildirilmiştir. Çalışmamızda Baermann-Wetzel yöntemi ile incelenen 100 kedi dışkısının 4 tanesinde (%4) *A. abstrusus* birinci dönem larvaları tespit edilmiştir. Bu sonuç dünyada yapılan çalışmalarla benzerlik göstermektedir. Ancak Bulgaristan ve Macaristan'daki çalışmalara göre çok daha düşük çıkmasının sebebi olarak çalışma yapılan bölgelerin epidemiyolojik koşullarının birbirinden farklı olması düşünülmüştür.

Çalışmamızda *A. abstrusus* oranı cinsiyete göre karşılaştırıldığında erkelerde dişilere oranla daha yüksek bulunmasına rağmen, istatistiki olarak anlamlı bir fark bulunmamıştır (P>0.05). Carruth ve ark. (2019) çalışmamıza benzer şekilde Baermann-Wetzel yönteminde *A. abstrusus* oranını erkeklerde dişilere oranla daha yüksek oranda bulmuş ancak cinsiyetler arasında istatistiki olarak anlamlı bir fark bulamamışlardır. Hansen ve ark. (2017) dişi kedilerde daha yüksek oranda *A. abstrusus* pozitifliği saptamış, fakat yine cinsiyete göre istatistiki olarak anlamlı bir fark tespit edememişlerdir.

Çalışmamızda 1 yaş ve altındaki kedilerde, 1 yaş üstü kedilere göre daha yüksek oranda *A. abstrusus* saptanmasına rağmen, yaş grupları arasında istatistiki olarak anlamlı bir fark bulunamamıştır. Ciopaşiu ve ark. (2018) 2 aylık- 1 yaş arası kedilerde *A. abstrusus* oranını 1-2 yaş ve 2 yaşından büyük kedilere göre daha yüksek oranda bulduklarını bildirmişlerdir. Carruth ve ark. (2019), 1-12 aylık kedilerde, 12 aylıktan büyük kedilere oranla daha yüksek oranda saptadıklarını ve yaş grupları arasında istatistiki olarak anlamlı bir fark bulunduğunu bildirmişlerdir. Hansen ve ark. (2017) en yüksek pozitifliği 11-51 haftalık kedilerde saptamışlardır ve 10 haftalıktan küçük, 1-3 yaş ve 3 yaşından büyük kedilerle karşılaştırdıklarında istatistiki olarak anlamlı bir fark bulmuşlardır. Giannelli ve ark. (2017) Avrupa'nın çeşitli ülkelerinde yaptıkları çalışmada *A. abstrusus* oranını 2 yaşından büyük kedilerde, 1-2 yaş, 6-12 aylık ve 6 aylıktan küçük kedilere oranla daha yüksek bulduklarını bildirmişlerdir. Ancak bahsedilen tüm çalışmalarda etkenin genç kedilerde yaşlılarda daha yüksek olduğu dikkati çekmektedir.

Çalışmamızda kedi ve köpeklerde antiparaziter tedavi durumuna göre akciğer kıl kurdu larvalarının yaygınlıkları araştırılmıştır. *A. abstrusus*, rutin olarak düzenli bir şekilde kliniklerde antiparaziter tedavi (fenbendazole, ivermektin, selamektin ve miks etken maddeli antihelmintik) uygulanan kedilerde antiparaziter tedavi uygulanmayanlara göre daha yüksek bulunmuştur. Ancak gruplar arasında istatistiki olarak anlamlı bir fark bulunmamıştır. Hansen ve ark. (2017) çalışmamızın aksine tedavi uygulanmayan kedilerde daha yüksek oranda *A. abstrusus* pozitifliği saptamışlardır. Ancak söz konusu çalışmada da tedavi uygulanan ve uygulanmayan kediler arasında istatistiki olarak anlamlı bir fark tespit edemediklerini bildirmişlerdir.

Çalışmaya alınan kediler melez ırklar ve saf ırklar olarak değerlendirilmiş ve sonuçlar buna göre karşılaştırılmıştır. Melez ırk kedilerde, saf ırk kedilere göre daha yüksek oranda saptanmasına rağmen, yaş, cinsiyet ve tedavi durumunda olduğu gibi yine istatistiki olarak anlamlı bir fark bulunmamıştır. Melez ırk kedilerin daha çok sahipsiz olması, bunların ara konaklarla karşılaşma olasılığını arttırmaktadır. Bu nedenle melez kedilerde saf ırk kedilere oranla daha yüksek düzeyde *A. abstrusus* larvası saptanmış olabileceği düşünülmüştür.

Türkiye'de yapılan çalışmalarda kedilerde akciğer kıl kurtlarından *A. abstrusus* ve *T. brevior*'a rastlanmıştır (Tüzer ve ark. 2002; Burgu ve Sarımehmetoğlu 2004; Atasever ve Yazar 2009; Gökpınar ve Yıldız 2010; Yıldız ve Gökpınar 2011; Umur ve ark. 2020).

Çalışmamızda kedilerde %4 oranında *A. abstrusus*'a rastlanmış, *T. brevior* ise tespit edilememiştir. Türkiye'de daha önce yapılan çalışmalarda ve vaka bildirimlerinde *A. abstrusus*'un daha yaygın olduğu, *T. brevior*'a sadece 1 vakada rastlandığı görülmektedir. Bu bilgiler ışığında Türkiye'de evcil kedilerde akciğer kıl kurtları arasında baskın türün *A. abstrusus* bir kez daha ortaya konmuştur.

Türkiye'de köpeklerde dışkı bakısına göre *F. hirthi* %6 ve *A. vasorum* %2 olarak tespit edilmiştir (Doğanay 1983). *O. osleri* ise nekropside %0.6 oranında bildirilmiştir (Pamukçu ve Ertürk 1961). Çalışmamızda ise köpeklerde dışkı bakısına göre akciğer kıl kurdu larvalarına rastlanmamıştır.

Sonuç olarak çalışmamızda Baermann-Wetzel yöntemi ile yapılan dışkı bakısına göre akciğer kıl kurtlarından kedilerde *A. abstrusus*'un birinci dönem larvalarına rastlanırken, köpeklerde herhangi bir paraziter etkene rastlanmamıştır. Solunum sistemi ile ilgili klinik belirti gösteren kedilerin *A. abstrusus* varlığı yönünden de değerlendirilmesi gerektiği düşünülmektedir. Köpeklerde akciğer kıl kurtlarının yaygınlığı çalışmalarında dışkı bakısının tek başına yeterli olamayacağı, ayrıca hayvanların salyasının da incelenmesi gerektiği kanaatine varılmıştır. Söz konusu çalışma, Türkiye'de kedi ve köpeklerde akciğer kıl kurtlarının yaygınlığının belirlendiği ilk çalışma olması bakımından önemlidir.

ÇIKAR ÇATIŞMASI

Yazarlar bu çalışma için herhangi bir çıkar çatışması olmadığını beyan ederler.

TEŞEKKÜR VE BİLGİLENDİRME

Bu çalışma 21st International Veterinary Medicine Students Scientific Research Congress isimli kongrede sözlü sunu olarak sunulmuş, kongre kitabına özet metin olarak basılmıştır.

YAZAR KATKILARI

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

KAYNAKLAR

Anderson RC (2000). Nematode Parasites of Vertebrates: Their Development and Transmission. CABI, Wallingford.

Atasever A, Yazar S (2009). İmmun sistemi baskılanmış bir kedide *Aelurostrongylus abstrusus* pnömonisi. *Türkiye Parazitol Derg,* 33 (1), 89-91.

Bowman D, Hendrix C, Lindsay D, Barr S (2002). Feline Clinical Parasitology. I. Edition. Iowa State University Press, Iowa.

Brianti E, Giannetto S, Dantas-Torres F, Otranto D (2014). Lungworms of the genus Troglostrongylus (Strongylida: Crenosomatidae): neglected parasites for domestic cats. *Vet Parasitol*, 202, 104–112.

Burgu A, Sarımehmetoğlu O (2004). *Aelurostongylus abstrusus* infection in two cats. *Vet Rec,* 154, 602-604.

Carruth AJ, Buch JS, Braff JC, Chandrashekar R, Bowman DD (2019). Distribution of the feline *Aelurostrongylus abstrusus* in the USA based on fecal testing lungworm. *JMSF Open Reports,* 1-6.

Cervone M, Giannelli A, Rosenberg D, Perrucci S, Otranto D (2018). Filaroidosis infection in an immunocompetent adult dog from France. *Helminthologia*, 55 (1), 77-83.

Ciopaşiu RM, Berbece EF, Ionita M, Mitrea IL (2018). Clinical and epidemiological study on *Aelurostrongylus abstrusus* infection in cats, Romania; Preliminary data. *Agriculture for Life Life for Agriculture" Conference Proceedings*, 1(1), 428-431.

Colella V, Knaus M, Lai O et al. (2019). Mice as paratenic hosts of *Aelurostrongylus abstrusus*. *Parasit Vector,* 12, 49.

Diakou A, Di Cesare A, Barros LA et al. (2015). Occurrence of *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* in domestic cats in Greece. *Parasit Vector*, 8, 590.

Di Cesare A, Di Francesco G, Di Regalbono AF et al. (2015). Retrospective study on the occurrence of the feline lungworms *Aelurostrongylus abstrusus* and *Troglostrongylus* spp. in endemic areas of Italy. *Vet J*, 203 (2), 233-238.

Doğanay A (1983). Ankara sokak köpeklerinde görülen helmint türleri, bunların yayılışı ve halk sağlığı açısından önemi. *Ankara Üniv Vet Fak Derg*, 30, 550-561.

Doğanay A, Öge H, Öge S, Yıldız K (2018). Nenatoda (Yuvarlak helmintler). Doğanay A (Ed). Helmintoloji (s. 149-340). Ankara Nobel Tıp Kitabevleri Ltd Şti, Ankara.

Elsheikha HM, Wright I, Wang B, Schaper R (2019). Prevalence of feline lungworm *Aelurostrongylus abstrusus* in England. *Vet Parasitol Reg Stud*, 16, 100271.

Falsone L, Colella V, Napoli E, Brianti E, Otranto D (2017). The cockroach *Periplaneta americana* as a potential paratenic host of the lungworm *Aelurostrongylus abstrusus*. *Exp Parasitol*, 182, 54-57.

Giannelli A, Brianti E, Varcasia A et al. (2015). Efficacy of Broadline_ spot-on against *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* lungworms in naturally infected cats from Italy. *Vet Parasitol*, 209, 273–277.

Giannelli A, Capelli G, Joachim A et al. (2017). Lungworms and gastrointestinal parasites of domestic cats: a European perspective. *Int J Parasitol*, 47 (9), 517-528.

Gökpınar S, Yıldız K (2010). Çeşitli sıcaklık derecelerinin kedi dışkısındaki *Aelurostrongylus abstrusus* 1. dönem larvasının canlılığına etkisi. *Turkiye Parazitol Derg*, 34 (2), 102-105.

Hansen AP, Skarbye LK, Vinther LM et al. (2017). Occurrence and clinical significance of *Aelurostrongylus abstrusus* and another endoparasites in Danish cats. *Vet Parasitol*, 234, 31-39.

Jezewski W, Buńkowska-Gawlik K, Hildebrand J, Perec-Matysiak A, Laskowski Z (2013). Intermediate and paratenic hosts in the life cycle of *Aelurostrongylus abstrusus* in natural environment. *Vet Parasitol*, 198, 401–405.

Kiszely S, Gyurkovszky M, Solymosi N, Farkas R (2019). Survey of lungworm infection of domestic cats in Hungary. *Acta Vet Hung*, 67 (3), 407-417.

Latrofa MS, Lia RP, Giannelli A et al. (2015). *Crenosoma vulpis* in wild and domestic carnivores from Italy: a morphological and molecular study. *Parasitol Res*, 114, 3611-3617.

Lopez-Osorio S, Navarro-Ruiz JL, Rave A et al. (2021). *Aelurostrongylus abstrusus* infections in domestic cats (*Felis silvestris catus*) from Antioquia, Colombia. *Pathogens,* 10, 337.

Miro G, Montoya A, Jimenez S et al. (2004). Prevalence of antibodies to *Toxoplasma gondii* and intestinal parasites in stray, farm and household cats in Spain. *Vet Parasitol*, 126, 249-255.

Morgan E, Shaw S (2010). *Angiostrongylus vasorum* infection in dogs: continuing spread and developments in diagnosis and treatment. *J Small Anim Pract,* 51 (12), 616-621.

Nabais J, Alho AM, Gomes L et al. (2014). *Aelurostrongylus abstrusus* in cats and *Angiostrongylus vasorum* in dogs From Lisbon, Portugal. *Acta Parasitol Port,* 20 (1/2), 35-40.

Olsen CS, Willesen JL, Pipper CB, Mejer H (2015). Occurrence of *Aelurostrongylus abstrusus* (Railliet, 1898) in Danish cats: A modified lung digestion method for isolating adult worms. *Vet Parasitol*, 210, 32-39.

Pamukçu AM, Ertürk E (1961). 1933-1960 yılları arasında Ankara ve yöresinde köpeklerde görülen hastalıklara toplu bir bakış. *Ankara Univ Vet Fak Derg*, 8, 323-346.

Payo-Puente P, Botelho-Dinis M, Urueña AMC et al. (2008). Prevalence study of the lungworm *Aelurostrongylus abstrusus* in stray cats of Portugal. *J Feline Med Surg*, 10, 242-246.

Robben SR, Le Nobel WE, Döpfer D et al. (2004). Infections with helminths and/or protozoa in cats in animal shelters in the Netherlands. *Tijdschr Diergeneeskd*, 129, 2-6.

Stoichev I, Sherkov S, Halachev M (1982). Pathology of cats from a region of Bulgaria with human endemic nephropathy. *J Comp Pathol,* 92, 99-107.

Tiğin Y (1972). Ankara'da bir köpekte tespit edilen *Angiostrongylus vasorum* Baillet, 1866 olayı. *Ankara Univ Vet Fak Derg,* 19, 76-84.

Traversa D, Lia RP, Iorio R et al. (2008). Diagnosis and risk factors of *Aelurostrongylus abstrusus* (Nematoda, Strongylida) infection in cats from Italy. *Vet Parasitol*, 153, 182–186.

Traversa D, Di Cesare A, Conboy G (2010). Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. *Parasit Vector,* 3, 62.

Tüzer E, Toparlak M, Gargılı A, Keleş V, Ulutaş Esatgil M (2002). A Case of *Aelurostrongylus abstrusus* infection in a cat in İstanbul, Turkey and its treatment with moxidectin and levamisole. *Turk J Vet Anim Sci*, 26, 411-414.

Umur Ş, Barılı Ö, Gencay Topçu EB, Gürler AT (2020). Report of a *Troglostrongylus brevior* case in a domestic cat in Turkey. *Turkiye Parazitol Derg*, 44 (3), 176-178.

Waap H, Gomes J, Nunes T (2014). Parasite communities in stray cat populations from Lisbon, Portugal. *J Helminthol,* 88, 389–395.

Woods M, Mcdonald R, Harris S (2013). Predation of wildlife by domestic cats Felis catus in Great Britain. *Mamm Rev*, 33, 177–184.

Yıldız K, Gökpınar S (2011). *Aelurostrongylus abstrusus* ile doğal enfekte kedi akciğerlerinin taramalı elektron mikroskobu ile incelenmesi. *Kafkas Univ Vet Fak Derg*, 17 (2), 315-318.

Zajac AM, Conboy GA (2012). Veterinary Clinical Parasitology. 8th Edition. John Wiley & Sons, Inc.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Doğan E, Merhan O, Erdağ D, Doğan E, Bozukluhan K, Doğan ANC (2021).** The Effect of Vitamin C on Oxidant and Antioxidant Parameters in Anthrax Vaccine Administered Cattle. *Van Vet J*, 32 (3), 109-113. DOI: https://doi.org/10.36483/vanvetj.958358

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

The Effect of Vitamin C on Oxidant and Antioxidant Parameters in Anthrax Vaccine Administered Cattle

Ertan DOĞAN[1,*] Oğuz MERHAN[2] Dinçer ERDAĞ[3] Ege DOĞAN[4] Kadir BOZUKLUHAN[5] Ali Nazmi Can DOĞAN[4]

[1] *University of Ardahan, Department of Laborant and Veterinary Health, Vocational School of Gole Nihat Delibalta, 75700, Ardahan, Turkey*
[2] *University of Kafkas, Department of Biochemistry, Faculty of Veterinary Medicine, 36100, Kars, Turkey*
[3] *University of Kafkas, Department of Medical Services and Techniques, Atatürk Vocational School of Health Services, 36100, Kars, Turkey*
[4] *Ministry of Health, Department of internal medicine, State Hospital of Gole, 75700, Ardahan, Turkey*
[5] *University of Kafkas, Kars School of Higher Vocational Education, 36100, Kars, Turkey*

Received: 27.06.2021 Accepted: 31.08.2021

ABSTRACT In this study, the effects of Vitamin C on oxidative stress in anthrax vaccinated cattle were investigated. The research was performed on 40 healthy cattle without any race and gender difference, and aged 6-8 months that were not anthrax vaccinated. The cattle were divided into four equal groups. The first group was determined as the control group. 1 ml anthrax vaccine was administered to the second group, 1 ml anthrax vaccine and 5 mg/kg dose of vitamin C was administered to the third group, and vitamin C was administered to the fourth group at a dose of 5 mg/kg. Blood collection were collected and serum samples were extracted just before the vaccination and drug administration (0^{th} day) and on the 2^{nd}, 14^{th} and 28^{th} days after the administration. Nitric Oxide (NO), Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase (CAT) levels of serum samples were evaluated. It was found that the MDA and NO levels significantly increased ($P<0.05$), and the amount of SOD and CAT decreased in the vaccine group on the 2^{nd}, 14^{th} and 28^{th} days compared to the control group. It was determined that the MDA and NO levels in the vaccine-vitamin groups on the 2^{nd}, 14^{th} and 28^{th} days decreased insignificantly ($P> 0.05$) compared to the vaccine group. It can be asserted that the use of vitamin C in combination with anthrax vaccine in cattle would be beneficial in terms of reducing oxidative stress.

Keywords: *Anthrax vaccine, Cattle, Lipid peroxidation, Oxidative stress, Vitamin C.*

ÖZ

Şarbon Aşısı Uygulanan Sığırlarda Vitamin C'nin Oksidan ve Antioksidan Parametreler Üzerine Etkisi

Bu çalışmada şarbon aşısı uygulanan sığırlarda oksidatif stres üzerine Vitamin C'nin etkileri araştırıldı. Araştırma, şarbon aşısı yapılmamış, ırk ve cinsiyetleri farklı, yaşları 6-8 ay olan 40 adet sağlıklı sığırlarda yürütüldü. Sığırlar dört eşit gruba ayrıldı. Birinci grup kontrol grubu olarak tutuldu. İkinci gruba 1 ml şarbon aşısı, üçüncü gruba 1 ml şarbon aşısı ve 5 mg/kg dozda C vitamini, dördüncü gruba ise 5 mg/kg dozda C vitamini uygulandı. Aşı ve ilaç uygulanmadan hemen önce (sıfırıncı gün) ve uygulama sonrası 2., 14. ve 28. günlerde kan alınarak serumları çıkartıldı. Serum örneklerinde Nitrik Oksit (NO), Malondialdehit (MDA), Süperoksit Dismutaz (SOD) ve Katalaz (CAT) düzeyleri ölçüldü. Aşı grubunda 2., 14. ve 28. günlerde MDA ve NO miktarının kontrol grubuna göre önemli oranda arttığı ($P<0.05$), SOD ve CAT miktarlarının ise azaldığı tespit edildi. Aşı-vitamin grubunda 2., 14. ve 28. günlerde MDA ve NO miktarlarının aşı gruba göre önemsiz ($P>0.05$) miktarda azaldığı belirlendi. Sığırlarda şarbon aşısı ile birlikte C vitamininin kullanılması oksidatif stresi azaltması açısından faydalı olacağı ileri sürülebilir.

Anahtar Kelimeler: *Antraks aşısı, C vitamini, Lipid peroksidasyonu, Oksidatif stres, Sığır.*

INTRODUCTION

Anthrax is a zoonotic infection caused by the bacterium *Bacillus anthracis* (Lewerin et al. 2010; Suchitra et al. 2010; Parlak et al. 2015; Brawand et al. 2019). The bacterium is gram-positive, and when contacting oxygen after leaving the body, it produces spores (Lewerin et al. 2010). The spore form of the bacterium is highly resistant to high and low temperature, dry air, pH changes and various chemicals. Spores survive in the environment for a long time and maintain their ability to cause disease (Suchitra et al. 2010; Dettwiler et al. 2018; Brawand et al. 2019).

✉ *Corresponding author: ertandogan@ardahan.edu.tr

Anthrax occurs in both domestic and wild animals. Animal species have different sensitivities, and the most susceptible species are reported to be herbivores (Brawand et al. 2019). Animal species susceptible to the disease are cattle, sheep, goats, horses, humans, and pigs. Carnivores, such as cats and dogs, are reported to be more resistant to the disease (Lewerin et al. 2010; Suchitra et al. 2010; Brawand et al. 2019).

Cattle get bacterial spores from contaminated pasture or water. Spores of the agent enter the body mostly through the digestive tract. Although rare, it can be taken from the respiratory tract. The minimal dose of spores that can infect humans and ruminants through the respiratory tract is estimated to be between 8000-50.000 (Dettwiler et al. 2018). The incubation period of the disease is around 1-14 days. Animals often die suddenly without showing any clinical symptoms (Lewerin et al. 2010; Brawand et al. 2019).

Spores taken by digestive and respiratory tracts are captured by macrophages and carried to lymph nodes. They take vegetative form in lymph nodes. When they reproduce, they generate a variety of toxins. Rapidly reproducing bacteria, enter the bloodstream and cause systemic anthrax, which usually results in death (Hanna et al. 1994; Lewerin et al. 2010). Meningoencephalomyelitis is not common in this disease. But if it does occur, mortality rate rises dramatically. Anthrax-induced meningoencephalomyelitis can also occur in humans. It has been reported that cases of anthrax are common in people living in some African, South American, Central Asian and the Middle Eastern countries (Parlak et al. 2015).

Oxygen and nitrogen species are released during the normal metabolic processes in organisms. These groups are known as reactive oxygen (ROS) and reactive nitrogen (RNS) species. Of these species, nitric oxide (NO) and superoxide (O_2^-) participate in intercellular transmission (Abd Ellah et al. 2009; Heidarpour et al. 2013). Reactive oxygen species, also known as free radicals, include superoxide (0^2), hydroxyl radical (OH^-), singlet oxygen (O^-), hydrogen peroxide (H_2O_2), and peroxyl radical (ROO-) (Nockels 1996). It is reported that the amount of these radicals increases when organisms are exposed to infection, heavy exercise, mastitis, pregnancy, metritis and tissue damage at birth (Nockels 1996; Sordillo and Aitken 2009). These substances, especially produced by defense cells, play an essential role in the defense system (Abd Ellah et al. 2009; Łuszczak et al. 2011). If oxidant species are produced in excess quantities, they also damage the somatic cells (Łuszczak et al. 2011). They cause a decrease or damage to the biological function of the cell membrane, DNA, and enzymes. The harmful effects of free radicals are eliminated by antioxidants. However, when the antioxidant defense system is insufficient or when oxidants called ROS and RNS are produced in excess, the cells are damaged due to oxidative stress (Abd Ellah et al. 2009; Jaguezeski et al. 2018). Damage to the cell membrane is caused by ROS. Free radicals generate the lipid peroxidation process by interacting with unsaturated fatty acids in the cell membrane. The final toxic product of this reaction, which indicates the degradation of the cell membrane, is malondialdehyde (MDA) (Bahrami et al. 2014; Esmaeilnejad et al. 2018).

There is an antioxidant defense mechanism that eliminates the harmful effects of oxidizers in the organism. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) form the enzymatic defense system, while trace elements such as albumin, ceruloplasmin, transferrin, vitamin A, E and C, iron, copper, and zinc form the non-enzymatic antioxidant defense system (Heidarpour et al. 2013).

Vitamin C, known as an antioxidant and reducing agent, acts as a cofactor in reactions catalyzed by copper dependent monooxygenases and iron dependent dioxygenases (Linster and Van Schaftingen 2007). Vitamin C is mostly synthesized from glucose in the liver of mammalians by the enzyme gulonolactone oxidase. Since this enzyme is not present in humans and pigs, they must take the vitamin externally. Vitamin C acts as an antioxidant by releasing electrons. Consequently, it protects somatic cells against oxidation. It forms the ascorbyl radical by binding free radicals. Ascorbyl radical is shorter-lived compared to other free radicals and does not possess reactive properties. Ascorbic acid reacts with free radicals, reducing its amount. Ascorbic acid has therefore been called a free radical scavenger (Padayatty et al. 2003).

Anthrax spores maintain long-term viability in the soil and the ability to cause disease. The disease in cattle progresses rapidly and it usually results in sudden death. Thus, the primary way to protect against the disease is to vaccinate healthy animals. It is thought that the administration of ascorbic acid may lead to a reduction in oxidative stress that may be seen as a result of vaccination. In this study, it is aimed to determine the oxidant and antioxidant status in the peracute (0-2 days), acute (2-14 days) and subacute (14-28 days) periods in cattle vaccinated against anthrax disease and to determine the effects of Vitamin C on them.

MATERIAL AND METHODS

This study has been approved by the Ethics Committee of Kafkas University (decision date 27.08.2020 and numbered 2020-119) and Ministry of Agriculture and Forestry of Turkey (letter dated 21.08.2020 and numbered E-2331704). Animal experiments were carried out in a cattle business located in Ardahan province. In the study, 40 healthy cattle, which are 6-8 months old, with different breeds and genders that had never been vaccinated against anthrax disease were used. The animals were divided into four even groups and fed ad libitum with meadow grass and tap water obtained from the same source during the course of the experiment. The first group was determined as the control group. 1 ml of anthrax vaccine (Basilax®-Vetal-Turkey) was intradermally injected to the second group. 1 ml of anthrax vaccine and 5 mg/kg dose of vitamin C (Maxivit-C®-baVET-Turkey) were intradermally injected in one shot at different body parts in the third group. The fourth group was given only vitamin C at a dose of 5 mg/kg. 10 ml of blood was obtained from the *vena jugularis* of the animals just before the drug and vaccine administration (day zero) and on the 2^{nd}, 14^{th} and 28^{th} days following the administration. The samples were centrifuged at 3000 rpm for 15 minutes, blood serums were separated and stored at -20^0C until analyzed. The activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in serum were determined by ELISA (Epoch, Biotek, USA) using commercial kits (Cayman Chemical Company, USA). MDA analysis was determined colorimetrically (Epoch, Biotek, USA) according to the method reported by Yoshioka et al. (1979) and NO by Miranda et al. (2001).

Statistical analysis

Microsoft Windows SPSS 20.0 software was used for the statistical evaluation of the research results. Normal distribution curve was checked with the Shapiro-Wilk test.

In comparison of group averages, one-way analysis of variance (ANOVA) and multiple comparisons were performed using Tamhane's T2 test. The results were given as mean (X) and standard deviation (SD). In the study, P<0.05 was considered statistically important.

RESULTS

In the vaccinated group, an increase in oxidant parameters and a decrease in antioxidant parameters were found compared to the control group (P<0.05). In the study, the amount of MDA and NO did not show any change in the control and vitamin-administered group on the 0th, 2nd, 14th and 28th days, whereas it increased significantly in the vaccine and vaccine-vitamin administered group on the 0th, 2nd, 14th and 28th days. While the amount of SOD and CAT, which are antioxidant parameters, did not show any change in the control and vitamin administered group on the 0th, 2nd, 14th and 28th days, it significantly decreased in the vaccine and vaccine-vitamin administered group on the 2nd, 14th and 28th days compared to the 0th day.

In the study, it was stated that the amount of MDA and NO evaluated on the 2nd, 14th and 28th days in the vaccine-vitamin administered group decreased insignificantly compared to the vaccine group. In the vaccine-vitamin administered group, the SOD level increased significantly on the 2nd and 14th days and increased insignificantly on the 28th day compared to the vaccine group. CAT levels in the vaccine-vitamin administered group were found to be insignificantly increased on the 2nd day and significantly increased on the 14th and 28th days compared to the vaccine administered group. Analyzes and analysis results of the serums obtained from cattle groups are given in Table 1 below.

Table 1. Some oxidative and antioxidant parameters by day.

Parameters	Groups	Day 0	Day 2	Day 14	Day 28
MDA (µmol/L)	Control	2.14 ± 0.1 [1]	2.16 ± 0.54 [1]	2.08 ± 0.52 [1]	2.12 ± 0.28 [1]
	Vaccine	2.14 ± 0.58 [1]	4.70 ± 0.89 [2]	5.14 ± 0.53 [2]	4.54 ± 0.89 [2]
	Vitamin C	2.17 ± 0.57 [1]	1.96 ± 0.46 [1]	2.01 ± 0.30 [1]	1.94 ± 0.48 [1]
	Vac- Vit.C	2.17 ± 0.32 [1]	4.48 ± 1.09 [2]	4.97 ± 1.20 [2]	4.37 ± 0.67 [2]
NO (µmol/L)	Control	32.28 ± 5.10 [1]	35.21 ± 5.52 [1]	36.13 ± 2.74 [1]	32.54 ± 4.69 [1]
	Vaccine	30.77 ± 4.31 [1]	47.62 ± 7.20 [2]	46.85 ± 7.05 [2]	44.59 ± 4.83 [2]
	Vitamin C	29.32 ± 7.25 [1]	26.54 ± 7.03 [1]	25.26 ± 5.87 [1]	27.34 ± 10.52 [1]
	Vac- Vit.C	30.27 ± 7.75 [1]	44.63 ± 15.45 [2]	44.43 ± 15.50 [2]	41.94 ± 4.43 [2]
SOD (U/mL)	Control	253.27 ± 5.35 [1]	254.20 ± 7.07 [1]	252.15 ± 32.60 [1]	253.98 ± 8.92 [1]
	Vaccine	250.70 ± 7.82 [1]	134.63 ± 17.90 [2]	143.40 ± 8.62 [2]	193.31 ± 38.16 [2]
	Vitamin C	249.81 ± 12.92 [1]	247.70 ± 12.21 [1]	241.28 ± 28.09 [1]	242.38 ± 21.89 [1]
	Vac- Vit.C	251.26 ±12.20 [1]	210.37 ± 38.65 [2]	199.66 ± 14.64 [2]	218.01 ± 21.57 [2]
CAT (nmol/min/mL)	Control	37.95 ± 5.04 [1]	39.16 ± 6.98 [1]	39.16 ± 7.81 [1]	37.81 ± 6.09 [1]
	Vaccine	38.85 ± 7.83 [1]	21.34 ± 6.15 [2]	19.89 ± 3.56 [2]	21.69 ± 4.50 [2]
	Vitamin C	37.29 ± 4.39 [1]	35.16 ± 4.41 [1]	36.72 ± 3.66 [1]	39.83 ± 6.61 [1]
	Vac- Vit.C	38.14 ± 7.16 [1]	28.17 ± 7.67 [2]	28.53 ± 8.97 [2]	30.15 ± 5.20 [2]

DISCUSSION AND CONCLUSION

Anthrax often causes sudden death in cattle. High fever, bleeding in the mucous membranes, edema in the head, neck and abdomen, noncoagulating dark red bleeding in the mouth, ear or nose are noted as clinical symptoms seen in some patients (Lewerin et al. 2010; Suchitra et al. 2010). In some patients, anthrax was isolated in the stillbirth, placenta and sublumbal lymph nodes following high fever (Brawand et al. 2019). For this reason, anthrax should be considered in cases of high fever and abort that may occur in cattle. Taking necessary measures in such cases is of great importance in terms of human and animal health. Because the disease is of importance in terms of public health, regular vaccination of healthy animals is necessary for protection against the disease. Disease and inoculations can affect oxidative stress levels in the body.

The increase of free oxygen radicals in the body initiates a series of reactions in the cell membrane. As a result of these reactions, MDA occurs as the final product. The formation of MDA is considered an important indicator of lipid peroxidation. Changes in oxidant and antioxidant balance after the administration of anthrax vaccine to cattle can change the expected results of vaccination. Therefore, a great number of researches have been conducted on oxidant and antioxidant balance in diseases. Studies have shown that there is an increase in MDA level in diseases such as Tuberculosis (Kizil and Keltek 2017), Brucellosis (Perin et al. 2017), Listeriosis (Jaguezeski et al. 2018), Foot-and-mouth disease (Mousa and Galal 2013; Khoshvaghti et al. 2014; Uzlu et al. 2016), Babesiosis (Saleh 2009), Coccidiosis (Tufan and Çam 2008; Yilmaz and Issi 2014), Theileriosis (Kızıl et al. 2011), Anaplasmosis (Esmaeilnejad et al. 2018), Hydatidosis (Heidarpour et al. 2013), Fascioliasis (Bahrami et al. 2014) and Cutaneous Papillomatosis (Arslan et al. 2018), Mastitis (Deveci and Güven 2008), in cattle infected with Neosporiosis (Glombowsky et al. 2017) and *Dictyocaulus viviparus* (Değer et al. 2008), calves with omphalitis (Bozukluhan et al. 2016), and also in cattle transferred from one region to another (Chirase et al. 2004). In the study, it was determined that the amount of MDA evaluated on the 2nd, 14th and 28th days in the vaccine group increased significantly (P<0.05) compared to the control group. These findings show similarities with the research results given above. This can be explained by the fact that in anthrax vaccinated cattle, the antigen is recognized by macrophages, the free radicals released during the process interact with the unsaturated fatty

acids in the cell membrane and cause the formation of MDA as a result of these reactions.

Nitric oxide (NO) is a compound of the reactive nitrogen species responsible for intercellular transmission (Abd Ellah et al. 2009; Heidarpour et al. 2013). Studies have shown that NO levels increased in cattle with omphalitis (Bozukluhan et al. 2016), mastitis (Atakisi et al. 2010) and foot-and-mouth disease (Bozukluhan et al. 2013; Mousa and Galal 2013; Uzlu et al. 2016). As a result of this study, it was stated that the amount of NO in the vaccine group on the 2nd, 14th and 28th days was significantly increased ($p < 0.05$) compared to the control group. These results are in line with the research results. A significantly higher level of NO in the anthrax vaccine group compared to the control group may cause the vaccine to stimulate cellular damage.

The harmful effects of free oxygen radicals formed in the organism on cells are either reduced or eliminated by antioxidant substances. Deficiency of these antioxidant substances or excessive production of oxidant substances (such as infection, inflammation, and tissue damage) causes cellular damage (Nockels 1996; Abd Ellah et al. 2009; Sordillo and Aitken 2009; Łuszczak et al. 2011; Jaguezeski et al. 2018). SOD is an antioxidant and transforms the cellular superoxide radical to hydrogen peroxide (H_2O_2). The resulting H_2O_2 is transformed into water and oxygen by catalase enzyme and neutralized (Aslankoç et al. 2020). In some studies, it has been reported that the SOD level is decreased in calves with *Dictyocaulus viviparus* (Değer et al. 2008) and coccidiosis (Tufan and Çam 2008), and cattle infected with Bovine Leukemia Virus (Ali et al. 2019), Foot-and-Mouth Virus (Khoshvaghti et al. 2014), *Anaplasma marginale* (Esmaeilnejad et al. 2018), *Echinococcus granulosus* (Heidarpour et al. 2013) and *Fasciola gigantica* (Bahrami et al. 2014). In the study, it was stated that the SOD level significantly ($P < 0.05$) decreased on the 2nd, 14th and 28th days in the group that was administered anthrax vaccine compared to the control group. These findings share similarities with the research results stated above. This similarity can be attributed to the rise in use of SOD in order to eliminate the harmful effects of increasing oxidants in the anthrax vaccine group. Contrary to these findings, it has been reported that there is an increase in SOD level in calves with brucella (Perin et al. 2017) and septicemia (Meral et al. 2017) and in cattle infected with *Taenia saginata* (Łuszczak et al. 2011), as well as ethionine administered cattle (Abd Ellah et al. 2009). Consequently, it can be suggested that there may be other factors affecting SOD level.

It has been reported that CAT levels are decreased in cattle infected with *Theileria annulata* (Kızıl et al. 2011), *Anaplasma marginale* (Esmaeilnejad et al. 2018), as well as ethionine administered cattle (Abd Ellah et al. 2009). It has been reported that there is a decrease in the number of total antioxidants in transferred cattle (Chirase et al. 2004), milk of cattle with mastitis (Atakisi et al. 2010), as well as coccidiosis (Yilmaz and Issi 2014) and foot-and-mouth disease (Mousa and Galal 2013) and in cattle diagnosed with *Anaplasma marginale* (Esmaeilnejad et al. 2018). As a result of the research, it was determined that the amount of CAT in the group administered the Anthrax vaccine decreased significantly on the 2nd, 14th and 28th days compared to the control group. This outcome share similarities with the research results stated above. The reason for the decrease in CAT level can be attributed to its reaction with the increased oxidant substances in anthrax vaccinates cattle. In other studies, it has been reported that there is no significant change in the CAT level in cattle with foot-and mouth disease (Khoshvaghti et al. 2014) and cutaneous papillomatosis (Arslan et al. 2018), but there is an increase in the amount of CAT in Food-and-Mouth Disease vaccinated cattle (Kızıl and Gül 2004). The difference in the CAT level may be due to the characteristics of the administered vaccine (bacterial, viral, activated, attenuated, inactive, adjuvant substance, etc.). Bacterial and activated vaccines were administered in the study.

Vitamin C shows antioxidant effect by reacting with free radicals and forming ascorbyl radicals. As a result of this reaction, the number of free radicals present in the medium decreases (Padayatty et al. 2003). In the research, it can be stated that the anthrax vaccine increases oxidant parameters and decreases antioxidant parameters starting from the second day. It was stated that vitamin C administration showed an antioxidant effect by decreasing the amounts of MDA and NO and increasing the amounts of SOD and CAT. In the study, the decrease in the concentrations of NO and MDA in the vitamin C administered group can be explained by the reaction of ascorbic acid with these radicals.

Kızıl and Gül (2004) found that after the administration of FMD vaccine, FMD vaccine-vitamin C, FMD vaccine-vitamin AD3E and FMD vaccine-vitamin C-vitamin AD3E, the amount of CAT increased in the only vaccine administered group, that SOD level was not affected by vitamin administration and that MDA level did not increase on the 3rd day in the vitamin C administered group but decreased after the 14th day. The decrease in MDA level in this study is consistent with the research of Kızıl and Gül (2004). In a study conducted by Kızıl and Gül (2004), it was found that there was an increase in the amount of CAT in the vaccinated group, and the level of SOD did not change. In this study, a decrease in CAT and SOD levels was confirmed. The findings of SOD and CAT are in line with the findings of Kızıl and Gül (2004).

As a result, it can be stated that the amount of NO and MDA increases in anthrax vaccinated cattle, the concentration of SOD and CAT decreases, as well as vitamin C administration reduces the increased oxidant parameters, and recovers the antioxidant parameters. On that account, it can be suggested that it would be useful to administer vitamin C in combination with the anthrax vaccine to cattle.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: ED[1]
Supervision / Consultancy: KB
Data Collection and / or Processing: DE, ANCD
Analysis and / or Interpretation: OM
Literature Review: ED[1]
Critical Review: ED[4]

REFERENCES

Abd Ellah MR, Okada K, Goryo M, Oishi A, Yasuda J (2009). Superoxide dismutase activity as a measure of hepatic oxidative stress in cattle following ethionine administration. *Vet J*, *182*(2), 336–341.

Ali AF, Selim A, Manaa EA, Abdelrahman A, Sakr A (2019). Oxidative state markers and clinicopathological findings associated with bovine leukemia virus infection in cattle. *Microb Pathog, 136*, 1-4.

Arslan HH, Tarhan D, Cenesiz S ve ark. (2018). Evaluation of trace element levels and antioxidant metabolism in cattle with cutaneous papillomatosis. *Acta Sci Vet,* 46(1), 1–5.

Aslankoç R, Demirci D, İnan Ü ve ark. (2020). Oksidatif stres durumunda antioksidan enzimlerin rolü - Süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GPX). *SDÜ Tıp Fak Derg*, *26*(3), 362–369.

Atakisi O, Oral H, Atakisi E ve ark. (2010). Subclinical mastitis causes alterations in nitric oxide, total oxidant and antioxidant capacity in cow milk. *Res Vet Sci,* 89(1), 10–13.

Bahrami S, Esmaeilzadeh S, Oryan A (2014). Role of oxidative stress in concomitant occurrence of Fasciola gigantica and leiomyoma in cattle. *Vet Parasitol,* 203(1–2), 43–50.

Bozukluhan K, Merhan O, Ögün M, Cihan M, Gökçe G (2016). Omfalitisli Buzağılarda Bazı Oksidatif Stres Parametre Düzeylerinin Belirlenmesi. *FÜ Sağ Bil Vet Derg*, *30*(2), 79–81.

Bozukluhan K, Atakisi E, Atakisi O (2013). Nitric oxide levels, total antioxidant and oxidant capacity in cattle with foot-and-mouth-disease. *Kafkas Üniv Vet Fak Derg*, *19*(1), 179–181.

Brawand SG, Kittl S, Dettwiler M et al. (2019). An unusual case of bovine anthrax in the canton of Jura, Switzerland in 2017. *BMC Vet Res,* 15(1), 1–9.

Chirase NK, Greene LW, Purdy CW et al. (2004). Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. *Am J Vet Res,* 65(6), 860–864.

Değer S, Değer Y, Ertekin A ve ark. (2008). Dictyocaulus viviparus ile Enfekte Sığırlarda Lipit Peroksidasyon ve Antioksidan Durumunun Saptanması. *Türkiye Parazitol Derg, 32*(3), 234–237.

Dettwiler M, Mehinagic K, Gobeli Brawand S et al. (2018). Bacillus anthracis as a cause of bovine abortion – A necropsy case requiring special biosafety measures. *Schweiz Arch Tierheilkd,* 160(9), 547–552.

Deveci HA, Güven A (2008). Investigation of blood MDA and GSH levels in cow with mastitis. *Kafkas Üniv Vet Fak Derg*, *14*(1), 63–66.

Esmaeilnejad B, Tavassoli M, Samiei A et al. (2018). Evaluation of oxidative stress and antioxidant status, serum trace mineral levels and cholinesterases activity in cattle infected with Anaplasma marginale. *Microb Pathog,* 123, 402–409.

Glombowsky P, Bottari NB, Klauck V et al. (2017). Oxidative stress in dairy cows seropositives for Neospora caninum. *Comp Immunol Microbiol Infect Dis, 54*, 34–37.

Hanna PC, Kruskal BA, Ezekowitz RA, Bloom BR, Collier RJ (1994). Role of macrophage oxidative burst in the action of anthrax lethal toxin. *J Mol Med*, *1*(1), 7–18.

Heidarpour M, Mohri M, Borji H, Moghdass E (2013). Oxidant/antioxidant status in cattle with liver cystic echinococcosis. *Vet Parasitol*, *195*(1–2), 131–135.

Jaguezeski AM, Baldissera MD, Rhoden LA et al. (2018). Listeria monocytogenes impairs enzymes of the phosphotransfer network and alters antioxidant/oxidant status in cattle brain structures. *Microb Pathog, 124*(11), 284–290.

Khoshvaghti A, Askari A, Nazifi S, Ghane M (2014). Evaluation of some antioxidant enzymes in cattle infected with foot and mouth virus. *İstanbul Üniv Vet Fak Derg*, *40*(1), 70–75.

Kizil Ö, Keltek Y (2017). Tüberkülozisli Sığırlarda Antioksidan Parametrelerdeki Değişimler. *FÜ Sağ Bil Vet Derg*, *31*(1), 1–4.

Kızıl M, Baydar E, Kızıl Ö (2011). Tayleriyozisli Sığırlarda Antioksidan Parametrelerdeki Değişiklikler. *FÜ Sağ Bil Vet Derg*, *25*(2), 53–56.

Kızıl Ö, Gül Y (2004). Şap aşısı uygulanan besi sığırlarında antioksidan vitaminlerin klinik ve bazı hematolojik parametreler ile antioksidan enzim ve lipit peroksidasyon düzeylerine etkileri. *FÜ Sağ Bil Derg*, *18*(2), 97–106.

Lewerin SS, Elvander M, Westermark T et al. (2010). Anthrax outbreak in a Swedish beef cattle herd - 1st case in 27 years: Case report. *Acta Vet Scand, 52*(1), 1–8.

Linster CL, Van Schaftingen E (2007). Vitamin C: Biosynthesis, recycling and degradation in mammals. *FEBS J*, *274*(1), 1–22.

Łuszczak J, Ziaja-Sołtys M, Rzymowska J (2011). Anti-oxidant activity of superoxide dismutase and glutathione peroxidase enzymes in skeletal muscles from slaughter cattle infected with Taenia saginata. *Exp Parasitol, 128*(2), 163–165.

Meral Ö, Ercan N, Fidanci UR (2017). Septisemili buzağılarda lipid peroksidasyon düzeyi ve antioksidan enzim aktiviteleri. *Ankara Üniv Vet Fak Derg*, *64*(3), 161–164.

Miranda KM, Espey MG, Wink DA (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, *5*(1), 62–71.

Mousa SA, Galal MK (2013). Alteration in Clinical , Hemobiochemical and Oxidative Stress Parameters in Egyptian Cattle Infected with Foot and Mouth Alteration in Clinical , Hemobiochemical and Oxidative Stress Parameters in Egyptian Cattle Infected with Foot and Mouth Disease FMD. *J Anim Sci Adv, 3*(9), 485–491.

Nockels CF (1996). Antioxidants improve cattle immunity following stress. *Anim Feed Sci Technol*, *62*(1), 59–68.

Padayatty SJ, Katz A, Wang Y et al. (2003). Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. *J Am Coll Nutr*, *22*(1), 18–35.

Parlak E, Parlak M, Atli SB (2015). Unusual cause of fatal anthrax meningitis. *Cutan Ocul Toxicol*, *34*(1), 77–79.

Perin G, Fávero JF, Severo DRT et al. (2017). Occurrence of oxidative stress in dairy cows seropositives for Brucella abortus. *Microb Pathog, 110*, 196–201.

Saleh MA (2009). Erythrocytic oxidative damage in crossbred cattle naturally infected with Babesia bigemina. *Res Vet Sci, 86*(1), 43–48.

Sordillo LM, Aitken SL (2009). Impact of oxidative stress on the health and immune function of dairy cattle. *Vet Immunol Immunopathol, 128*(1–3), 104–109.

Suchitra BR, Anil Kumar MC, Kalmath GP, Prakash L (2010). Outbreak of Anthrax and its management in Bangalore rural district. *Vet World*, *3*(3), 135–136.

Tufan A, Çam Y (2008). Tayleriyozisli Sığırlarda Antioksidan Parametrelerdeki Değişiklikler. *J Health Sci*, *17*(3), 131–136.

Uzlu E, Karapehlivan M, Erdoğan HM ve ark. (2016). Serum and saliva sialic acid and oxidative stress parameters changes in bulls with foot and mouth disease. *Kafkas Üniv Vet Fak Derg*, *22*(3), 321–325.

Yilmaz S, Issi M (2014). Malondialdehyde and Total Antioxidant Levels and Hematological Parameters of Beef Cattle with Coccidiosis. *Van Vet J*, *25*(2), 41–45.

Yoshioka T, Kawada K, Shimada T, Mori M (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am J Obstet Gynecol*, 135(3), 372–376.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Cellat M, Aydın T (2021).** The Effect of *Smilax excelsa* L. Ethanol Extract in an Experimentally Anemia Model induced by Cyclophosphamide in Rats. *Van Vet J*, 32(3), 114-118. DOI: https://doi.org/10.36483/vanvetj.972147

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Ratlarda Siklofosfamid ile Deneysel Olarak Oluşturulan Anemi Modelinde *Smilax excelsa* L. Etanol Ekstresinin Etkisi

Mustafa CELLAT[1,*] Tuba AYDIN [2]

[1] *Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Physiology, 31060, Hatay, Turkey*
[2] *Ağrı İbrahim Çeçen University Faculty of Pharmacy, Department of Pharmacognosy, 04100, Ağrı, Turkey*

Received: 26.07.2021 Accepted: 15.10.2021

ÖZ

Bu çalışmada, ratlarda siklofosfamid (CP) ile deneysel olarak oluşturulan anemi modelinde *Smilax excelsa* L. etanol ekstresinin etkisi araştırıldı. Çalışma toplam 4 gruptan oluştu. Grup 1 ve 2'deki ratlara oral gavaj yöntemi kullanılarak 28 gün süre ile günlük 1 ml serum fizyolojik, grup 3 ve 4'deki ratlara ise 400 mg/kg dozda *Smilax excelsa* L. etanol ekstresi uygulandı. Ayrıca grup 2 ve 4'deki ratlara haftada bir kez olmak üzere toplam dört doz 50 mg/kg CP uygulaması intramusküler olarak yapıldı. Çalışmanın 28. günü bütün ratlardan anestezi altında kan örnekleri alındı ve daha sonra ötanazi işlemi uygulandı. Kan örneklerinde eritrosit sayısı (RBC), lökosit sayısı (WBC), hemoglobin konsantrasyonu (HGB), hematokrit değer (Hct), ortalama eritrosit hemoglobini (MCH), ortalama eritrosit hacmi (MCV) ve ortalama eritrosit dağılım genişliği (RDW) gibi hematolojik parametreler incelendi. Serum malondialdehit (MDA) ve indirgenmiş glutatyon (GSH) düzeyleri ile katalaz (CAT) ve glutatyonperoksidaz (GSH-Px) enzim aktivitelerine spektrofotometrik olarak bakıldı. Grup 4'de *Smilax excelsa* L. etanol ekstresi tedavisinin serum MDA düzeyini düşürdüğü, CAT ve GSH-Px enzim aktivitelerini ise arttırdığı belirlendi. Ayrıca bu tedavinin RBC, HGB ve Hct gibi hematolojik parametrelerde iyileşmelere sebep olduğu, RDW değerlerini ise azalttığı gözlemlendi. Sonuç olarak *Smilax excelsa*'nın sahip olduğu güçlü antioksidan etki ile CP'nin kemik iliğindeki baskılayıcı etkisini azalttığı ve anemi şekillenmesini önlediği görüldü.

Anahtar Kelimeler: *Aplastik anemi, Oksidatif stres, Siklofosfamid, Smilasagiller.*

ABSTRACT

The Effect of *Smilax excelsa* L. Ethanol Extract in an Experimentally Anemia Model İnduced by Cyclophosphamide in Rats

In this study, the effect of *Smilax excelsa* L. ethanol extract on anemia model experimentally induced by Cyclophosphamide (CP) in rats was investigated. The study consisted of 4 groups, The rats in groups 1 and 2 were administered 1 ml of physiological saline for 28 days using the oral gavage method, and the rats in groups 3 and 4 were administered *Smilax excelsa* L. ethanol extract at a dose of 400 mg/kg. In addition, four doses of 50 mg/kg CP were administered intramuscularly once a week to the rats in groups 2 and 4. On the 28th day of the experiment, blood samples were taken from all rats under anesthesia and then euthanasia was performed. Hematological parameters such as red blood cell count (RBC), leukocyte count (WBC), hemoglobin concentration (HGB), hematocrit value (Hct), mean erythrocyte hemoglobin (MCH), mean erythrocyte volume (MCV), and mean erythrocyte distribution width (RDW) were examined in blood samples. Serum malondialdehyde (MDA) and reduced glutathione (GSH) levels and catalase (CAT) and glutathione peroxidase (GSH-Px) enzyme activities were measured spectrophotometrically. In group 4, it was determined that *Smilax excelsa* L. ethanol extract treatment decreased the serum MDA level and increased the CAT and GSH-Px enzyme activities. In addition, it was observed that this treatment caused improvements in hematological parameters such as RBC, HGB and Hct, and decreased RDW values. As a result, it was observed that *Smilax excelsa,* with its strong antioxidant effect, reduced the suppressive effect of CP in the bone marrow and prevented the formation of anemia.

Keywords: *Aplastic anemia, Cyclophosphamide, Oxidative stress, Smilacaceae.*

✉ *Corresponding author: mcellat@mku.edu.tr

GİRİŞ

Aplastik anemi (AA), kemik iliği hematopoietik hücrelerinin hipoplazisi ve periferik kan hücrelerinin hematopenisi ile karakterizedir (Jia ve ark. 2020). Kemoterapi, AA'nin en önemli nedenleri arasında yer almaktadır (Chen ve ark. 2014). Radyoterapi veya kemoterapinin en önemli yan etkileri miyelosupresyondur ve kanser tedavisinde kemoterapi ve radyoterapinin klinik etkinliğini geliştirmek için miyelosupresyonun hafifletilmesi büyük önem taşımaktadır (He ve ark. 2021). Kemik iliği baskılanması veya miyelotoksisite olarak da bilinen miyelosupresyonda kemik iliği aktivitesi, RBC, WBC ve PLT sayılarında azalma olur (Brigle ve ark. 2017). Siklofosfamid (CP) hepatokarsinom, akciğer kanseri, beyin tümörü, akut miyeloid lösemi ve otoimmün hastalıklara karşı güçlü etki gösteren antikanser ve immünosupresan bir ilaçtır (Ashry ve ark. 2013). Klinik uygulamada CP, oksidatif stres, kemik iliği supresyonu, anemi ve sitotoksisite dahil olmak üzere birçok yan etkiye sebep olur (Woo ve ark. 2008). Kemik iliğinin toksisitesi daha sonra anemi, lökopeni ve trombositopeni gibi hematopoietik işlev bozukluğuna yol açar (Raj 2009). Kemoterapötik bir ajan olan CP tedavisi, kemik iliğindeki kök hücreleri ve dolaşan periferik kan hücrelerini tüketerek anemi ve immünodensite ile sonuçlanabilir (Xu ve ark. 2014). CP, karaciğerde fosforamid mustard ve akroleine metabolize olan bir ön ilaçtır. Fosforamid mustard, CP'nin terapötik etkisinden, akrolein ise toksik etkisinden sorumludur (Khan ve ark. 2014; Khorwal ve ark. 2017). Bu toksik metabolitler, metabolizma sırasında oksidatif stresle sonuçlanan reaktif türleri oluşturur (Goudarzi ve ark. 2017). Bu reaktif türler normal sıçan hücrelerinde mitokondriyal membran geçirgenliği geçişini indükleyerek apoptoza sebep olur (Anyasor ve ark. 2012). CP, oksidatif strese sebep olarak sitotoksik etkiler göstermektedir (El-Sebaey ve ark. 2019). Tıbbi bitkilerin toksik maddelere karşı kemoprotektif aktiviteye sahip olduğu bilinmektedir (Kalantar ve ark. 2016). İngilizce'de "greenbrier" veya "sarsaparilla" olarak adlandırılan Smilax, çok yıllık ve tırmanıcı bir bitkidir (Yıldız ve ark. 2018). S. excelsa L. yaprak ekstrelerinin fenolik ve flavonoid maddeler bakımından zengin olduğu ve bu maddelerin lipid peroksidasyonunu inhibe etme, radikalleri temizleme ve demir şelatlama gibi aktiviteleri sayesinde güçlü bir antioksidan etki gösterebileceği ifade edilmektedir (Özsoy ve ark. 2008).

Bu çalışmada, ratlarda CP ile deneysel olarak oluşturulan anemi modelinde *Smilax excelsa L*. bitkisinin yapraklarından elde edilen etanol ekstresinin anemiye karşı koruyucu etkisi araştırıldı.

MATERYAL VE METOT

Bu çalışma, Hatay Mustafa Kemal Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan 18.03.2021 tarihinde 2021/02-15 sayılı izin alınarak yapılmıştır.

Bitki Temini

Bu çalışmada kullanılan *Smilax excelsa* L. bitkisinin yaprakları 2020 yılı haziran ayında Hatay il merkezinden toplandı. Bitkinin tür teşhisi HMKÜ Ziraat Fakültesi'nde yapıldıktan sonra bitki oda sıcaklığında gölgede kurutuldu ve Ağrı İbrahim Çeçen Üniversitesi Merkezi Araştırma ve Uygulama Laboratuvarı'nda muhafaza edildi.

Ekstraksiyon

Smilax excelsa L. bitkisinin kurutulmuş yaprakları, öğütücü yardımıyla toz haline getirildi. Etanol ekstresini hazırlamak için 50 g bitki tozu ve %96'lık etanol (1 L) kullanıldı. Bitki, soxhlet ekstraktöründe 50 C°'de ısıtılarak dört saat boyunca ekstrakte edildi. Ekstraksiyon işlemi tamamlandıktan sonra süzüntü ayrıldı ve bir evaporatör yardımıyla etanol uzaklaştırıldı. Elde edilen etanol ekstresi miktarı 7.27 gram, verim %14.5 olarak belirlendi. *Smilax excelsa* L. etanol ekstresinin miktarını artırmak amacıyla aynı işlemler tekrarlandı.

Hayvan Materyali

Çalışmada; HMKÜ Deney Hayvanları Merkezi'nden temin edilen 180-250 g ağırlığında 28 adet wistar albino ırkı dişi rat kullanıldı. Deneysel uygulamalar laboratuvar hayvanlarının bakım ve kullanım şartlarına (12 saat aydınlık-12 saat karanlık ve 21±1 °C) uygun olarak yürütüldü. Deneysel uygulamalar süresince ratlara standart ticari yem (pelet yem) ve musluk suyu ad-libitum olarak sağlandı.

Deney Grupları

Ratlarda CP ile oluşturulan deneysel anemi modeli El-Sebaey ve ark.'nın (El-Sebaey ve ark. 2019), *Smilax excelsa* L. etanol ekstresinin kullanım dozu ise Özsoy ve ark.'nın (Özsoy ve ark. 2016) makaleleri esas alınarak planlandı. Çalışmada grup 1 (Kontrol grubu), grup 2 (CP), grup 3 (Smilax) ve grup 4 (CP + Smilax) olmak üzere toplam 4 grup oluşturuldu. Her grupta 7 rat olmak üzere 4 grupta toplam 28 adet wistar albino ırkı dişi rat kullanıldı.

Grup 1 (Kontrol Grubu): Bu gruptaki ratlara günde bir defa ve oral gavaj yöntemi kullanılarak 28 gün boyunca 1 ml serum fizyolojik uygulaması yapıldı.

Grup 2 (CP): Bu gruptaki ratlara 50 mg/kg dozda, haftada bir kez ve 4 hafta süre ile kas içi CP uygulaması yapıldı.

Grup 3 (Smilax): Bu gruptaki ratlara 28 gün süre ile günde bir defa, 1 ml serum fizyolojik içerisinde ve 400 mg/kg dozda oral gavaj yöntemi kullanılarak *Smilax excelsa* L. etanol ekstresi uygulandı.

Grup 4 (CP + Smilax): Bu gruptaki ratlara 4 hafta süre ile haftada bir kez ve 50 mg/kg dozda kas içi CP uygulaması yapıldı. Ayrıca bu gruba 28 gün süre ile günde bir defa, 1 ml serum fizyolojik içerisinde ve 400 mg/kg dozda oral gavaj yöntemi kullanılarak *Smilax excelsa* L. etanol ekstresi uygulandı.

Çalışmanın 28. gününde ratlar ketamin (60 mg/kg İM) + ksilazin (10 mg/kg İM) kullanılarak anesteziye alındı. Kuyruk veninden usulüne uygun olarak antikoagulanlı (Etilendiamin tetraasetik asit) ve antikoagulansız (serum) kan tüplerine kan örnekleri alındı. Serum tüplerine alınan kan örnekleri 3000 rpm ve 15 dakika santrifüj edilerek kan serumları hazırlandı. Hazırlanan serum örnekleri epondorf tüplere konuldu ve biyokimyasal analizler yapılıncaya kadar -80 °C 'de derin dondurucuda saklandı. Anestezi altında kan örnekleri alınan bütün ratlara dekapitasyon yöntemi kullanılarak ötönazi işlemi uygulandı.

Hematolojik Analizler

Veteriner Fakültesi Merkez Laboratuvarında bulunan Mindray BC2800 marka otomatik kan sayım cihazı ile taze kan örneklerinden hematolojik analizler yapıldı. Hematolojik analizlerde WBC, RBC, HGB, Hct, MCV, MCH ve RDW gibi parametreler incelendi.

Biyokimyasal Analizler

Derin dondurucudan (-80°C) çıkarılan serum örneklerinde oksidatif hasar ve antioksidan aktivite durumunu ortaya koyabilmek için spektrofotometrik olarak MDA ve GSH düzeyleri ile CAT ve GSH-Px enzim aktiviteleri analiz edildi. Lipit peroksidasyonu ve antioksidan etkinlik için

alınan serum örnekleri homojenize edildikten sonra analizler spektrofotometre yardımıyla gerçekleştirildi. Lipid peroksidasyonu seviyesi, tiyobarbitürik asit reaktif maddeler konsantrasyonuna göre ölçüldü ve elde edilen MDA miktarı, lipid peroksidasyonunun bir indeksi olarak kullanıldı. 532 nm'de MDA seviyesi protein gramı başına nanomol cinsinden ifade edildi (Placer ve ark. 1966). GSH düzeyi, Sedlak ve Lindsay tarafından tanımlanan yöntem kullanılarak ölçüldü (Sedlak ve Lindsay 1968). 412 nm'de GSH seviyesi protein gramı başına nanomol olarak ifade edildi. Glutatyon peroksidaz (GSH-Px, EC 1.11.1.9) aktivitesi, Lawrence ve Burk tarafından tanımlanan metoda göre belirlendi (Lawrence ve Burk 1976). 340 nm'de GSH-Px enzim aktivitesi, gram protein başına uluslararası birimler olarak ifade edildi. Katalaz (CAT, EC 1.11.1.6) aktivitesi, 240 nm'de hidrojen peroksit (H_20_2) ayrışımının ölçülmesi ile belirlendi ve kg/protein olarak ifade edildi (Aebi 1983). Protein analizleri için Lowry (Lowry ve ark. 1951) metodu kullanıldı.

İstatistiksel Analizler

Elde edilen verilerin normal dağılım gösterip göstermediğini belirlemek için Shapiro-Wilk normallik analizi yapıldı ve analiz tüm parametrelerin normal bir dağılım izlediğini gösterdi. Grup ortalamalarını karşılaştırmak için Tek Yönlü Varyans Analizi (ANOVA) ve gruplar arasındaki farklılıkları belirlemek için Tukey testi kullanıldı. İstatistiksel analiz, IBM SPSS sürüm 23.0 yazılımı kullanılarak yapıldı ve P<0.05 istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Hematolojik Bulgular

CP uygulanan gruptaki ratların periferik kan RBC (P:0.001), WBC (P:0.001), HGB (P:0.005) ve Hct (P:0.003) seviyeleri kontrol grubu ile karşılaştırıldığında istatistiki açıdan anlamlı düşüşlerin meydana geldiği belirlendi. CP'nin MCV (P:0.239) ve MCH (P:0.061) değerleri üzerine istatistiki açıdan önemli bir etkisinin olmadığı gözlendi. Ayrıca CP grubunun RDW değerleri (P:0.001) kontrol grubu ile kıyaslandığında önemli düzeyde artışlar görüldü. *Smilax excelsa* L. etanol ekstresi tedavisinin periferik kan RBC (P:0.001), HGB (P:0.005) ve Hct (P:0.003) değerlerindeki düşüşleri önlediği ve bu parametrelerin kontrol grubuna yakın olduğu tespit edildi. Ayrıca bu tedavinin artmış olan RDW değerlerini (P:0.001) kontrol grubuna yakın seviyelere indirdiği, MCV (P:0.239) ve MCH (P:0.061) seviyeleri üzerinde ise anlamlı bir etkisinin olmadığı gözlemlendi. WBC değerleri açısından diğer gruplarla kontrol grubu kıyaslandığında diğer grupların WBC değerlerinin (P:0.001) kontrol grubuna göre anlamlı düzeyde düştüğü tespit edildi. WBC değerleri açısından kontrol grubu dışındaki gruplar arasında istatistiki açıdan önemli farklılıklar saptanmadı. Hematolojik bulgular Tablo 1'de verildi.

Tablo 1. CP ve *Smilax excelsa*'nın Kan Parametreleri Üzerine Etkileri.

Table 1. Effects of CP and *Smilax excelsa* on Blood Parameters.

Grup / Parametre	Kontrol	CP	Smilax	CP + Smilax	Önemlilik
RBC ($1X10^6$)	7.885 ± 0.19^a	5.448 ± 0.34^b	8.456 ± 0.59^a	7.554 ± 0.40^a	0.001
HGB (gr/dl)	14.625 ± 0.73^a	10.240 ± 0.41^b	14.560 ± 1.25^a	12.980 ± 0.53^a	0.005
Hct (%)	43.875 ± 0.87^a	33.280 ± 1.11^b	48.320 ± 3.88^a	42.200 ± 1.91^a	0.003
MCV (fL)	55.775±0.97	55.020±0.74	59.940±1.98	58.260±2.61	0.239
MCH (%)	17.550±0.24	16.960±0.36	16.080±0.19	17.840±0.71	0.061
RDW (%)	11.175 ± 0.27^a	15.780 ± 1.16^b	10.960 ± 0.20^a	13.260 ± 0.81^a	0.001
WBC ($1x10^3$)	8.875 ± 1.14^a	3.980 ± 0.72^b	4.240 ± 0.26^b	4.660 ± 0.62^b	0.001

1 mm3 kanda; Eritrosit (RBC), hemoglobin (HGB), hematokrit (Hct), ortalama eritrosit hacmi (MCV), ortalama eritrosit hemoglobini(MCH), retikulosit dağılım genişliği (RDW) lökosit sayısı (WBC). (Veriler Ort ± SE şeklinde verilmiştir). Sütunlardaki farklı harflendirme istatistiksel olarak anlamlı farklılık ifade etmektedir.

Biyokimyasal Bulgular

Ratlarda CP uygulamasının serum MDA düzeylerini (P:0.00) arttırdığı, GSH-Px ve CAT enzim aktivitelerini (P:0.01) ise düşürdüğü ve oksidatif strese sebep olduğu tespit edildi. Serum GSH düzeyi üzerine de inhibe edici etkisinin olduğu fakat bu etkinin istatistiki açıdan (P:0.076) önemli olmadığı belirlendi. *Smilax excelsa* L. etanol ekstresi tedavisinin, serum MDA düzeylerini (P:0.00) düşürerek oksidatif hasarı azalttığı belirlendi. GSH-Px ve CAT enzim aktivitelerini ise istatistiki açıdan (P:0.01) anlamlı derecede yükselttiği gözlendi. Serum GSH düzeyinde artışa neden olduğu fakat bu etkinin istatistiki açıdan (P:0.076) anlamlı olmadığı görüldü. Serum MDA ve GSH düzeyleri ile CAT ve GSH-Px enzim aktiviteleri seviyeleri Tablo 2'de verildi.

Tablo 2. CP ve *Smilax excelsa*'nın Serum MDA ve GSH düzeyleri ile CAT ve GSH-Px Enzim Aktivite Düzeyleri Üzerine Etkileri.

Table 2. Effects of CP and *Smilax excelsa* on Serum MDA and GSH levels and CAT and GSH-Px Enzyme Activity Levels.

Grup / Parametre	Kontrol	CP	Smilax	CP + Smilax	Önemlilik
MDA (nmol/mL)	18.286 ± 0.47^a	23.516 ± 1.06^b	17.576 ± 0.63^a	19.725 ± 0.42^a	0.00
GSH (nmol/mL)	3.265±0.16	2.856±0.12	3.520±0.05	3.275±0.25	0.076
GSH-Px (IU/gr prot)	46.910 ± 2.15^a	30.497 ± 3.31^b	52.418 ± 3.79^a	44.749 ± 3.01^a	0.01
CAT (U/ml)	75.498 ± 1.70^a	65.385 ± 1.28^b	80.510 ± 3.78^a	78.961 ± 1.12^a	0.01

Serum, malondialdehit (MDA), redukte glutatyon (GSH) seviyeleri ve glutatyon peroksidaz (GSH.Px) ve katalaz (CAT) enzim aktiviteleri (Veriler Ort ± SE şeklinde verilmiştir). Sütunlardaki farklı harflendirme istatistiksel olarak anlamlı farklılık ifade etmektedir.

TARTIŞMA ve SONUÇ

CP ve ilişkili nitrojen mustard türevi alkilleyici ajanlar, meme kanseri, akciğer karsinomu, sistemik lupus eritematozus, romatoid artrit ve multipl skleroz gibi çeşitli maligniteler ve bozuklukların tedavisinde (Zhang ve ark. 2006; Perini ve ark. 2007) ve kemik iliği nakli sırasında bağışıklık sistemini baskılamak amacıyla yaygın olarak kullanılmaktadır (Khorwal ve ark. 2017). Sıçanlarda CP tedavisinin kemik iliğini baskıladığı, hematokrit değeri düşürdüğü ve aplastik anemiye sebep olduğu ifade edilmektedir. Ayrıca immünosupressif etki gösterdiği ve WBC seviyesini önemli oranda azalttığı vurgulanmaktadır (Anokwuru ve ark. 2019). Kanser tedavisinde CP'nin kullanımı, hastalarda anemi riskini arttırır ve tedavi sırasında immünosupresyonu önleyebilmek için tedbirlerin alınması gerekir (Ikumawoyi ve ark. 2016). CP uygulamasının karaciğerde ve böbreklerde eritropoietinin mRNA sentezini azalttığı bildirilmektedir (Dame ve ark. 2006). Sıçan ve farelerde yapılan araştırmalarda değişik doz ve sürelerde uygulanan CP tedavisinin kan WBC, RBC ve Platelet sayıları ile HGB konsantrasyonunda (Chukwuemeka ve ark. 2015; Elhalim ve ark. 2017; Li ve ark. 2017; Zhang ve ark. 2017; Eltantawy ve ark. 2018; Ayhancı ve ark. 2019; Kulshrestha ve ark. 2019; Han ve ark. 2020; Iqubal ve ark. 2020), kemik iliği çekirdekli hücre sayılarında (Ayhancı ve ark. 2019) ve Hct (Elhalim ve ark. 2017; Zhang ve ark. 2017; Kim ve ark. 2018) seviyelerinde istatistiki açıdan anlamlı derecede azalmalara sebep olduğu bildirilmektedir. Bu çalışmada CP uygulamasının WBC, RBC, HGB ve Hct seviyelerinde önemli düzeylerde azalmalara sebep olduğu ve anemi şekillendiği görüldü. CP grubunun RDW değerleri kontrol grubu ile kıyaslandığında anlamlı artışlar gözlendi. Fakat MCV ve MCH parametreleri üzerinde istatistiki açıdan önemli etkisinin olmadığı belirlendi. Sonuçlarımız yukarıda verilen literatür bilgileriyle uyumluluk göstermektedir. CP + Smilax grubunun hematolojik bulguları incelendiğinde *Smilax excelsa* L. etanol ekstresi tedavisinin CP'nin sebep olduğu anemiye karşı koruyucu etki gösterdiği, RBC, HGB ve Hct seviyelerindeki azalmaları engellediği ve bu değerlerin kontrol grubuna benzer olduğu tespit edildi. Ayrıca RDW değerlerinde istatistiki açıdan anlamlı düşüşlere sebep olduğu saptandı. *Smilax excelsa* L. etanol ekstresi tedavisinin WBC değerlerinde hafif düzeyde artışlara sebep olduğu fakat bu etkinin istatistiki açıdan anlamlı olmadığı görüldü. MCV ve MCH seviyeleri açısından gruplar arasında bir fark bulunamadı. *Smilax excelsa* L. etanol ekstresi tedavisinin hemopoetik sistemi uyardığı, sahip olduğu güçlü antioksidan etki ile CP'nin kemik iliği üzerindeki baskılayıcı etkisini engellediği ve anemiyi azalttığı tespit edildi.

Oksidatif stres CP toksisitesinin patofizyolojisinde rol oynar. Akrolein, dokuların antioksidan sistemine müdahale eder ve reaktif oksijen türleri (ROS) üretir (Mythili ve ark. 2004). CP tedavisinin serum MDA seviyesinde artışa (Ramadan ve ark. 2012; Zhang ve ark. 2017; Eltantawy ve ark. 2018), GSH düzeyi (Ramadan ve ark. 2012; Eltantawy ve ark. 2018) ile GSH-Px (Ramadan ve ark. 2012) ve CAT (Ramadan ve ark. 2012; Rostampur ve ark. 2018) enzim aktivitelerinde ise azalmaya sebep olduğu ifade edilmektedir. CP tedavisi plazma GSH düzeyi ile CAT enzim aktivitelerinde önemli düzeyde azalmalara sebep olmaktadır (Anokwuru ve ark. 2019). Bu çalışmada CP uygulamasının serum MDA düzeyini arttırdığı, GSH-Px ve CAT enzim aktivitelerini ise azalttığı ve oksidatif strese sebep olduğu belirlendi. Serum GSH düzeyini azalttığı fakat bu azalmanın istatistiki açıdan önemli olmadığı gözlendi. GSH sonuçlarındaki uyumsuzluğun CP'nin kullanım dozundan kaynaklanabileceği değerlendirildi. CP'nin kemik iliğini baskılayıcı etkisinin oksidatif stres artışından kaynaklandığı görüldü.

Zararlı etkileri olmayan ve kimyasal ilaçların yan etkilerini ciddi anlamda ortadan kaldırabilen şifalı bitkiler dünyanın pek çok ülkesinde yaygın olarak kullanılmaktadır (Murthy ve ark. 2015). Doğal ürünlerin, zarar görmüş bağışıklık sistemini ve CP'nin sebep olduğu bağışıklık yetmezliğini onarma kabiliyetine sahip olduğu bildirilmektedir (Bai ve ark. 2018). Antioksidanlar, kemoterapötik ajanların toksik yan etkilerini en aza indirebilir ve daha yüksek dozlarda antikanser ilaç kullanımına izin verebilir (Süzek ve ark. 2017). Antikanser özelliklerine müdahale etmeden bu ilaçların yan etkilerini azaltabilen spesifik adjuvan tedavilere ihtiyaç vardır. Günümüzde, önemli ölçüde antioksidan, anti-inflamatuar ve anti-apoptotik potansiyele sahip oldukları için doğal olarak oluşan biyoaktif moleküllere daha fazla önem verilmektedir (Wang ve ark. 2018). Karbontetraklorür ile deneysel olarak karaciğer ve böbrek hasarı oluşturulan ratlarda S. excelsa L. yapraklarından elde edilen su ekstresinin yükselmiş olan MDA düzeyi ve miyeloperoksidaz (MPO) enzim aktivitesini düşürdüğü, düşmüş olan CAT ve GSH-Px enzim aktiviteleri ile GSH düzeyini ise artırdığı ifade edilmektedir. Aynı çalışmada S. excelsa L. yapraklarının güçlü bir antioksidan etki göstermek suretiyle karaciğer ve böbrek hasarına karşı koruyucu etkiye sahip olduğu vurgulanmaktadır (Özsoy ve ark. 2016; Özsoy ve ark. 2013). CP + *Smilax excelsa* L. etanol ekstresi grubu ratların serum MDA düzeylerinde istatistiki açıdan anlamlı düzeyde düşüşlerin görüldüğü, GSH-Px ve CAT enzim aktivitelerinde ise önemli artışların olduğu belirlendi. Serum GSH düzeylerinde hafif düzeyde artışların olduğu fakat bu artışın istatistiki açıdan önemli olmadığı saptandı. *Smilax excelsa* L.'nın sahip olduğu güçlü antioksidan etki ile CP' nin neden olduğu kemik iliği baskılayıcı etkisini azalttığı ve anemik durum gelişimine karşı koruyucu etkiye sahip olduğu görüldü.

Bu çalışmada özellikle çeşitli kanser türlerinin tedavisinde kullanılan ve alkilleyici bir antikanser ilaç olan CP uygulamasının serum MDA düzeyini arttırmak ve GSH-Px ve CAT enzim aktivitesini azaltmak suretiyle oksidatif etki gösterdiği ve bu etkinin kemik iliğini baskıladığı ve periferik kan WBC, RBC, HGB ve Hct değerlerinde önemli düzeylerde azalmalara sebep olduğu tespit edildi. *Smilax excelsa* L. etanol ekstresi tedavisinin güçlü antioksidan etki gösterdiği ve CP'nin kemik iliği üzerindeki oksidatif stres kaynaklı inhibe edici etkisini azalttığı ve böylece periferik kan RBC, HGB ve Hct değerlerindeki azalmaları önleyerek anemi gelişimini engellediği görüldü. *Smilax excelsa* L. etanol ekstresinin etkinliğine yönelik değişik doz ve sürelerde daha ileri çalışmaların yapılmasının faydalı olabileceği düşünülmektedir.

ÇIKAR ÇATIŞMASI

Yazarlar bu çalışma için herhangi bir çıkar çatışması olmadığını beyan ederler.

YAZAR KATKILARI

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

KAYNAKLAR

Aebi H (1983). Catalase. In H. U. Bergmeyer (Ed.), Methods of enzymatic analysis. Verlag Chemie. pp. 273-286, Weinhem, Germany.

Anokwuru CP, Anyasor GN, Shokunbi OS et al. (2019). Chemoprotective activity of aqueous leaf extract of Acalypha wilkesiana against cyclophosphamide-induced toxicity in rats. *Asian Pac J Trop Med*, 12 (9), 409-415.

Anyasor GN, Oyewole IO, Ogunwenmo KO, Ayomide A. (2012). Coartemether induced oxidative and hepatic damage in Plasmodium berghei strain Anka infected mice. *Bull Environ Contam Toxicol*, 1, 108-111.

Ashry NA, Gameil NM, Suddek GM (2013). Modulation of cyclophosphamide- induced early lung injury by allicin. *Pharm Biol*, 51, 806-811.

Ayhancı A, Heybeli N, Kulcanay Şahin İ, Cengiz M (2019). Myelosuppression and oxidative stress induced by cyclophosphamide in rats: The protective role of selenium. *Adıyaman Univ J of Sci*, 9 (2), 252-265.

Bai Y, Jiang Y, Liu T et al. (2018). Xinjiang herbal tea exerts immunomodulatory activity via TLR2/4-mediated MAPK signaling pathways in RAW264.7 cells and prevents cyclophosphamideinduced immunosuppression in mice. *J Ethnopharmacol*, 228,179-187.

Brigle K, Pierre A, Finley-Oliver E et al. (2017). Myelosuppression, bone disease, and acute renal failure: evidence-based recommendations for oncologic emergencies. *Clin J Oncol Nurs*, 21 (5 Suppl. l), 60-76.

Chen YF, Zhao ZQ, Wu ZM et al. (2014). The role of RIP1 and RIP3 in the development of aplastic anemia induced by cyclophosphamide and busulphan in mice. *Int J Clin Exp Pathol*, 7 (12), 8411-8420.

Chukwuemeka I, Utuk GS, Ugwu Okechukwu PC et al. (2015). The effect of ethanol leaf extract of Jatropha curcas on some haematological parameters of cyclophosphomide induced anaemia in wister albino rats. *EJAS*, 7 (1), 17-20.

Dame C, Kirschner KM, Bartz KV et al. (2006). Wilms tumor suppressor, Wt1, is a transcriptional activator of the erythropoietin gene. *Blood*, 107, 4282-4290.

Elhalim SAA, Sharada HM, Abulyazid I, Aboulthana WM, Elhalim STA (2017). Ameliorative effect of carob pods extract (Ceratonia siliqua L.) against cyclophosphamide induced alterations in bone marrow and spleen of rats. *J Appl Pharm Sci*, 7 (10), 168-181.

El-Sebaey AM, Abdelhamid FM, Abdalla OA (2019). Protective effects of garlic extract against hematological alterations, immunosuppression, hepatic oxidative stress, and renal damage induced by cyclophosphamide in rats. *Environ Sci Pollut Res*, 26, 15559-15572.

Eltantawy FM, Sobh MAA, EL-Waseef AM, Ibrahim RAA, Saad MAA (2018). Protective effect of Spirulina against cyclophosphamide-induced urotoxicity in mice. *Egypt j basic appl sci*, 5, 191-196.

Goudarzi M, Khodayar MJ, Mohammad S et al. (2017). Pretreatment with melatonin protects against cyclophosphamide-induced oxidative stress and renal damage in mice. *Fundam Clin Pharmacol*, 31, 625-635.

Han J, Dai M, Zhao Y et al. (2020). Compatibility effects of ginseng and Ligustrum lucidum Ait herb pair on hematopoietic recovery in mice with cyclophosphamide-induced myelosuppression and its material basis. *J Ginseng Res*, 44, 291-299.

He M, Wang N, Zheng W et al. (2021). Ameliorative effects of ginsenosides on myelosuppression induced by chemotherapy or radiotherapy. *J Ethnopharmacol*, 268, 113581.

Ikumawoyi VO, Awodele O, Rotimi K, Fashina AY (2016). Evaluation of The Effects of the hydro-ethanolic root extract of Zanthoxylum zanthoxyloides on hematological parameters and oxiative stress in cyclophospamide treated rats. *Afr J Tradit Complement Altern Med*, 13 (5), 153-159.

Iqubal A, Syed MA, Haque MM et al. (2020). Effect of nerolidol on cyclophosphamide-induced bone marrow and hematologic toxicity in swiss albino mice. *Exp Hematol*, 82, 24-32.

Jia W, Zhen M, Li L et al. (2020). Gadofullerene nanoparticles for robust treatment of aplastic anemia induced by chemotherapy drugs. *Theranostics*, 10 (15), 6886-6897.

Kalantar M, Goudarzi M, Khodayar MJ et al. (2016). Protective effect of the hydroalcoholic extract of Capparis spinose L. against cyclophosphamide-induced nephrotoxicity in mice. *Jundishapur J Nat Pharm Prod*, 11 (4), 37240.

Khan JA, Shahdad S, Makhdoomi MA et al. (2014). Effect of cyclophosphamide on the microanatomy of liver of albino rats. *Int J Res Med Sci*, 2 (4), 1466-1469.

Khorwal G, Chauhan R, Nagar M (2017). Effect of cyclophosphamide on liver in albino rats: A comparative dose dependent histomorphological study. *Int J Biomed Adv Res*, 8 (3), 102-107.

Kim JW, Choi JS, Seol DJ, Choung JJ, Kwang Ku SK (2018). Immunomodulatory effects of Kuseonwangdogo-based mixed herbal formula extracts on a cyclophosphamide-induced immunosuppression mouse model. *eCAM*, Article ID 6017412, 14 .

Kulshrestha MK, **Mahapatra KB, Das AK (2019).** Haematopoietic activity of Mandoor parpati on cyclophosphamide induced anaemia in wistar strain albino rats. *Bull Env Pharmacol Life Sci*, 8 (3), 98-102.

Lawrence RA, Raymond FB (1976). Glutathione peroxidase activity in selenium deficient rat liver. *Biochem Biophys Res*, 71 (4), 952-958.

Li F, Tang R, Chen LB et al. (2017). Effects of Astragalus combined with angelica on bone marrow hematopoiesis suppression induced by cyclophosphamide in mice. *Biol Pharm Bull*, 40, 598-609.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193, 265-275.

Murthy HN, Park SY, Lee EJ, Paek KY (2015). Biosafety and toxicological evaluation of tissue-cultured Echinacea purpurea adventitious roots. *Korean J Hortic Sci Technol*, 33 (1), 124-132.

Mythili Y, Sudharsan PT, Selvakumar E, Varalakshmi P (2004). Protective effect of DL-alpha-lipoic acid on cyclophosphamide induced oxidative cardiac injury. *Chem Biol Interact,* 151 (1), 13-19.

Özsoy N, Okyar A, Arda Pirinçci P et al. (2016). The Protective effect of an aqueous extract from *Smilax excelsa L.* against carbon tetrachloride-induced liver in rats. *Afr J Tradit Complement Altern Med*, 13 (3), 203-208.

Özsoy N, Okyar A, Arda Pirinçci P et al. (2013). Evaluation of *Smilax excelsa* L. use in experimentally induced nephrotoxicity. *Kafkas Univ Vet Fak Derg*, 19 (5), 807-814.

Özsoy N, Can A, Yanardag R, Akev N (2008). Antioxidant activity of *Smilax excelsa* L. leaf extracts. *Food Chem*, 110 (3), 571-583.

Perini P, Calabrese M, Rinaldi L, Gallo P (2007). The safety profile of cyclophosphamide in multiple sclerosis therapy. Expert Opin Drug Safety, 6, 183-90.

Placer ZA, Cushman LL, Johnson BC (1966). Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. *Anal Biochem*, 16 (2), 359-364

Raj SV (2009). Management of chemotherapy-induced thrombocytopenia: current status of thrombopoietic agents. *Semin Hematol*, 46 (2), 26-32.

Ramadan G, El-Beih NM, Zahra MM (2012). Egyptian sweet marjoram leaves protect against genotoxicity, immunosuppression and other complications induced by cyclophosphamide in albino rats. *Br J Nutr*, 108, 1059-1068.

Rostampur S, Feizi MAH, Khojasteh SMB, Daluchi F (2018). Heracleum persicum extract improves cyclophosphamide-induced liver toxicity and oxidative stress in male rats. *Adv Herb Med*, 4 (3), 34-44.

Sedlak J, Lindsay RH (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*, 25, 192-205.

Süzek H, Çelik I, Doğan A (2017). Nephroprotective hepatoprotective potential and antioxidant role of carob pods (*Cerotonia siliqua L.*) against carbon tetrachloride-induced toxicity in rats. *Indian J Pharm Educ Res*, 51 (2), 312-320.

Wang Z, Qi F, Cui Y et al. (2018). An update on Chinese herbal medicines as adjuvant treatment of anticancer therapeutics. *Biosci Trends*, 12, 220-239.

Woo S, Krzyzanski W, Jusko WJ (2008). Pharmacodynamic model for chemotherapy-induced anemia in rats. *Cancer Chemother Pharmacol*, 62, 123-133.

Xu M, He RR, Zhai YJ, Abe K, Kurihara H (2014). Effects of carnosine on cyclophosphamide-induced hematinic suppression in mice. *Am J Chin Med*, 42, 131-142.

Yıldız ÖŞ, Ayanoğlu F, Bahadırlı NP (2018). Saparna (*Smilax aspera* L., *Smilax excelsa* L.) Türlerinin Bazı Morfolojik ve Kimyasal Özellikleri. *MKÜ Ziraat Fakültesi Dergisi*, 23 (2), 254-261.

Zhang J, Tian Q, Zhou S (2006). Clinical pharmacology of cyclophosphamide and ifosfamide. *Curr Drug Ther*, 1, 55-84.

Zhang L, Gong AGW, Riaz K et al. (2017). A novel combination of four flavonoids derived from Astragali Radix relieves the symptoms of cyclophosphamide-induced anemic rats. *FEBS Open Bio*, 7, 318-32

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Ölmez M, Makav M (2021).** The effect of Pennyroyal (*Mentha pulegium L.*) on growth performance and some serum biochemical parameters in New Zealand rabbits. *Van Vet J*, 32(3), 119-124.
DOI: https://doi.org/10.36483/vanvetj.995024

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

The Effect of Pennyroyal (*Mentha Pulegium L.*) on Growth Performance and Some Serum Biochemical Parameters in New Zealand Rabbits

Mükremin ÖLMEZ[1,*] Mustafa MAKAV[2]

[1] *Department of Animal Nutrition and Nutritional Diseases, Kafkas University, 36100, Kars, Turkey*
[2] *Department of Physiology, Faculty of Veterinary Medicine, Kafkas University, 36100, Kars, Turkey*

Received: 14.09.2021 Accepted: 01.11.2021

ABSTRACT

This study was to identify the effects of dried aerial parts powder pennyroyal added to rabbit diets on growth performance and some serum biochemical parameters. The research utilized 15 New Zealand rabbits weighing 2-2.1 kg on average. It employed control and two experimental groups with five rabbits each. All rabbits were hosted in individual cages. While basal diet was provided to the control group, pennyroyal (*Mentha pulegium L.*) powder was given at 0.1% (P1) and 0.2% (P2) levels to the treatment groups, respectively. The trial lasted for 28 days. It was measured body weight and feed consumption of animals at the beginning and end of the experiment. Blood samples were taken from rabbits on days 0, 14 and 28 of the study and the changes of serum biochemical parameters throughout the study were analyzed. There were no differences between the groups in terms of performance parameters (P>0.05). It was observed that the high point result was that the pennyroyal lowered the serum cholesterol level (P<0.05). However, pennyroyal did not affect other biochemical parameters (P>0.05). In conclusion, it was noted that pennyroyal might be used as an alternative growth promoter and cholesterol regulator to rabbit diets. In particular, up to 0.2% pennyroyal may utilize in rabbit diets.

Keywords: *Biochemical parameters, Cholesterol, Mentha pulegium, Performance, Rabbit.*

ÖZ

Yarpuzun (*Mentha pulegium L.*) Yeni Zelanda tavşanlarında büyüme performansı ve bazı serum biyokimyasal parametreleri üzerine etkisi

Bu çalışma, tavşan diyetlerine ilave edilen yarpuzun (*Mentha pulegium L.*) kurutulmuş yeşil kısımlarının büyüme performansı ve bazı serum biyokimyasal parametreleri üzerindeki etkilerini belirlemek amacıyla yapılmıştır. Araştırmada ortalama 2-2.1 kg ağırlığında 15 Yeni Zelanda tavşanı kullanıldı. Her birinde beş tavşan bulunan kontrol ve iki deneme grubu dizayn edildi. Tüm tavşanlar bireysel kafeslerde barındırıldı. Kontrol grubuna bazal diyet verilirken, deneme gruplarına sırasıyla %0.1 (P1) ve %0.2 (P2) seviyelerinde yarpuz tozu verildi. Deneme 28 günde tamamlandı. Canlı ağırlık ve yem tüketimleri deneme başı ve sonunda yapılan tartımlarla belirlendi. Çalışmanın 0, 14 ve 28. günlerinde tavşanlardan kan örnekleri alınarak serum biyokimyasal parametrelerinin çalışma boyunca değişimi analiz edildi. Performans parametreleri açısından gruplar arasında fark bulunmadı (P>0.05). Yarpuzun serum kolesterol seviyesini düşürdüğü belirlendi (P<0.05). Ancak yarpuzun diğer biyokimyasal parametreler üzerine etkisinin olmadığı belirlendi (P>0.05). Sonuç olarak, yarpuzun tavşan diyetlerine alternatif bir büyüme destekleyici ve kolesterol düzenleyici olarak kullanılabileceği belirlendi. Özellikle tavşan diyetlerinde %0.2'ye kadar yarpuz kullanılabileceği kanaati oluşmuştur.

Anahtar Kelimeler: *Biyokimyasal parametreler, Kolesterol, Mentha pulegium, Performans, Tavşan.*

INTRODUCTION

Disease due to the deterioration of microflora in rabbits' digestive system can pave the way for problems that can result in death (Bäuerl et al. 2014). Antibiotics have been used for many years as feed additives to prevent such ailments (Cesari et al. 2008). Following the European Union's prohibition of antibiotics as feed additives, the pursuit for alternative feed additives have increased, and many products such as antibiotics have been employed in trials (Wahyuni et al. 2019). Researchers support those aromatic plants can be a natural alternative among growth

✉ *Corresponding author: mükremin.olmez@hotmail.com

promoters for animal production (Losa and Kohler 2001; Şahin et al. 2012; Ölmez et al. 2020). Such plants secrete various secondary metabolites (volatile network, flavonoid, alkaloid, saponin) during their development (Şahin et al. 2020). Many biological and pharmaceutical effects of such metabolites have been identified, including antioxidant, antimicrobial and anti-inflammatory actions (Zengin et al. 2018). Besides, we observe that they demonstrate effects that protect the physiological health of living things with their appetite-enhancing, digestive system-regulating, and beneficial microorganisms-supporting properties (El-kaiaty et al. 2020).

Labiatae (Lamiaceae) family, one of the nearly 300 plant families that grow in nature, is known to be one of the most widely used aromatic plants for many purposes in the World (Ishtiaq et al. 2014). Mentha, a member of the Labiatae family, is widely used in medicine, food, and cosmetics while spreading from the Mediterranean to Central Asia (Elshafie and Camele 2017). Pennyroyal (*Mentha pulegium L.*), one of the twenty species of Mentha herbs is a perennial aromatic herbaceous plant that can grow than half a meter (Gruenwald et al. 2000). The dried parts and the essential oil obtained from them are widely used in traditional medicine (respiratory system ailments, digestion, liver and gallbladder disorders), gastronomy (spices, snacks), aromatherapy, and cosmetics (Ahmed et al. 2018). It has also been known that pennyroyal has many physiological, biochemical, and pharmacological effects, thanks to its phenolics. Linalool and Menthone have been identified to be the main constituent of Pennyroyal (Velpandian et al. 2001). In previous research, it has been reported that pennyroyal protects the welfare of animals and increases their growth performance by showing growth-promoting, regulating intestinal flora and antioxidant action (Abedini et al. 2017; Reyan Mohasesi et al. 2020; Makav and Ölmez 2021; Ölmez et al. 2021).

This study aimed to identify the pennyroyal's effect on performance and some serum biochemical parameters in rabbits.

MATERIAL AND METHODS

Ethics committee permission

This research was conducted with the permission of the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK/2020-123).

Animals and Trial Design

A total of fifteen New Zealand rabbits of age with an initial body weight of 2-2.1 kg were utilized in the experiment. The rabbits were obtained from the Atatürk University Experimental Animal Breeding Unit, which is officially authorized to breed and sell experimental animals. Rabbits were housed individually in species-specific cages and fed ad libitum. The environment in which the cages were located was kept at a comfortable temperature (24 ° C). The environment was illuminated with a 12-hour light-12-hour dark system. The rabbits were fed with a diet prepared per their needs in Table 1 (Halls 2010). The rabbits were divided into three experimental groups (Control, 0.1% pennyroyal (P1) and 0.2% pennyroyal (P2)), with five rabbits in the group. While the rabbits in the control group were provided with the basal diet, pennyroyal powder to the groups' feed was added at a level of 0.1% (P1) and 0.2% (P2), respectively. The trial was completed in 28 days.

Table 1. Nutrient content and chemical analysis of the diet.

Ingredients	%
Barley	14.00
Wheat	21.00
Maize	16.00
Wheat bran	15.00
Oak husk	4.00
Soybean meal	8.00
Cotton seed meal	10.00
Clover flour	5.00
Vegetable oil	2.00
Molasses	1.00
Dicalcium phosphate	1.80
Marble dust	1.60
Salt	0.35
Vit-min mix	0.25
Analysis	
Dry matter, %	89.79
Crude protein, %	15.83
Metabolizable energy (Kcal/kg)	2500.00
Crude fibre, %	8.98
Crude fat, %	4.48
Ash, %	7.83

Pennyroyal (*Mentha pulegium L.*)

The pennyroyal was collected during the season from Boğatepe village (40 ° 48'21.2 "N 42 ° 53'37.8" E) of Kars province. The plant was dried and powdered without solarizing at room temperature. The essential oil was obtained via the water vapor distillation method and performed GC/MS analysis. The analysis reveals that the pennyroyal's major compounds were Linalool (13.61%) and Menthone (10.56%). The powder of pennyroyal was used in the trial.

Performance Parameters

All rabbits were individually weighed every week during the trial, and body weight (BW) and body weight gains (BWG) were calculated and recorded. Also, feed consumption (FC) was identified by removing the remaining feed. The feed conversion ratio (FCR) was identified by dividing the resulting feed consumption by increasing body weight.

Sampling and analysis

Blood samples were taken from rabbits' ear veins on the 0th, 14th, and 28th days. Blood samples were centrifuged at 3000 rpm for 5 minutes. All samples were stored at -20 °C until the analysis day. Glucose, cholesterol, triglyceride, total protein, urea, calcium, and phosphorus levels in serum samples thawed at +4 °C on the day of analysis identified by the kit procedure by using Enzyme-Linked ImmunoSorbent Assay (ELISA-Elabscience® UK) commercial kits.

Statistical Analysis

The data were analyzed using the One-Way ANOVA in statistical software GraphPad Prism 8 (San Diego, CA). The post-hoc test (Tukey) measured differences of means. Statistical significances were adopted to be $P<0.05$.

RESULTS

Table 2 illustrated the effect of pennyroyal supplementation to rabbit diet on performance values such as BW, BWG, FC, and FCR. No differences were among the groups in the initial and final BW's (P> 0.05). The effect of pennyroyal on BWG was limited (P> 0.05). Considering the FC, there were no differences among all groups (P> 0.05). When the FCR's were calculated, no significant differences were discerned among the results (P> 0.05).

Table 2. The effect of Pennyroyal on performance parameters.

Parameters	Control	P1	P2	p
Initial BW (g)	2049.33 ±30.02	2051.67 ±15.41	2054.42 ±8.97	P>0.05
Final BW (g)	2201.00 ±31.24	2205.67 ±13.54	2212.75 ±9.82	P>0.05
BWG (g/day)	5.42 ±0.09	5.50 ±0.11	5.65 ±0.15	P>0.05
FC (g/day)	23.02 ±0.61	23.49 ±0.41	24.37 ±0.73	P>0.05
FCR (g/g)	4.25 ±0.05	4.27 ±0.04	4.31 ±0.05	P>0.05

P1: 0.1% Pennyroyal, P2: 0.2% Pennyroyal, BW: Body weight, BWG: Body weight gain, FC: Feed consumption, FCR: Feed conversion ratio.

The results reveal that the pennyroyal's effect on glucose, total protein, urea and triglyceride levels were not significant (P>0.05). Cholesterol level was significantly decreased in all the pennyroyal groups on the 14th and 28th days of study (P<0.05). The lowest cholesterol level was determined in P2 group last (28th) day of the study. The pennyroyal did not influence serum calcium and phosphorus level (P>0.05) (Figure 1).

DISCUSSION AND CONCLUSION

This study was carried to evaluate pennyroyal's impact on performance and some blood biochemical parameters in rabbits. No change was found in the groups to which 0.1% and 0.2% pennyroyal was added compared to the control group. Findings reveal that the effect of adding pennyroyal powder to rabbit diets on growth performance was limited. Similar performance results have been reported in studies with Labiatae plants (Abd El-Hady et al. 2013; Zeweil et al. 2017). Benlemlih et al. (2020) reported that the supplementation of thyme and oregano to diets of growing rabbits had no effect on performance parameters. Also, these results were consistent with the literature research of Alagawany et al. (2016) that 2, 4, 6 g/kg turmeric addition and of Peiretti et al. (2011) that 3 g/kg turmeric supplementation to rabbit diets did not affect the growth performance. Similarly, Basavaraj et al. (2011) stated that dietary turmeric did not change BWG and FC in rabbits. Consistent with the current study, it was identified that adding 100 and 200 mg/kg oregano extract and 0.15% rosemary oil to rabbit diets did not affect performance (Botsoglou et al. 2004; Erdelyi et al. 2008).

Some studies differed from the results of the present study (Rotolo et al. 2013; Cardinali et al. 2015). Pebriansyah et al. (2019) observed that the addition of phytogenic feed additives significantly affects BWG and FC parameters. Similarly, the addition of thyme oil to rabbit diets has been reported to improve body weight gain (Placha et al. 2013). We note that aromatic herbs affect feed intake and digestion of nutrients and increase performance by regulating the digestive system (Adibmoradi et al. 2006). It was observed in another study that the addition of thyme to the diet of New Zealand rabbits positively affected growth performance, especially in the group with 5% thyme extract, body weight was higher than in the control group (Ibrahim et al. 2000; Alagawany et al. 2016). In another study, it was reported that the supplementation of thyme oil reduced body weight gain but did not show any effect on feed consumption (El-Azeem et al. 2019). Differences of the results are considered to be caused by changes related to the kind, derivatives, harvest time, from of the aromatic plants. It could not be found any literature on the supplementation of pennyroyal to rabbit diets. Therefore, it has been discussed in studies with plants in the same family and genus. We believe that it will shed light on future studies within this scope.

Pennyroyal powder added to rabbit diets at different levels had no overall effect on blood parameters. Serum glucose level was not affected by pennyroyal's addition during the whole trial (P>0.05). In a similar study, El-kaiaty et al. (2020) reported that the addition of thyme oil to rabbit diets did not affect the serum glucose level. These results were consistent with the results of El-Gogary et al. (2018) that adding rosemary to rabbit diets and of Al-Jamal and Alqadi (2011) that adding rosemary to rat diets did not alter serum glucose levels. Unlike this study, Abd-El-Hady (2014) reported that the herbal supplement to the rabbit diets significantly increased the serum glucose level in the middle of the study compared to the control group. Similarly, Ibrahim et al. (2000) described that the addition of 0.5% peppermint and thyme to male New Zealand rabbit diets significantly increased serum glucose level. It was identified that aromatic herbs and their products could increase pancreatic activity and affect glucose levels (Moore 2010).

Serum total protein results at the end of the trial are compatible with the results of Abd-El-Hady (2014) that it was not affected by adding 300 and 400 g/ton of herbal additives to rabbit diets. Tousson et al. (2011) reported that 2 kg/ton black seed and thyme in the rabbit diets did not change the total protein values compared to the control. In a different study, it was observed that thyme oil significantly decreased total protein value in growing rabbits compared to the control group (El-kaiaty et al. 2020). It is believed that the added aromatic herbs' differences in glucose and protein results may be affected depending on the feed consumption.

Blood urea results were not affected by pennyroyal's supplementation (P>0.05). Contrary to the present trial, studies reported that the addition of thyme and oregano oil significantly reduced urea, one of the kidney function parameters, as a positive indicator compared to the control group (Tousson et al. 2011; El-kaiaty et al. 2020).

Serum cholesterol level decreased significantly in the groups that added 0.1% and 0.2% pennyroyal to the rabbit diets compared to the control group. However, the serum triglyceride level did not alter among the groups. Thus, with supplementation of 2 g/ton thyme to the rabbit diets, the cholesterol level decreased significantly, while the triglyceride level did not change (Tousson et al. 2011). Cholesterol level decreased, while triglyceride level did not change significantly in the study investigating the effect of oregano oil added to drinking water at the level of 1ml/L in growing rabbits on performance and blood parameters (El-kaiaty et al. 2020). While El-Gogary et al. (2018)

reported no significant change in cholesterol and triglyceride levels by giving rosemary oil to rabbit diets, Abd-El-Hady (2014) reported that the phytogenic feed additive decreased cholesterol and triglyceride levels in rabbits during the growing period. The phenolic ingredient of aromatic herbs is known to inhibit cholesterol absorption in the digestive system. It was also attributed that pennyroyal can decrease cholesterol by hindering 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the enzyme involved in cholesterol synthesis (Elson and Qureshi 1995).

It has also been reported that polyphenols with high antioxidant activity show cholesterol-lowering activity. Phenolic compounds of pennyroyal have also been observed to have high antioxidant activity (Sunarno et al. 2019; Makav and Ölmez 2021). The pennyroyal acts a crucial role in lowering cholesterol levels, and the difference between some research varies depending on the use, dose, and application of aromatic herbs with different components. The supplementation of pennyroyal did not significantly affect serum calcium and phosphorus levels during the whole trial. No literature data is available to analyze the effects of aromatic herbs and products on serum calcium and phosphorus levels in New Zealand rabbits. The available data are consistent with some research on monogastric animals and analyze aromatic herbs and products (Khaksar et al. 2012; Cengiz et al. 2016). In another study, the essential oil mixture reduced the serum calcium level but did not change the broiler's serum phosphorus level (Chen et al. 2013). However, the effect of aromatic herb mixtures on serum biochemical parameters in laying hens reveals that serum calcium and phosphorus levels increased and that herbal components increased the absorption of these minerals from the intestine into the bloodstream (Sakthi Priya et al. 2017). It is believed that the differences observed are animal species, diet composition, variety, aromatic plants, and feeding conditions.

As a result of the study, cruces of the trial were the decrease in serum cholesterol level with the supplementation of pennyroyal. So, it is believed that the pennyroyal may be safely used as an alternative growth promoter, as it is observed that pennyroyal has some benefits on health of rabbits.

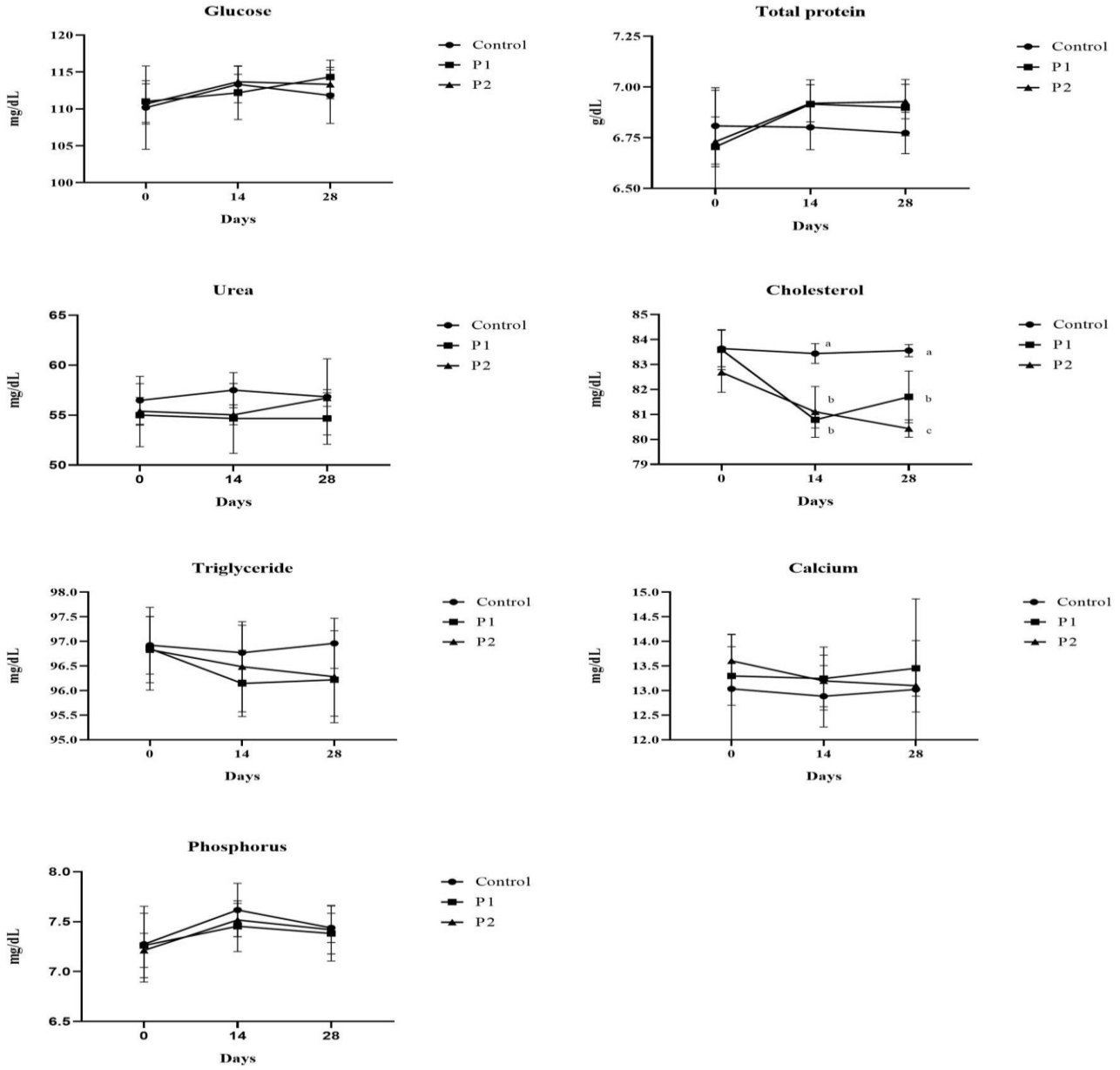


Figure 1. The effect of Pennyroyal on biochemical parameters.

P1: 0.1% Pennyroyal; P2: 0.2% Pennyroyal a,b,c: Means in the same column with different superscripts differ (P<0.05)

CONFLICTS OF INTEREST

No potential conflict of interest was reported by the authors.

AUTHOR CONTRIBUTIONS

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

REFERENCES

Abd-El-Hady A (2014). Performance, physiological parameters and slaughter characteristics in growing rabbits as affected by a herbal feed additives (DIGESTAROM). *J Int Sci Publ Agric Food*, 2, 353-365.

Abd El-Hady A, El-Ghalid O, El-Raffa A (2013). Influence of a herbal feed additives (Digestarom®) on productive performance and blood constituents of growing rabbits. *Egypt J Anim Prod*, 50 (1), 27-37.

Abedini A, Hassanabadi A, Afzali N, Kermanshahi H (2017). The effect of different dietary levels of Pennyroyal (Mentha Pulegium L.), probiotic and antibiotic on performance, carcass characteristics and, sellected nutrients digestibility in broiler chickens. *Arch Med Lab Sci*, 3 (2), 15-22.

Adibmoradi M, Navidshad B, Seifdavati J, Royan M (2006). Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. *J Poult Sci*, 43 (4), 378-383.

Ahmed A, Ayoub K, Chaima AJ, Hanaa L, Abdelaziz C (2018). Effect of drying methods on yield, chemical composition and bioactivities of essential oil obtained from Moroccan Mentha pulegium L. *Biocatal Agric Biotechnol*, 16, 638-643.

Al-Jamal A-R and Alqadi T (2011). Effects of rosemary (Rosmarinus officinalis) on lipid profile of diabetic rats. *Jordan J Biol Sci*, 147 (622), 1-5.

Alagawany M, Ashour EA, Reda FM (2016). Effect of dietary supplementation of garlic (Allium sativum) and Turmeric (Curcuma longa) on growth performance, carcass traits, blood profile and oxidative status in growing rabbits. *Ann Anim Sci*, 16 (2), 489-505.

Basavaraj M, Nagabhushana V, Prakash N, et al. (2011). Effect of dietary supplementation of Curcuma longa on the biochemical profile and meat characteristics of broiler rabbits under summer stress. *Vet World*, 4 (1), 15.

Bäuerl C, Collado MC, Zúñiga M, et al.(2014). Changes in cecal microbiota and mucosal gene expression revealed new aspects of epizootic rabbit enteropathy. *PloS one*, 9 (8), e105707.

Benlemlih M, Barchan A, Aarab A, Bakkali M, Arakrak A et al. (2020). Effect of Dietary Dried Fennel and Oregano and Thyme Supplementation on Zootechnical Parameters of Growing Rabbits. *World*, 10 (3), 332-337.

Botsoglou N, Florou-Paneri P, Christaki E, Giannenas I, Spais A (2004). Performance of rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with oregano essential oil. *Arch Anim Nutr*, 58 (3), 209-218.

Cardinali R, Cullere M, Dal Bosco A, et al. (2015). Oregano, rosemary and vitamin E dietary supplementation in growing rabbits: Effect on growth performance, carcass traits, bone development and meat chemical composition. *Livest Sci*, 175, 83-89.

Cengiz S, Yesilbag D, Eren M, et al. (2016). Effects of volatile oil additives on growth, carcass performances, and calcium and phosphorus concentrations in serum and bone of broilers. *Rev Med Vet (Toulouse)*, 167 (7-8), 230-239.

Cesari V, Toschi I, Pisoni A, Grilli G, Cesari N (2008). Effect of dietary acidification on growth performance and caecal characteristics in rabbits. Proc 9th World Rabbit Congress.

Chen G, Wu J, Li C (2013). The effect of different selenium levels on production performance and biochemical parameters of broilers. *Ital J Anim Sci*, 12 (4), e79.

El-Azeem A, Ahmed E, Al-Sagheer AA, Daader A, Bassiony S (2019). Effect of dietary supplementation with betaine, thyme oil and their mixtures on productive performance of growing rabbits. *Zagazig J Agric Res*, 46 (3), 815-828.

El-Gogary M, El-Said E, Mansour A (2018). Physiological and immunological effects of rosemary essential oil in growing rabbit diets. *J Agric Sci*, 10, 485-491.

El-kaiaty AM, El-Moghazy GM, El-Manylawi MAF, Abdel-Mageed MGY (2020). Impact of thyme oil and lactobacillus acidophilus as natural growth promoters on performance, blood parameters and immune status in growing rabbits. *J World's Poult Res*, 10 (1), 1-11.

Elshafie HS and Camele I (2017). An overview of the biological effects of some mediterranean essential oils on human health. *Biomed Res Int*, 2017, ID9268468.

Elson C and Qureshi A (1995). Coupling the cholesterol-and tumor-suppressive actions of palm oil to the impact of its minor constituents on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. *Prostaglandins Leukot Essent*, 52 (2-3), 205-208.

Erdelyi M, Matics Z, Gerencsér Z et al. (2008). Study of the effect of rosemary (Rosmarinus officinalis) and garlic (Allium sativum) essential oils on the performance of rabbit. 9th World Rabbit Congress–June.

Gruenwald J, Brendler T, Jaenicke C (2000). Physician's desk reference (pdr) for herbal medicines. Medical Economics Company.

Halls AE (2010). Nutritional requirements for rabbits. *Retrieved September*, 21, 2014.

Ibrahim SA, El-Ghamry A, El-Mallah G (2000). Effect of some medicinal plants of Labiatae family as feed additives on growth and metabolic changes of rabbits. *Egyptian J Rab Sci*, 10 (1), 105-120.

Ishtiaq S, Ahmad M, Hanif U, Akbar S, Kamran SH (2014). Phytochemical and in vitro antioxidant evaluation of different fractions of Amaranthus graecizans subsp. silvestris (Vill.) Brenan. *Asian Pac J Trop Med*, 7, 342-347.

Khaksar V, Van Krimpen M, Hashemipour H, Pilevar M (2012). Effects of thyme essential oil on performance, some blood parameters and ileal microflora of Japanese quail. *J Poult Sci*, 49 (2), 106-110.

Losa R and Kohler B (2001). Prevention of colonisation of Clostridium perfringens in broilers intestine by essential oils. Proc 13th Eur Symp Poult Nutr, Blankenberge (Belgium).

Makav M and Ölmez M (2021). Effects of Pennyroyal (Mentha pulegium L.) on angiotensin converting enzyme-2 (ace-2) and antioxidant parameters in rabbits. *J VetBio Sci Tech*, 6 (1), 9-14.

Moore JE (2010). Natural standard herb and supplement guide: An evidence-based reference. *Ulster Med J*, 79 (3), 152-153.

Ölmez M, Adıgüzel Işık S, Şahin T, Yörük M (2021). The effects of Pennyroyal (Mentha pulegium L.) on performance, carcass and meat quality in Japanese quails (Coturnix coturnix japonica). *Ankara Üniv Vet Fak Derg*, 68 (4), 2343-2348.

Ölmez M, Şahin T, Karadağoğlu Ö, et al. (2020). The impact of an essential oil mixture on growth performance and intestinal histology in native Turkish geese (Anser anser). *Kafkas Üniv Vet Fak Derg*, 26 (5), 625-631.

Pebriansyah A, Lukešová D, Knížková I, Silberová P, Kunc P (2019). The effect of natural phytoadditive silybum marianum on performance of broiler rabbits. *Sci Agric Bohem*, 50 (1), 40-45.

Peiretti P, Masoero G, Meineri G (2011). Effects of replacing palm oil with maze oil and Curcuma longa supplementation on the performances, carcass characteristics, meat quality and fatty acid profile of the perirenal fat and muscle of growing rabbits. *Anim*, 5 (5), 795-801.

Placha I, Chrastinova L, Laukova A, et al. (2013). Effect of thyme oil on small intestine integrity and antioxidant status, phagocytic activity and gastrointestinal microbiota in rabbits. *Acta Vet Hung*, 61 (2), 197.

Reyan Mohasesi A, Darmani Koohi H, Naseri Harsini R, Mirza Aghazadeh A, Kioumarsi H (2020). Effect of Pennyroyal (Mentha Pulegium L.) on Performance, Carcass Characteristics and Some of the Blood Parameters in Broilers. *J Environ Res Technol*, 6 (6), 71.

Rotolo L, Gai F, Nicola S, et al. (2013). Dietary supplementation of oregano and sage dried leaves on performances and meat quality of rabbits. *J Integr Agric*, 12 (11), 1937-1945.

Sakthi Priya M, Gopala Krishna Murthy T, Vijayanand T (2017). Effect of blend herbal supplement on haematology and serum biochemistry in commercial layer chicken. *J World Poult Res*, 7 (2), 48-56.

Sunarno S, Zubaedah S, Rini ANS, Sekarsari E, Rahadian R (2019). Cinnamon and gotu kola supplementation to produce high antioxidant and low cholesterol of quail pectoral meat. *Biosaintifika J Biol Biol Educ*, 11 (1), 148-155.

Şahin T, Karadağoğlu Ö, Sarı M (2012). Effects of Ground Echinacea (Echinacea purpurea)Supplementation Quail Diets on Growth Performance and Carcass Traits. *Kafkas Univ Vet Fak Derg*, 18 (1), 15-19.

Şahin T, Sural T, Ölmez M, Karadağoğlu Ö (2020). Effects of herbal extract mixtures on performance, carcass yield and some ınternal organ weight in broilers. *Vet Hek Der Derg*, 91 (2), 137-146.

Tousson E, El-Moghazy M, El-Atrsh E (2011). The possible effect of diets containing Nigella sativa and Thymus vulgaris on blood parameters and some organs structure in rabbit. *Toxicol Ind Health*, 27 (2), 107-116.

Velpandian T, Jasuja R, Bhardwaj R, Jaiswal J, Gupta S (2001). Piperine in food: interference in the pharmacokinetics of phenytoin. *Eur J Drug Metab Pharmacokinet*, 26 (4), 241-247.

Wahyuni AETH, Prakasita VC, Nahak TEM, et al. (2019). Peluang imbuhan pakan herbal-probiotik komersial "promix®" sebagai pengganti antibiotic growth promoter (agp) pada ayam pedaging yang diberi vaksin ND. *J Sain Vet*, 37 (2), 180-184.

Zengin G, Aumeeruddy-Elalfi Z, Mollica A, Yilmaz MA, Mahomoodally MF (2018). In vitro and in silico perspectives on biological and phytochemical profile of three halophyte species—A source of innovative phytopharmaceuticals from nature. *Phytomedicine*, 38, 35-44.

Zeweil H, Zahran S, Ahmed M, Morshedy S, El-Mabrok B (2017). Effects of essential oils supplementation on growth performance, digestibility coefficients and carcass characteristics of growing rabbits. *J Adv Agric Res*, 22 (4), 650-660.

Van Vet J, 2021, 32 (3) 125-129

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Raouf S, Ağan UB, Uzun B, Dalğın D (2021).** The Insidious Disorder Hiding Behind Aging: Canine Cognitive Disorder. *Van Vet J*, 32(3), 125-129. DOI: https://doi.org/10.36483/vanvetj.935024

ISSN: 2149-3359 **Review** e-ISSN: 2149-8644

The Insidious Disorder Hiding Behind Aging: Canine Cognitive Disorder

Saba RAOUF* Umut Burak AĞAN Büşra UZUN Duygu DALĞIN

Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Internal Medicine, 55270, Samsun, Turkey

Received: 08.05.2021 Accepted: 10.10.2021

ABSTRACT Canine cognitive disorder (CDS) is a neurodegenerative, progressive, and irreversible disorder of senior dogs, generally over eight years old. CDS is characterized by a decrease in cognitive functions. Clinically significant changes in dog's behavior and daily routines including disorientation, decrease in social interactions, changes in sleep-wake cycles, loss of household habits, poor communication, increased anxiety, and changes in activity levels with the potential to create significant problems and discomfort in the lifestyle of the owners, deteriorating pet-owner attachment and generally be frustrating for the owner. The definite diagnosis depends on brain biopsies, and there is no confirmed clinical diagnosis method developed, meeting the whole criteria of CDS. The owner assessment using several scales available is the primary tool to rely on, which has a high potential to be subjective. Although it still has many dark sides, CDS is a disease with convenient diagnostic and therapeutic strategies available. Veterinarians need to consider this disease in geriatric patients and cooperate by increasing owner awareness, stopping or at least slowing down the disease in elderly dogs, and making the geriatric period more comfortable for both the dog and the owner. This review aims to introduce current information in the diagnosis and treatment of CDS.

Keywords: *Cognitive behavioral therapy, Cognitive disorder, Cognitive dysfunction, Cognitive therapy, Dementia, Dogs.*

ÖZ

Köpeklerin Yaşlanmaya Bağlı Bilişsel Bozukluğu

Köpek bilişsel bozukluğu (CDS), genellikle 8 yaşın üzerindeki yaşlı köpeklerde görülen nörodejeneratif, progresif ve geri dönüşümsüz bir bozukluktur. CDS, klinik olarak bilişsel işlevlerde azalma, günlük rutinlerinde önemli değişiklikler ve davranışlarda oryantasyon bozukluğu, sosyal etkileşimlerde azalma, uyku-uyanma döngülerinde değişiklikler, ev alışkanlıklarının kaybı, zayıf iletişim, artan anksiyete ve aktivite düzeylerinde değişiklikler dahil olmak üzere değişiklikler ile karakterizedir. Bu değişiklikler sahiplerin yaşam tarzlarında önemli sorunlar, ciddi rahatsızlık oluşturma, köpek ve sahip arası iletişimin bozulması potansiyeli barındırmasının yanı sıra bazen köpek sahibi için dayanılmaz olma durumu vardır. Kesin tanı beyin biyopsilerine dayanmaktadır ve CDS'nin tüm kriterlerini karşılayan doğrulanmış bir klinik tanı yöntemi geliştirilmemiştir. Mevcut olan bir dizi ölçeğin sahiplerin görüşü doğrultusunda değerlendirilmesi, güvenilecek birincil araçtır ve öznel olma potansiyeli yüksektir. Hala birçok karanlık tarafı olmasına rağmen, CDS, kullanışlı tanı ve tedavi stratejileri olan bir hastalıktır. Veteriner hekimlerin geriatrik hastalarda bu hastalığı göz önünde bulundurması ve sahiplerin hastalık konusunda farkındalığını artırarak iş birliği yapması, yaşlı köpeklerde hastalığı durdurması veya en azından yavaşlatması ve geriatrik dönemi hem köpek hem de sahibi için daha konforlu hale getirmesi önemlidir. Bu derleme, CDS'nin tanı ve tedavisinde güncel bilgileri sunmayı amaçlamaktadır.

Anahtar Kelimeler: *Bilişsel davranışçı terapi, Bilişsel bozukluk, Bilişsel tedavi, Demans, Köpekler.*

INTRODUCTION

Canine cognitive disorder (CDS) describes the progressive neurodegenerative disorder of older dogs due to a gradual decline in cognitive function in the veterinary literature. CDS is characterized by a decrease in cognitive functions and clinically significant changes in dog's behavior and daily routines due to progressive neurodegenerative changes in the cerebral cortex and hippocampus and affected dogs are generally over eight years of age (Landsberg 2012). The awareness of the owners for pet health, the increase in pet household companionship, the advances in the veterinary field, and preventive medicine enabled a longer life span for pets. The prolongation of life brings along an increase in the incidence of geriatric problems and degenerative diseases (Landsberg 2012).

Changes associated with CDS are very likely to affect the dog-human bond, as pet owners are often unaware of the changes that come with aging. Considering that these

✉ *Corresponding author: seba.rauf@gmail.com

changes include disorientation, decrease in social interactions, changes in sleep-wake cycles, loss of household habits, poor communication, increased anxiety, and changes in activity levels with the potential to create significant problems and discomfort in the lifestyle of the owners may be frustrating for some individuals (Rosado et al. 2012; Larsen and Farcas 2014).

Awareness of the disease and supportive interventions will enable the pet owner to comprehend the pathology, get rid of confusion, and have a healthy attachment in the geriatric period.

Similar to neuropathological aging in humans, a part of the aged dog population shows signs of cognitive dysfunction and dementia. It's critical to differentiate between physiological, cognitive aging, and pathological brain aging at this stage. Behavioral changes in older dogs can be the first indication of deteriorating health and wellbeing, and they can help determine if the animal is aging physiologically or pathologically. (Landsberg 2012; Toepper 2017).

Physiologic Aging Process

Aging is a natural process that affects every component of the body. In the physiology of aging, there are many metabolic events such as rusting effects of free radicals, telomere shortening, gene effects, and molecular events. Free radical production causes oxidative damage to proteins, lipids, and nucleotides, leading to neuronal dysfunction and eventually death. Oxidative stress is essential for brain function, as it maintains a delicate balance between the development of free radicals and the defense or harm of the brain. The aging of the brain can result in a variety of pathological behavioral and cognitive changes (Rusbridge et al. 2018). Exploring strategies to reduce the harm caused by free radical reactions in humans and animals will reduce morbidity and mortality due to degenerative changes associated with the aging process.

As in humans and all other animals, different behavioral and cognitive features accompany different stages of aging, including changes in the ability to play, concentrate and learn, in dogs according to various life stages and ages (Bature et al. 2017). Studies in laboratory dogs, similar to humans, have reported a decrease in learning, memory, and routine activities with increasing age. The last few studies in pet dogs have reported a reduction in social sensitivity, curiosity for new objects, attention, learning and memory, as age progresses (Adams et al. 2000; Wallis et al. 2014; Wallis et al. 2016). It's a matter of when aging starts in dogs, given such lifelong changes in temperament and cognition. Because there is a large variation in life spans of different dog breeds, aging begins depending on the dog's breed, size, and weight (O'Neill et al. 2013; Szabó et al. 2016). Over the past 200 years, dog breeds are highly inbred, so significant genetic variations have emerged between breeds (Ostrander et al. 2017). Dogs are known to be the most phenotypically diverse mammal species and this difference applies to life span as well as traits such as behavior, morphology, congenital anomalies, disease propensity (Hoffman et al. 2018). So much so that up to two times the difference between species in terms of life span can be observed. Another point that should not be overlooked is the variety of environments where dogs live, food, and care. However, to take an average model, beagles are defined as old middle-aged at 5 to 9 years (human age 40-60) and young before five years old. Although many studies are suggesting that aging begins at the age of seven for Laboratory Beagles, it has been reported that short-term memory disorders start to appear after the age of 6 (Studzinski et al. 2006). On the other hand, (Salvin et al. 2011) reported that even though the aging process progresses along the natural path, in some dogs older than eight years, there is a decrease in response to commands, play, and activity, and even an increase in fear and phobia during these six months. It had been well documented that fear of noises affects the cognitive state in dogs (Karen et al. 2019)

Physiopathology and Clinical Presentation of CDS

Similar to humans, cognitive dysfunction in dogs results in neuron loss and hyperphosphorylated tau protein (HTP) with amyloid (Aβ) accumulation produced by the breakdown of an excessive Aβ amyloid precursor protein (APP), in the brain parenchyma and blood vessels (Condello and Stöehr 2018). A deposits' volume and extent are related to the degree of cognitive impairment and cause oxidative damage. (DeVos et al. 2018) The histopathological structure in dogs with CDS includes neuropathology with Aβ pathology, decreased brain volume, neuronal loss, and impaired neurogenesis. These deposits, which occur mostly from the age of 8, settle as widespread plaques in the cortical areas, starting with the prefrontal cortex followed by temporal and occipital cortexes with the hippocampus (Landsberg et al. 2012). Pathological changes in CDS include meningeal calcification, significant demyelination, cerebral cortex and subcortical white matter reduction, lipofuscin augmentation, ventriculomegaly resulting in brain atrophy due to neuronal loss (Heath et al. 2007).

Alzheimer's disease (AD) has closely similar pathogenesis in dogs with cognitive dysfunction. Cortical atrophy, amyloid-B plaque deposition, neuronal loss, altered neurotransmitter function, and reduced neural regeneration have all been observed in dogs. Along with these pathologies altered sleep-wake cycles, social interactions and activity levels are the most common signs that observed (Nešić et al. 2017)

Clinical signs are symbolized under the term DISHA, which refers to the basic symptoms such as disorientation, social deterioration, changes in sleep habits, elimination problems, increased anxiety with changes in activity levels (Landsberg 2012). In experiments testing spatial learning and memory, dogs with cognitive impairment demonstrated a substantial reduction in cognitive abilities (Studzinski et al. 2006). In addition, changes in social sensitivity, such as erratic movement patterns, increased frequency of purposeless behavior, reduced response to the communication, mirror relation, aggression or apathy, anxiety, timidity, not recognizing the owners with circadian rhythm changes are also presented in dogs with CDS (Chapagain et al. 2017; Rosado et al. 2012).

Ozawa et al. (2019) reported new physical signs of CCD, including swaying or falling, smell disturbance, tremor, head ptosis, and vision impairment which must be considered in the geriatric examination (Makiko et al. 2019). The findings of this study demonstrate new signs in the early stage of the disease that may be misevaluated as the signs of physiologic aging.

Diagnosis: Differentiating Between Physiologic Aging and CDS

Since dog and owner between loyalty is an extremely close relationship, often the owners can detect behavioral differences in their dogs that come with aging, but they attribute them to old age. However, CDS is progressive. Although early diagnosis and treatment do not provide complete recovery, therapeutic attempts have significant

effects in slowing down the process, providing a higher life quality in the long term. Therefore, correct evaluation of CDS-related findings has prognostic importance. (Romanucci and Della Salda 2015)

The trouble in diagnosis is that there is no confirmed method developed meeting the whole criteria of CDS. The owner assessment is the primary tool to rely on, which has a high potential to be subjective. Several scales based on questionnaires are suggested or scales have been developed (Landsberg et al. 2012; Salvin et al. 2011; Rofina et al. 2006; Yu C-H et al. 2011; González et al. 2011;) but evaluation of the data in these methods are very distinct. (Szabó et al. 2016; Schütt et al. 2015)

According to us, Salvin et al. (2011) scale is the most practical and efficient tool, but the general opinion is that it reflects only severe stages. Canine Dementia Scale (Madari et al. 2015) was designed to describe different stages of CDS. Therefore, methods for laboratory dogs (Adams et al. 2000; Tapp et al. 2003; Wallis et al. 2016; Studzinski et al. 2006; Milgram et al. 2005) may be modified for household pet dogs. In addition, food-seeking (González et al. 2013), or place selection tests (Nagasawa et al. 2012) may be preferred as (Rosado et al. 2012) had demonstrated the effect of CDS stage on locomotion, exploratory behavior, corner-directed (aimless) behaviors, sniffing episodes directed towards the objects and social responsiveness in pet dogs.

In our clinical experience, the scale of (Salvin et al. 2011) is easy to apply for practitioners and provides elementary data. Further scales in combination may be used for the detailed evaluation.

Treatment

As the general rule, "earlier diagnosis – better prognosis," therefore awareness of the practitioner for CDS in geriatric patients is crucial. It is also very important to inform the owner in detail to obtain optimum collaboration targeting a satisfying treatment response. The owner must know that the signs are not reversible but can be paused or slowed. Indeed, each patient creates its own story, so general expectations may be frustrating.

The current treatment protocol combines three strategies: drug treatment, nutraceuticals and diet, and environmental enrichment. Strict and intense therapy with these strategies generally results in satisfactory clinical outcomes (Landsberg 2012).

Drug therapy

The main three drugs in the conventional therapy of CCD are Selegiline, Propentofylline, and Nicergoline. They restore the blood flow in brain tissue nearby other therapeutic effects.

Selegiline

Selegiline was the first drug approved by the FDA for dog cognitive disorders and is an irreversible and selective inhibitor of monoamine oxidase B (MAOB) in canines. Selegiline increases enhance dopamine and catecholamine function in the canine brain and improve cognitive function on its own. Improved synaptic impulse transmission could be possible with catecholamine enhancement. Selegiline has an amphetamine-like effect, increasing dopamine release and blocking dopamine reuptake. Selegiline can be the first choice due to its effects on enhancing sleep-wake cycles and social interactions (Landsberg et al. 2012). Recommended dosage in canine CDS is 0.5–1 mg/kg SID and effects may be expected in 2-8 weeks (Milgram et al. 2002). Augmented impacts could be achieved with a prescription diet. (Dodd et al. 2003)

Propentofylline

Propentofylline improves cerebral blood perfusion, spatial attention, enables nutrient entrance to brain cells, and increases adenosine, elementary for mitochondria. Helps with dullness, lethargy, and depression in dogs (Landsberg et al. 2012). The therapeutic dose for CDS is 2.5-5 mg/kg BID.

Nicergoline

Nicergoline acts as an α-1 and α -2 adrenergic antagonist to increase neuronal conduction, cerebral blood flow, and neuroprotective effects. The therapeutic dose for CDS is 0.25-0.5 mg/kg SID (Siwak et al. 2000).

Unfortunately, there is insufficient research on the effects of canine CDS, so Selegiline is suitable for conventional treatment.

Adrafinil and Modafinil

Adrafinil and Modafinil are noradrenergic systems stimulating drugs. The noradrenergic system aids in the maintenance of alertness, concentration, memory, and learning and neuroprotection. They are prescribed to maintain regular sleep-wake cycles, locomotion, and learning. In dogs, adrafinil introduces increased motility without stereotypic manners at and above 20 mg/kg. Alas, these doses may result in memory loss (Siwak et al. 2000; Dewey 2008; Kelly et al. 2017)

In a study comparing effects in immobility with propentofylline, nicergoline, and adrafinil, the latter was reported to have an improvement in motility, while nickergoline or propentophile did not. The therapeutic dose for adrafinil in CDS is 20 mg/kg SID. (Siwak et al. 2000).

Memantine

Memantine is an N-methyl-d-aspartate (NMDA) receptor antagonist, inhibiting glutamate development in CDS patients who have too much glutamine in their system. Memantine is used in AD in humans. In dogs, doses ranging from 0.3 - 1.0 mg/kg BID have been used to treat compulsive behaviors (Schneider et al. 2009).

Ropesalazine

In Alzheimer's disease, ropesalazine is used to prevent nerve cell death, the formation of amyloid plaques, and the formation of neurofibrillary tangles. A new drug for dog CDS is being developed by GNT pharma. They suggest that their preliminary studies revealed promising effects in canine dementia, but published data is not available yet.

Antidepressants and Anxiolytics

The use of antidepressants and anxiolytics such as valproic acid, amitriptyline, buspirone, benzodiazepines, fluoxetine, gabapentin, and paroxetine will be essential support in the treatment process since CDS-related neuropathologies may lead to behavioral changes such as nervousness, decreased sensitivity to stimuli, fear, agitation, changing sleep-wake cycles, mood variation, and anxiety. Clomipramine is also a good choice in dogs. (Landsberg 2012)

Dietary and Nutraceutical Treatment

Antioxidants, vitamin E, and C supplementations were demonstrated to have favorable clinical effects. Vitamins E and C fight with free radicals and prevents cell integrity. Also, a positive correlation of cognitive ability in CDS with vitamin E supplementation was reported. (Landsberg 2012)

Antioxidants include flavonoids, carotenoids, beta carotene, selenium, and dlalpha-lipoic acid.

L-carnitine and dl-alpha-lipoic enhance mitochondrial function. Omega-3 fatty promotes cell integrity with considerable anti-inflammatory effects. Hills Prescription diet R Canine b/d R provided these supplements and had been demonstrated to create improvement in cognitive signs in a couple of months (Dodd et al. 2003).

Thiamine (B1), cobalamin (B12), folate (B9) and pyridoxine (B6) are all essential for neurodevelopment and cognitive function. (Selhub et al. 2010). The same effects are determined in dogs and cats, but vitamin B deficiencies are rare in these species (May and Laflamme 2019).

Medium-chain triglycerides must be a part of the therapy providing glucose for neurons, deteriorating with age. Dietary medium-chain triglycerides (MCTs) can raise blood ketone levels, providing energy for cerebral activity, potentially providing up to 20% of the brain's energy needs. Diet including 5.5% MCT given to dogs with CDS resulted in improved cognitive abilities in 8 months (Pan et al. 2011).

L-arginine is a practical antioxidant nearby many other effects. It is metabolized in neurons and body cells, forming citrulline, yielding to nitric oxide (Pan et al. 2013). Nitric oxide modulates vascular tone and blood flow, immune responses, neural communication, and expression of antioxidant enzymes. In addition, the high metabolic activity of the brain requires good perfusion, primarily mediated by nitric oxide (Vauzour et al. 2017).

Phosphatidylserine is a naturally occurring phospholipid that is present in high concentrations in the brain and synapses. It improves memory, learning, and social behavior in dogs and cats by facilitating membrane-dependent neuronal processes, increasing acetylcholine release, inhibiting muscarinic receptor loss, activating dopamine synthesis and release, and possibly improving memory learning and social behavior. (Osella et al. 2008)

N-acetylcysteine and resveratrol are also very potent antioxidants.

Apoaequorin is a calcium-buffering protein with neuroprotective effects positively influencing learning and attention abilities (May and Laflamme 2019). A recent study demonstrated that 10 mg apoaequorin showed superior performance on cognitive tasks compared with dogs receiving 1 mg/kg selegiline (Milgram et al. 2015)

S-Adenosyl-l-Methionine (SAMe) is a product of methionine and is essential for liver functions. Exogenous SAMe causes a rise in serotonin turnover and dopamine and norepinephrine levels by raising endogenous glutathione production (May and Laflamme 2019).

Since 95% of serotonin is gut originated, the link between microbiota and anxiety is apparent, also there is evidence of the effect of gut microbiota in geriatric cognitive deterioration (Bastiaanssen et al. 2019). Therefore, properly capsulated probiotic supports will contribute to clinical satisfaction to the author's experience.

Environmental enrichment

Environmental enrichment is the irrevocable part of therapy. Studies displayed that enrichment of the environment, toys, exercises, increasing the time spent playing in addition to dietary supplements had a significant positive effect on preventing the symptoms (Milgram et al. 2002; Milgram et al. 2004). Maintaining a regular daily routine contributes to anxiety relief, maintaining brief orientation, and keeping the dog active during daylight hours and sleeping patterns. Adding aromatherapy, tactile and sound cues, such as vocal cues to remind rooms, bed, and routine areas, scent cues like scented candles, and tactile cues like textured matting become the right stimuli.

CONCLUSION

Geriatric problems are the most challenging period in the dog-owner relationship. For example, in a study, the primary reason for relinquishing dogs to the shelter for euthanasia was the incurable illness of aged dogs (25.77%) (Vučinić et al. 2009). Dementia and CDS are one of the major frustrating geriatric disorders.

Although it still has many dark sides, CDS is a disease with useful diagnostic and therapeutic strategies available. Veterinarians need to consider this disease in geriatric patients and cooperate by increasing owner awareness, stopping or at least slowing down the disease in elderly dogs, and making the geriatric period more comfortable for both the dog and the owner. As all animal lovers know, not only do they need us, but we also need them, and our mutual responsibility lasts until death.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: SR
Supervision / Consultancy: DD
Data Collection and / or Processing: SR
Analysis and / or Interpretation: SR, UBA, BU, DD
Writing the Article: SR
Critical Review: DD

REFERENCES

Adams B, Chan A, Callahan H, Milgram NW (2000). The canine as a model of human cognitive aging: recent developments. *Prog. Neuropsychopharmacol Biol Psychiatry*, 24 (5), 675–692.

Bastiaanssen T, Cowan C, Claesson MJ, Dinan TG, Cryan JF (2019). Making Sense of the *Microbiome in Psychiatry nt J Neuropsychopharmacol*, 22 (1), 37–52.

Bature, F, Guinn, B-A, Pang, D, Pappas, Y (2017). Signs and symptoms preceding the diagnosis of Alzheimer's disease: a systematic scoping review of literature from 1937 to 2016. *BMJ Open* 7:e015746.

Chapagain D, Virányi Z, Wallis LJ et al. (2017). Aging of attentiveness in border collies and other pet dog breeds: the protective benefits of lifelong training. *Front Aging Neurosci,* 9, 100.

Condello C, Stöehr J (2018). Aβ propagation and strains: Implications for the phenotypic diversity in Alzheimer's disease. *Neurobiol Dis,* 109, 191–200.

DeVos SL, Corjuc BT, Oakley DH, et al. (2018). Synaptic Tau seeding precedes Tau pathology in human Alzheimer's disease brain. *Front Neurosci,* 12:267.

Dewey CW (2008). Encephalopathies: Disorders of the brain. In A Practical Guide to Canine and Feline Neurology, 115-220, ed. C.W. Dewey. Iowa State University Press, Ames, Iowa.

Dodd CE, Zicker SC, Jewell DE (2003). Can a fortified food affect the behavioral manifestations of age-related cognitive decline in dogs? *Vet Med Anim Clin*, 98, 396.

González-Martínez Á, Rosado B, Pesini P et al. (2011). Plasma β-amyloid peptides in canine aging and cognitive dysfunction as a model of Alzheimer's disease. *Exp Gerontol*, 46, 590–596.

Heath SE, Barabas S, Craze PG (2007). Nutritional supplementation in cases of caninecognitive dysfunction-a clinical trial. *Appl Anim Behav Sci,* 105, 274–283

Hoffman JM, Creevy KE, Franks A, O'Neill DG, Promislow DEL (2018). The companion dog as a model for human aging and mortality. *Aging Cell;* 17, e12737.

Kelly SC, He B, Perez SE et al. (2017). Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. *Acta Neuropathol Commun,* 5 (1), 8.

Landsberg GM, Nichol J, Araujo JA (2012). Cognitive dysfunction syndrome: a disease of canine and feline brain aging. *Vet Clin North Am Small Anim Pract,* 42, 749–768.

Larsen JA, Farcas A (2014). Nutrition of aging dogs. *Vet Clin North Am Small Anim Pract*, 44 (4), 741–746.

Madari A, Farbakova J, Katina S et al. (2015). Assessment of severity and progression of canine cognitive dysfunction syndrome using the CAnine DEmentia Scale (CADES). *Appl Anim Behav Sci,* 171, 138–145.

May K, Laflamme DP (2019). Nutrition and the aging brain of dogs and cats. *J Am Vet Med Assoc*, 255 (11), 1245-1254.

Milgram NW, Landsberg G, Merrick D, Underwood MY (2015). A novel mechanism for cognitive enhancement in aged dogs with the use of a calcium-buffering protein. *J Vet Behav*, 10 (3), 217-222

Nagasawa M, Yatsuzuka A, Mogi K, Kikusui T (2012). A new behavioral test for detecting decline of age-related cognitive ability in dogs. *J Vet Behav Clin Appl Res*, 7, 220–224.

Nešić S, Kukolj V, Marinkoviæ D, Vuèiæeviæ I, Jovanoviæ M (2017). Histological and immunohistochemical characteristics of cerebral amyloid angiopathy in elderly dogs. *Vet Q,* 37, 1–7.

O'Neill DG, Church DB, McGreevy PD, Thomson PC, Brodbelt DC (2013). Longevity and mortality of owned dogs in England. *Vet J*, 198 (3), 638–643.

Osella MC, ReG, Badino P (2008). Phosphatidylserine (PS) as a potential nutraceutical for canine brain aging: a review. *J Vet Behav Clin Appl Res*, 3 (2), 41-51.

Ostrander EA Wayne RK, Freedman AH,Davis BW (2017). Demographic history, selection and functional diversity of the canine genome. *Nat Rev Genet*, 18, 705– 720.

Overall KL, Dunham AE, Scheifele P, Malowski KS (2019). Fear of noises affects canine problem-solving behavior and locomotion in standardized cognitive tests. *Appl Anim Behav Sci*, 221, 104863.

Ozawa M, Inoue M, Uchida K et al. (2019). Physical signs of canine cognitive dysfunction. *J Vet Sci*,81 (12), 1829–1834.

Pan Y (2011). Enhancing brain functions in senior dogs: a new nutritional approach. Top Companion Anim Med, 26 (1), 10–16.

Pan Y, Araujo JA, Burrows J et al. (2013). Cognitive enhancement in middle-aged and old cats with dietary supplementation with a nutrient blend containing fish oil, B vitamins, antioxidants and arginine. *Br J Nutr*, 110 (1), 40–49.

Rofina JE, van Ederen AM, Toussaint MJM et al. (2006). Cognitive disturbances in old dogs suffering from the canine counterpart of Alzheimer's disease. *Brain Res* 1069, 216–226.

Romanucci M, Della Salda L (2015). Oxydativestress and protein quality control systems in the aged canine brain as a model for human neurodegenerativedisorders. Oxidative Medicine and Cellular Longevity, Hindawi Publishing Corporation, London.

Rosado B, González-Martínez Á, Pesini P et al. (2012). Effect of age and severity of cognitive dysfunction on spontaneous activity in pet dogs. 1. Locomotor and exploratory behaviour. *Vet J*, 194, 189–195.

Rusbridge C, Salguero FJ, David MA et al. (2018). An aged Canid with behavioral deficits exhibits blood and cerebrospinal fluid amyloid beta oligomers. *Front. Aging Neurosci,* 10, 7.

Salvin HE, McGreevy PD, Sachdev PS, Valenzuela MJ (2011). Growing old gracefully – behavioral changes associated with "successful aging" in the dog, Canis familiaris. *J Vet Behav Clin Appl Res,* 6, 313–320.

Schmidt F, Boltze J, Jäger C et al. (2015). Detection and Quantification of β-Amyloid, Pyroglutamyl Aβ, and Tau in Aged Canines. *J Neuropathol Ex Neurol*, 74 (9), 912–923.

Schneider BM, Dodman NH, Maranda L (2009). Use of memantine in treatment of canine compulsive disorders. *J Vet Behav*, 4 (3), 118-126.

Schütt T, Toft N, Berendt M (2015). A comparison of 2 screening questionnaires for clinical assessment of canine cognitive dysfunction. *J Vet Behav Clin Appl Res*, 10, 452–458.

Selhub J, Troen A, Rosenberg IH (2010). B vitamins and the aging brain. *Nutr Rev,* 68, 112–118.

Siwak CT, Gruet P, Woehrle F et al. (2000). Behavioral activating effects of adrafinil in aged canines. *Pharmacol Biochem Behav,* 66, 293–300.

Studzinski CM, Christie LA, Araujo JA et al. (2006). Visuospatial function in the beagle dog: an early marker of cognitive decline in a model of human aging and dementia. *Neurobiol Learn Mem,* 86, 197–204.

Szabó D, Gee NR, Miklósi Á (2016). Natural or pathologic? Discrepancies in the study of behavioral and cognitive signs in aging family dogs. *J Vet Behav Clin Appl Res*, 11, 86– 98.

Tapp PD, Siwak CT, Estrada J, Holowachuk D, Milgram NW (2003). Effects of age on measures of complex working memory span in the beagle dog (Canis familiaris) using two versions of a spatial list learning paradigm. *Learn Mem*, 10, 148–160.

Toepper M. (2017). Dissociating Normal Aging from Alzheimer's Disease: A View from Cognitive Neuroscience. *Journal of Alzheimer's disease: JAD*, *57* (2), 331–352.

Vauzour D, Camprubi-Robles M, Miquel-Kergoat S et al. (2017). Nutrition for the ageing brain: Towards evidence for an optimal diet. *Ageing Res Rev*, 35, 222–240.

Vučinić M, Đorđević M, Teodorović R et al. (2009). Reasons for relinquishment of owned dogs in a municipal shelter in Belgrade. *Acta Vet-Beograd*, 59 (2-3), 309-317.

Wallis L, Range F, Müller C et al. (2014). Lifespan development of attentiveness in domestic dogs: drawing parallels with humans. *Front Psychol*, 5, 71.

Wallis LJ, Virányi Z, Müller CA et al. (2016). Aging effects on discrimination learning, logical reasoning and memory in pet dogs. *Age*, 38 (1), 6.

Yu CH, Song GS, Yhee JY et al. (2011). Histopathological and immunohistochemical comparison of the brain of human patients with Alzheimer's disease and the brain of aged dogs with cognitive dysfunction. *J Comp Pathol*, 145, 45–58.

VAN VETERINARY JOURNAL

Article Copyright Transfer Agreement Form

We, the undersigned researchers, certify that; the article we have sent; is original, wasn't sent to or disapproved of potential publication by any other journal, wasn't initially published, and we bear the responsibility concerning the Scientific content and Ethical values related to the article, and transfer any kind and form of copyright related to the Article to Van Veterinary Journal since it is published in the journal, and accept that we will not make any changes wholly or partly in the article and chose …….. as the authorized researcher.

Title of the article:

Authors Name	Date	Signature
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		

Authorized Researcher

Title, Name-Surname :

Full Address :

e- mail :

Tel, Fax :

Date and Signature :

INSTRUCTION FOR AUTHORS

1. Van Veterinary Journal (ISSN: 2149-3359/e-ISSN: 2149-8644) is a peer-reviewed scientific journal of the Van Yuzuncu Yil University Faculty of Veterinary Medicine. Our journal is published three times a year, in March, July and November. The abbreviated name of the journal is "**Van Vet J**".

2. Van veterinary journal is open access and publishes articles in Turkish or English. Unpublished, original manuscripts such as **research articles, short articles, case reports** and **reviews** are accepted for our journal.

3. In the field of **Veterinary Basic Sciences** (Anatomy, Biochemistry, Physiology, Histology, History of Veterinary Medicine and Deontology), **Veterinary Pre-Clinical Sciences** (Pharmacology and Toxicology, Microbiology, Parasitology, Pathology, Virology), **Veterinary Clinical Sciences** (Internal Medicine, Surgery, Obstetrics and Gynecology, Reproduction and Artificial Insemination), **Animal Science and Animal Nutrition** (Animal Nutrition and Nutrition Diseases, Animal Science, Animal Health Economics and Management, Biostatistics, Genetics), **Food Hygiene and Technology, Public Health, Exotic, Wild** and **Laboratory Animal Science** our journal publishes research articles, short articles, case reports, invited or editor-approved reviews with current and original value.

4. Our journal aims to publish quality articles and to be scanned in higher indexes. For this purpose, priority will be given to quality and **English-written** publications in article acceptance. Review type of publications are accepted only if it is editorial invited, current topic titled and written in English.

5. Our journal is free, and there is no article processing fee (evaluation fee or publishing fee) and no subscription fee for accessing the articles. In addition, the authors are not paid for copy rights.

6. Studies submitted to our journal must be original. All or most of the article should not have been published elsewhere or sent elsewhere for review. The author (s) are responsible for the accuracy of all data and results in the studies and for all legal, criminal and legal liabilities that may arise.

7. All articles submitted to our journal for publication should be reviewed using a professional plagiarism program (**Ithenticate** or **Turnitin**). The report must be uploaded to the system by the author. Articles with a similarity rate above 15% as a result of the review are rejected or a revision may be requested from the author(s) by our editorial board.

8. If the study sent to our journal is; supported by any national or international institution-organization; adapted from a master's / doctoral thesis; presented in any format in a national and / or international scientific (congress, symposium, panel, bineal, workshop, etc.), ınformation should be given under the heading "**Information**" on the title page.

9. Authors must include ORCID numbers, author contributions, conflict of interest and ethics committee information on the article title page.

10. Authors are obliged to accept all responsibilities of their articles submitted to our journal and to transfer the copyright / publication right to Van Veterinary Journal. The authors of the articles submitted to our journal must fill in the "**Article Copyright Transfer document**", sign it with wet signature and upload it to the system. Articles not submitted with a wet signatured Copyright Transfer Agreement document will not be taken into consideration.

11. All experimental, clinical, pharmaceutical or similar studies on animals must be carried out in accordance with the "*Animal Welfare Act, US Public Health Service Policy on the*

Humane Care and Use of Laboratory Animals, National Research Council's Guide for the Care and Use of Laboratory Animals, and Guide for the Care and Use of Agricultural Animals in Research and Teaching" guidelines.

12. In all research articles using animal material, "ethics committee approval certificate" or "the study doesn't require ethics committee approval " document from Animal Experiments Local Ethics Committee must be uploaded to the system at the application stage. For research in other fields, approval documents obtained from the relevant ethics committee must be uploaded to the system during the application. Research articles without an ethics committee approval will not be evaluated. Also, it is obligatory to include information on "**Ethics Committee Approval**" (Board name, date, number, and number) and decleration on "Local Ethics Committee approval and compliance with ethical principles" in the relevant section on the title page.

13. In the case reports, an "informed consent form" should be obtained from the patient with a wet signature. These documents must be uploaded to the system by the author at the time of application.

14. Articles submitted to our journal should be a academic study and in a format that can be reviewed by reviewers. For this reason, it is obligatory for the authors to send the "***Article Control Form***" together with the work they will submit.

15. For the articles on "Notifiable Diseases" specified by the Republic of Turkey Ministry of Agriculture and Forestry, a permit letter from the Ministry of Agriculture and Forestry must be submitted to our editor.

16. Submitted articles are pre-evaluated by our editor. The editor has the right to request a correction or reject the pre-evaluated article before it is sent to the reviewers. Manuscripts deemed appropriate by the editorial office are sent to double-blind reviewing. The work is sent to at least 2 reviewers for evaluation. When necessary, a new reviewer is appointed and a decision is made by taking the opinion of the editor or 3rd reviewer.

17. Article submission to our journal is made only through DergiPark. For this reason, all files and appendices should be uploaded to our journal through the DergiPark system. Article submissions via e-mail are not accepted.

18. Abbreviations to be used in submitted studies should be scientifically accepted abbreviations. Authors should make the explanation of their abbreviation at the first mention in the text. Latin genus and species names should be typed in italics (Example: Escherichia coli). All measurement units must be written according to SI (Systeme Internationale). A period (.) should be used in numerical and statistical data used in the article (Example: 0.36; 94.5%; P <0.05 etc.).

19. Author (s) and referees should pay attention to the confidentiality of personal and institutional information that will reveal the authors and or referees in the content of the main text file and the Word file properties. Responsibility on this subject belongs to the authors and reviewers.

PREPARATION OF THE ARTICLE

1. Articles should be written using Microsoft Word (Office 2016 and higher); A4 size, Times New Roman font, 12 point size, 1.5 line spacing; continuous line and page number; justified aligned and 2.5 cm margins from each side. Paragraphs should be started with a 1.25 cm indent.

2. Articles prepared as stated above, without Tables and Figures, should not exceed 15 pages in research articles, 7 pages in short articles and case reports, and 20 pages in reviews.

3. All publications submitted to our journal should consist of the following sections:

- **Research articles and short articles written in Turkish must consist of these sections:** Manuscript Title, Short Title, Turkish Abstract (ÖZ), Anahtar Kelimeler, English Title, Short Title, English ABSTRACT, Keywords,GİRİŞ, MATERYAL VE METOT, BULGULAR, TARTIŞMA VE SONUÇ, KAYNAKLAR.
- **Case reports written in Turkish must consist of these sections:** Manuscript Title, Short Title, Turkish Abstract (ÖZ), Turkish Keywords, English Title, Short Title, English ABSTRACT, Keywords, GİRİŞ, VAKANIN TAKDİMİ, TARTIŞMA VE SONUÇ, KAYNAKLAR
- **Review articles:** Subtitles are determined by the authors up to the **REFERENCES** section. In our journal, only reviews written in English are taken into consideration and published.
- **Research articles and short articles written in English must consist of these sections:** English Manuscript Title, Short Title, English ABSTRACT, Keywords, Turkish Title, Turkish Abstract (ÖZ), Keywords, INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION AND CONCLUSION, REFERENCES.
- **Case reports written in English must consist of these sections:** English Manuscript Title, Short Title, ABSTRACT, Keywords, Turkish Title, Short Title, Turkish Abstract (Öz), Keywords, INTRODUCTION, CASE DESCRIPTION, DISCUSSION AND CONCLUTION, REFERENCES

4. Title Page

- The title page will be downloaded from our magazine website. (click here for the sample title page).,
- Authors should make the necessary arrangements on the downloaded document. On this page, the article type (research article, short article, case report, review), the title of the study in Turkish and English, the names and surnames of the author (s), institution information (will be written only in English), ORCID numbers of all authors should be written.
- In the corresponding author section, the name and surname of the responsible author, institution information, telephone number and e-mail address should be written.
- "**Ethics Committee Information, Information, Acknowledgment, Conflict of Interest and Author Contributions**" sections located on the title page should be filled in in accordance with the examples given.
- The prepared Title Page should be uploaded to the system as a separate file.

5. Manuscript Page;

- Click for a sample "Manuscript Page" file.

- Care should be taken not to include any information about the authors and their institutions in the Manuscript Page file. This is necessary to ensure that the articles are evaluated objectively by the reviewers (blind review system).
- The Manuscript Page file sample should consist of the following sections:

6. Manuscript Sections:

ÖZ (Turkish Abstract)

- The "ÖZ" section should not be less than 150 words and more than 250 words. ABSTRACT should be written in a way to cover aim of the study, material and method, results and conclusion sections respectively.
- **Keywords:** minimum 3, maximum of 6 key words must be selected from Turkey Science Terms web page.
- Keywords should be listed alphabetically in Turkish, words should be separated with commas (,) and at the end a period (.) should be put.

ABSTRACT

- The ABSTRACT section should not be less than 150 words and more than 250 words. It should be written in English in the same way as Turkish ÖZ.
- Keywords: Should be the same as Turkish keywords and selected from "The Medical Subject Headings" (MeSH). Words should be sorted alphabetically in English, words should be separated by commas (,) and at the end a period (.) should be put.

INTRODUCTION

- In this section, after brief literature information about the study, the aim of the study should be given in the last paragraph of the introduction.
- The citations used in the study should be shown in the following ways.
    - If the citation is by a single author; should be written in the form of the author's surname and date. Example: (Özkan 2020)
    - When citing to a source with two authors, the surnames of both authors should be written with the "and" separator. Example: (Özkan and Kayıkcı 2019)
    - If the source has three or more authors, the surname of the first author should be written, the other authors should be indicated with the "et al." abbreviation. Example: (Özkan et al.2018).
    - If several sources are cited, these sources should be separated from each other with a semicolon ":" sign and these sources should be arranged chronologicaly from oldest to newest. Example: (Özkan et al.2000; Çakmak 2010; Karasu and Yaman 2014; Kuşcu 2020).
    - Publications with the same date should be listed in alphabetical order. Example: (Aksu 2010; Bulut et al.2010; Cenger and Kuzu 2010).
    - If it is citing to several sources of the same author belong the same year in the text, it should be stated as (Özkan 2008a; Özkan 2008b; Özkan 2008c) based on the order in the source section.

MATERIAL AND METHODS

In this section, material methods and statistical analysis methods should be included.

RESULTS

- Tables in the article should be named as "Table". All visual elements (graphic, photograph, figure, diagram, etc.) other than the table should be named as "Figure".
- Comma (,) should not be used between the numerical values in the table, a period (.) should be used in all values.
- The photographs and figures presented in the study must be in TIFF or JPEG format and at least 300 dpi resolution and must be uploaded as a separate file during application to the system. Figures should not be included in the main text file.
- In Turkish articles, the Turkish and English names of the tables and figures, and the necessary explanations must be written separately in both languages.
- For English articles, only the English names and necessary explanations of the tables and figures should be written.
- The table title should be given at the top of the table, and the abbreviations and necessary explanations used in the table should be given below the table. Figure title and explanations should be written in the lower part of the figure.
- Tables and figures in the main text file should be numbered in order of usage (Example: Table 1, Figure 1).

DISCUSSION AND CONCLUTION

- In the last paragraph of this section, the conclusions reached in the study should be given as a separate paragraph.

REFERENCES

- The number of references used should not be less than 10 for case reports, less than 20 for research articles and more than 45 for all article types.
- It should be noted that majority of the sources used for all articles belong to the last 5 years.
- Each source used in the article should be listed at the end of the article under the heading REFERENCES, in alphabetical order.
- The short name of the reference journal should be used in italics (The short name of the journals can be found from "Medline, CAplus Core Journal Coverage list, Clarivate or Pubmed Journals Database").
- The author (s) are responsible for the accuracy of all references used in the study.

According to the type of resource used, the references should be written as shown in the examples below.

1. If the source is a article;

- Source with 1 - 5 authors;
- **Keleş İ, Değer S, Altuğ N, Karaca M, Akdemir C (2001).** Tick-borne diseases in cattle: Clinical and haematological findings, diagnosis, treatment, seasonal distribution, breed, sex and age factors and the transmitters of the diseases. *YYU Vet Fak Derg*, 12 (1-2), 26-32.
- **Özkan C, Kaya A, Akgül Y (2012).** Normal values of hematological and some biochemical parameters in serum and urine of New Zealand white rabbits. *World Rabbit Sci*, 20 (4), 253-259
- Source with more than 6 authors;
- **Özkan C, Akgül Y, Altuğ N ve ark. (2013).** Doğal theileriozisli sığırlarda tedavi öncesi ve sonrası bazı klinik, hematolojik ve biyokimyasal parametreler. *Erciyes Üniv Vet Fak Derg*, 10 (2), 93-100.
- **Fleming DW, Cochi SL, MacDonald KL et al. (1985).** Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *The New England J Med*, 312 (7), 404-407.

2. If the source is a book;

- **Sağlam M, Aştı RN, Özer A (2001).** Genel Histoloji. 2. Baskı. Yorum Matbaacılık, Ankara.
- **Marrow DA (1986).** Current Therapy in Theriogenology. I. Edition. W.B. Saunders Company, Philadelphia.

3. If the source is a chapter in a book;

- **Şimşek H, Öksüzoğlu G (1999).** Akut Pankreatit. Kadayıfçı A, Karaaslan Y, Köroğlu E (Ed). Acil Durumlarda Tanı ve Tedavi (s. 116-126). Hekimler Yayın Birliği, Ankara.
- **Bahk J, Marth EH (1990).** Listeriosis and Listeria monocytogenes. Cliver DO (Ed). Foodborne Diseases (pp. 248-256). Academic Press, San Diego.

4. If the source is a thesis;

- **Mis L (2009).** Çinko Bakımından Yetersiz Diyet ve Çinko İlavesinin Deneysel Böbrek Yetmezliği Oluşturulan Ratlarda Lipid Peroksidasyonu ve Eritropoetin Seviyelerine Etkisi. Doktora tezi, Van Yüzüncü Yıl Üniversitesi, Sağlık Bilimleri Enstitüsü, Van, Türkiye.
- **Kay JG (2007).** Intracellular Cytokine Trafficking and Phagocytosis in Macrophages. PhD thesis, University of Queensland, Institute for Molecular Bioscience, St Lucia, Australia.

5. If the source is proceeding;

- **Özkan C, Altuğ N, Yüksek N, Kaya A, Akgül Y (2011).** Neonatal ishale bağlı hiperkalemi gelişen buzağılarda elektrokardiografik bulgular, serum nitrik oksit, kardiak troponin ve bazı enzim düzeylerinin değerlendirilmesi. IX. Ulusal Veteriner İç Hastalıkları Kongresi (Uluslararası Katılımlı), Antalya, Türkiye.

Oğuz B (2018). Molecular analysis of Echinococcus granulosus through amplification of

COX1 gene fragments from sheep in Van province. In: Proceeding of the 1st International GAP Agriculture and Livestock Congress, Sanliurfa, Turkey.

6. If the source is a software;

- **SAS (1990).** SAS user's guide: Statistics, 4th ed., Sas Institute, Cary.

7. If the source is a electronic material;

- Note: Web-based resources should not be used as possible as. **Atherton J (2010).** Behaviour modification. Erişim Tarihi: 10 Nisan 2010. Erişim Adresi: http://www.learningandteaching.info /learning/behaviour_mod.htm.
- **WHO (2018).** Flouride and arsenic in drinking water. Erişim Tarihi: 26 Nisan 2018. Erişim Adresi: http://www.who.int/ceh/publications/en/08fluor.pdf, 2016.

Click to download our journal's Writing Rules in PDF format.

MANUSCRİPT CONTROL FORM

Manuscript Title:			
Corresponding Author: **Date:** **Signature:**			
No	**Manuscript Control List**	**Yes**	**No**
1.	This study was written and prepared in accordance with the writing rules of the "**Van Veterinary Journal**".		
2.	The study was examined using one of the plagiarism software (Ithendicate, Turnitin), the obtained report was uploaded to the system and the similarity rate is below 15%.		
3.	Are all the required files completely uploaded to the DergiPark system?		
4.	Has the "**Copyright Transfer Agreement**" downloaded from the system filled in, signed by all authors, and uploaded to the system?		
5.	Has the **ethics committee approval document** or **the document indicating that there is a study not requiring an ethical committee** for research articles or **enlightened patient owner consent form** received from the patient for case reports, uploaded to the system?		
6.	The sample "Title Page" has been downloaded from our magazine website. Have the article type, the title of the study in Turkish and English, the name and surname of the author (s), the institution information in English, the ORCID numbers of the authors written on this page?		
7.	Have the author's name-surname, institution information, phone number and e-mail address written on the title page, in the corresponding author section?		
8.	Have the ethics committee information, information, acknowledgment, conflict of interest, and authors' contributions sections located on title page been filled in in accordance with the examples given?		
9.	Has the information on "Ethics Committee Approval" (Board name, date, number, and number) and the decleration on "Local Ethics Committee approval and compliance with ethical principles" included in the relevant section on the title page?		
10.	Is the prepared "Title Page" uploaded to the system as a separate file?		
11.	Has the "Manuscript File" sample been downloaded from the website and the article prepared in accordance with the sample from this document?		
12.	According to the spelling rules, was the article written on A4 paper, in Times New Roman font, 12 pt., justified, with 2.5 cm margins from each side? Are the paragraphs started from 1.25 cm fist indent?		

13.	Are line numbers (from first page) and page numbers (at the bottom and centered) included in "Main Text File"?		
14.	Was the page limitation considered in the prepared manuscript? (15 pages for research articles, 7 pages for short articles and case reports, and 20 pages for review articles without tables and figures).		
15.	Does the study include related section titles according to the article type (research article, short article, case report, review) and the language (Turkish or Englis) in which it was written?		
16.	Has it been paid attention that there is no personal and institutional information to identify the authors in the content of the "**Main Text File**"?		
17.	If the article type is review, is the writing language of the review English?		
18.	Do the ABSTRACT and ABSTRACT parts of the study consist of minimum 150 and maximum 300 words?		
19.	Have a minimum of 3 and a maximum of 6 keywords been written for the keywords? Did the keywords chosen from Türkiye Bilim Terimleri and MeSH). Are the words in Turkish and English alphabetically ordered, separated by commas and a full stop at the end?		
20.	Were the citations used in the text made according to the journal writing rules?		
21.	If statistical analysis is present, is it given under the heading "Statistical Analysis" at the end of the "MATERIAL AND METHOD" section of the article?		
22.	Are the tables in the article called as "Table", and all visual elements (graphic, photograph, figure, diagram, etc.) other than the table called as "Figure"?		
23.	Is a period (.) used among all numerical values in the table?		
24.	Is the title of the table given above it, and the abbreviations and the necessary explanations under the table? Were the figure titles and descriptions written under the figure?		
25.	Have Turkish and English names and required explanations of tables and figures in Turkish articles written in both languages, and in English articles, names of tables and figures, and necessary explanations have been written only in English?		
26.	Are tables and figures numbered in order of use in the main text file?		
27.	The photographs and figures presented in the study were uploaded to the system in TIFF or JPEG format and at a resolution of at least 300 dpi.		

28.	The figures were not included in the main text file. The figures were uploaded to the system as separate files.		
29.	Abbreviations were written after the normal word in the first usage, after the first usage of the abbreviation, it was used in the rest of the manuscript alone. Latin genus and species names are written in italics. Point was used in numerical and statistical data used in the article.		
30.	Have International Standard Units (SI) been used in the units and measures stated in the article?		
31.	Were the conclusions reached in the study given in a separate paragraph in the last paragraph of the DISCUSSION AND CONCLUSION section?		
32.	Each source used in the article was listed alphabetically under the heading REFERENCES at the end of the article and was written according to the spelling rules and examples given.		
33.	The number of references should not be less than 10 for case reports, less than 20 for research articles and more than 45 for all article types.		
34.	It was paid attention that at least 30% of the resources used in the article belong to the last 5 years.		
35.	Are the tables given after the REFERENCES section in a separate page for each table?		