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## CONTENTS

# Protective effect of L-carnitine against ethanol-induced gastric damage: Investigation of possible mechanisms of action

Research Article


## ABSTRACT

The underlying mechanisms of L-carnitine's (L-CAR) protective effect against ethanol (EtOH)-induced gastric mucosal damage were investigated in this study. The rats were randomly divided into four groups: control (CON), EtOH, EtOH + L-CAR50, and EtOH + LCAR100. Control group was given saline (5 mL/kg) twice at 1-hour interval. EtOH group was given 5 mL/kg saline 1-hour before absolute EtOH administration (5 mL/kg). EtOH + LCAR50 group received 50 mg/kg LCAR 1-hour before absolute EtOH administration (5 mL/kg). EtOH + LCAR100 group received 100 mg/kg LCAR 1-hour before absolute EtOH administration (5 mL/kg). All the rats were euthanized 1 hour after the administration of EtOH. The gastric lesion area was grossly examined, and gastric lesions were histopathologically evaluated. Real-time PCR was used to examine the expression of cyclooxygenase 1 and 2 (COX-1 and COX-2), inducible- and endothelial- nitric oxide synthase (iNOS and eNOS), tumor necrosis factor alpha (TNF-α), heat shock protein 70 (HSP70), and trefoil factor 2 (TFF2) mRNA in the gastric mucosa. Histopathological examination revealed that L-CAR treatment reduced the severity and extent of gastric lesions caused by EtOH administration, such as shedding of the superficial epithelium, glandular gland necrosis, intralesional hemorrhage, submucosal edema, and neutrophil infiltration. L-CAR administration was found to significantly reduce the mRNA levels of COX-2, iNOS, eNOS, and TNF-α in the gastric mucosa compared to EtOH administration alone. It was determined that L-CAR administration further increased the gastric mucosal HSP70 mRNA expression than EtOH administration alone. L-CAR treatment increased TFF2 expression which was decreased after EtOH administration. Finally, L-CAR administration was thought to protect against EtOH-induced gastric mucosal damage by regulating the expression of gastric mucosal COX and NOS systems, reducing the inflammatory cytokine levels, inducing a cellular stress response, and stimulating the expression of factors associated with mucus secretion and gastric epithelium restitution.


**Keywords:** Cyclooxygenase, ethanol, heat shock protein, L-carnitine, nitric oxide synthetase, trefoil peptides


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## INTRODUCTION

A mucus-bicarbonate-phospholipid barrier covering the surface of the gastric mucosa, epithelium, and gastric microcirculation, as well as gastroprotective factors (prostaglandins, nitric oxide, heat shock proteins, trefoil factor family peptides) secreted by epithelium and endothelial cells, maintain gastric mucosal integrity (Aihara et al., 2017; MacNaughton et al., 1989a; Martin and Wallace, 2006; Tsukimi and Okabe, 2001). Necrotizing agents, such as ethyl alcohol (EtOH), cause focal hemorrhagic lesions by destroying the gastric mucosal barrier via unknown mechanisms (Chen et al., 2021). Microscopically, EtOH-induced lesions show necrosis and exfoliation of the glandular glands and cells covering the gastric luminal surface and pits in addition to interstitial hemorrhage, capillary hyperemia, and thrombosis (Guth et al., 1984; Lacy and Ito, 1982).

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EtOH-induced gastric mucosal lesions are most likely associated with the microvascular lesions. Vascular changes are thought to cause gastric mucosal damage by decreasing regional blood flow (Oates and Hakkinen, 1988; Szabo et al., 1985). Gastric epithelium initiates a cellular stress pathway in response to EtOH-induced gastric damage. To reduce EtOH-related gastric damage in rodents, cyclooxygenase (COX) and nitric oxide synthetase (NOS) systems are activated, and heat shock protein 70 (HSP70) expression is increased (Bakalarz et al., 2021; Magierowska et al., 2015; Saika et al., 2000; Zhao et al., 2009).

L-carnitine (L-CAR), synthesized endogenously from lysine and methionine precursors, is essential for the transfer of long-chain fatty acids to mitochondria (Kart et al., 2006). L-CAR has been shown in animal studies to protect against gastric mucosal damage caused by various irritants. Studies indicate that L-CAR administration protects against EtOH-induced damage by decreasing gastric mucosal lipid peroxidation, and by increasing the glutathione, prostaglandin E2, and nitric oxide levels (Arafa and Sayed-Ahmed, 2003; Dokmeci et al., 2005; Erkin et al., 2006; Izgüt-Uysal et al., 2007; Madi and Al-Barr, 2014). However, only a few studies have investigated the underlying mechanisms to explain the gastroprotective effect of L-CAR, particularly those related to the gastric mucosal barrier (Izgüt-Uysal et al., 2007; Madi and Al-Barr, 2014). In this study, we investigated the underlying mechanisms of gastroprotective effect of L-CAR against EtOH-induced gastric mucosal damage in rats. To this end, COX-1, COX-2, inducible- and endothelial- NOS (iNOS and eNOS), HSP70, pro-inflammatory cytokine TNF-α, and TFF2 levels were evaluated to understand the potential role of L-CAR in gastroprotection.

## MATERIALS AND METHODS

### *Animals*

A total of thirty-two 8-weeks-old male Sprague-Dawley rats weighing 200–250 g obtained from Adnan Menderes University Veterinary Faculty Experimental Animals Production and Research Center were used in this study. Throughout the experiment, the rats were housed in transparent polycarbonate cages at 22°C ± 2°C, 50%–70% humidity, and 12/12 hours light/dark conditions, and were fed ad libitum with standard rat feed. All the experimental procedures were approved by the local ethics committee of Aydın Adnan Menderes University (Approval no. 64583101/2020/009).

After two-weeks, the rats were randomly assigned to one of four groups: control (CON), EtOH, EtOH + LCAR50, and EtOH + LCAR100. Before the chemical application, all rats were subjected to fasting for 24 hours. The detailed applications for each group are given below:

1. Control group – rats were given saline (5 mL/kg) twice at 1-hour interval.
2. EtOH group – 5 mL/kg saline 1-hour before absolute EtOH administration (5 mL/kg).
3. EtOH + LCAR50 – 50 mg/kg LCAR 1-hour before absolute EtOH administration (5 mL/kg).
4. EtOH + LCAR100 – 100 mg/kg LCAR 1-hour before absolute EtOH administration (5 mL/kg).

The orogastric tube was used to administer all chemicals intragastrically. The L-CAR doses to be administered were determined based on studies that found protective effects against EtOH-induced gastric mucosal damage (Madi and Al-Barr, 2014). We obtained absolute EtOH (CAS no.: 64-17-5) from Merck KgaA (Darmstadt, Germany) and L-CAR (CAS no.: 541-15-1) from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical purity. All the rats were euthanized by cervical dislocation under general anesthesia induced by intraperitoneal injections of 50 mg/kg ketamine (Ketasol: Richter Pharma AG, Wels, Austria) and 5 mg/kg xylazine (Ksilazin: IPM Pharmaceuticals, İstanbul, Türkiye) 1-hour after the administration of absolute EtOH. Following systemic necropsy, all rats' stomachs were removed. An incision along the greater curvature of the stomach was made, and the gastric contents

were cleaned with physiological saline. After photographing the stomachs fixed on a flat surface with bulk needles, samples were taken for histopathological examination and gene expression analysis. The area of the gastric lesion ($mm^2$) was calculated by analyzing photographs with the Image J program (Wayne Rasband National Institutes of Health, USA).

### *Histopathological examination*

Gastric tissue samples were fixed in 10% neutral-buffered formalin overnight. After dehydrating the gastric samples in increasing concentrations of ethanol, they were equilibrated in xylene and embedded in paraffin. Then, 4–5-µm-thick serial sections were cut with a microtome, stained with hematoxylin-eosin, and examined with a light microscope (BX51, Olympus) and a digital camera (SC180, Olympus). Gastric lesions were evaluated using a modified scoring system recommended by Magierowska et al. (2018). In terms of severity and extent, gastric lesions were graded as 0= no lesion, 1= mild, 2 = moderate, and 3= severe.

### *Real-time PCR*

Quantitative real-time PCR was used to determine the expression of *COX-1*, *COX-2*, *iNOS*, *eNOS*, *TNF-α*, *HSP70*, and *TFF2* mRNA in the gastric mucosa. To summarize, total RNA was extracted using a Riboex GeneAll Biotechnology kit (South Korea) based on guanidium isothiocyanate / phenol chloroform method. Spectrophotometry was used to determine the concentration and purity of RNA. Then, using a cDNA Synthesis Kit with RNase Inh (High Capacity) from A.B.T.™ Laboratory Industry (Türkiye), 2-µg total RNA was reverse-transcribed into complementary DNA (cDNA). Table 1 shows the primer sequences used to amplify the specific mRNAs. cDNAs were amplified in the CFX Connect Real-Time PCR Detection System (Biorad, USA) using the A.B.T.™ 2X qPCR SYBR-Green MasterMix (with ROX) kit (A.B.T.™ Laboratory Industry, Türkiye) under the following cycling conditions: 95°C hold for 3 min, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at corresponding temperatures (see Table 1) for 20 seconds, and extension at 60°C for 20 seconds were performed. Melt curves were used to test the specificity of amplicons. The mRNA expression of the genes was normalized against *β-actin*, and fold changes were calculated using the ΔΔCt method against the intact gastric mucosa as a reference (Livak and Schmittgen, 2001).

**Table 1.** Sequences of primers used to amplify specific mRNAs by quantitative real-time polymerase chain reaction

| Target gene | Primer sequences (5'-3') | Annealing temperature (°C) | Accession number |
|---|---|---|---|
| ***COX-1*** | F: CTGCTCACAGATGCTGGG<br>R: ATGAGTACTTCTCGGATGAAGGT | 60 | NM_017043.4 |
| ***COX-2*** | F: TGTCAAAACCGTGGTGAATG<br>R: CCGAAGGAAGGGAATGTTGT | 60 | NM_017232.3 |
| ***eNOS*** | F: AGAACTCTTCACTCTGCCCC<br>R: GGTCCCTCATGCCAATCTCT | 60 | NM_021838.2 |
| ***iNOS*** | F: ACCACCCTCCTTGTTCAACT<br>R: AGCCTCTTGTCTTTGACCCA | 58 | NM_012611.3 |
| ***TNF-α*** | F: TTCATCCGTTCTCTACCCA<br>R: TTCAGCGTCTCGTGTGTTTC | 58 | NM_012675.3 |
| ***TFF2*** | F: TCTCTTGGTAGTGGTCCTTGTCT<br>R: CAGGTTGGAAAAGCAGCA | 58 | NM_053844.2 |
| ***HSP70*** | F: GACGACGGCATCTTCAAG<br>R: GTTCTGGCTGATGTCCTTC | 60 | NM_031971.2 |
| ***β-Actin*** | F: ATGGTGGGTATGGGTCAGAA<br>R: GGTCATCTTTTCACGGTTGG | 60 | NM_031144.3 |

*COX-1*: Cyclooxygenase 1; *COX-2*: Cyclooxygenase 2; *eNOS*: Endothelial nitric oxide synthase; *iNOS*: Inducible nitric oxide synthase; *TNF-α*: Tumor Necrosis Factor-α; *TFF2*: Trefoil Factor 2; *HSP70*: Heat Shock Protein 70.

### *Statistical analysis*

The Statistical Package for the Social Sciences (SPSS version 22.0; IBM Corp., NY, USA) was used to apply one-way analysis of variance on the data. Normality of the data was checked, and non-normalized traits were normalized using the logarithmic or square root transformation method. Based on the homogeneity of variances, Tukey's, or Tamhane tests were chosen as post-hoc tests. Assumptions of 95% probability ($P < 0.05$) were made to separate significantly different means. The results are shown as mean ± standard errors.

## RESULTS

### *Macroscopic findings*

Figure 1 depicts the macroscopic appearance of gastric lesions and the mean gastric lesion area of rats given L-CAR at doses of 50 mg/kg or 100 mg/kg in combination with absolute EtOH, absolute EtOH, and saline. There were no macroscopic lesions in the control group. Absolute EtOH administration was found to cause linear hemorrhagic lesions in rats' stomachs. The lesions were more visible in the gastric folds. Submucosa was quite thickened by gelatinous edema.

Figure 1 shows that 50 mg/kg L-CAR administration significantly reduced the gastric lesion area caused by EtOH administration. However, it was determined that L-CAR administration at a dose of 100 mg/kg had no effect on the gastric lesion size induced by EtOH.

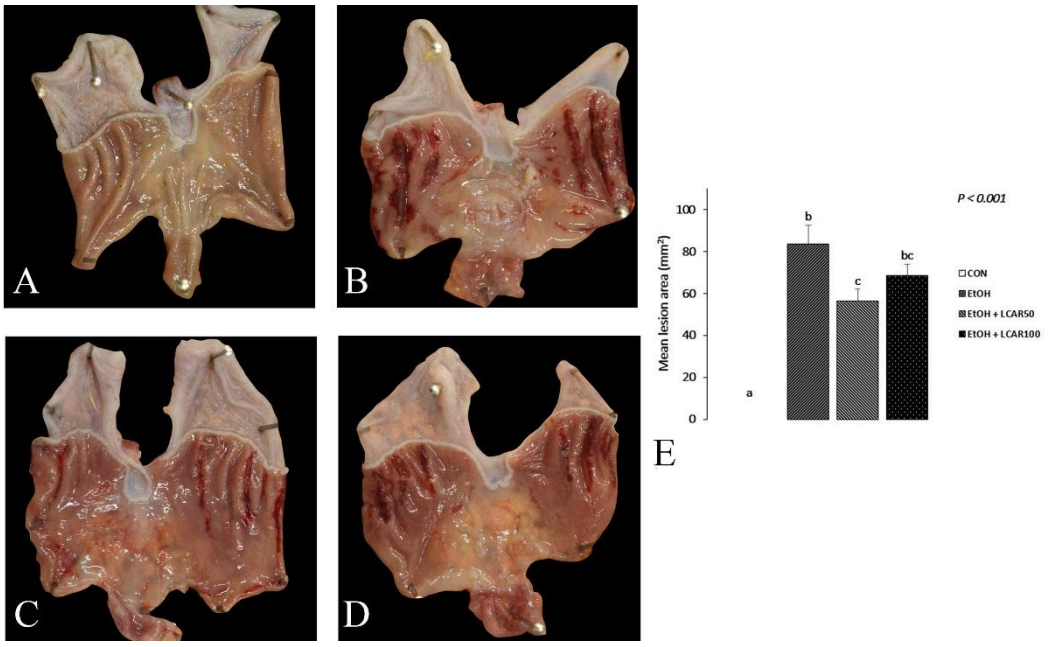


**Figure 1.** Gastric lesions in situ (A-D) and mean mucosal lesion area (E) in rats given saline (A), absolute ethanol (B), or L-CAR at 50 mg/kg (C) or 100 mg/kg (D) in combination with absolute EtOH. a, b, c Bars with different superscripts differed significantly

### *Histopathological findings*

Figure 2 depicts the histopathological findings in rats given saline, absolute EtOH, or absolute EtOH in combination with L-CAR at doses of 50 mg/kg or 100 mg/kg. The control group showed no signs of pathological changes. Absolute EtOH administration resulted in wedge-shaped gastric erosions with multifocal distribution in rat gastric mucosa, characterized by exfoliation of the superficial epithelium and necrosis of the glandular glands. The lesions in the stomach extended deep into the mucosa. In the lesion areas, there was extensive hemorrhage with hyalinized vessel walls. Mild to moderate neutrophil infiltrations were seen around the vessels at the base of gastric mucosa. The submucosa was significantly enlarged with abundant edema accompanied by neutrophils. As shown in Table 2, gastric mucosal

damage was more superficial in rats given L-CAR before EtOH, and extent of gastric lesion was lower compared to rats administered EtOH. Furthermore, L-CAR administration reduced intralesional hemorrhage, submucosal edema, and neutrophil infiltrations. There was no statistically significant difference between L-CAR doses in terms of severity or lesion extent.

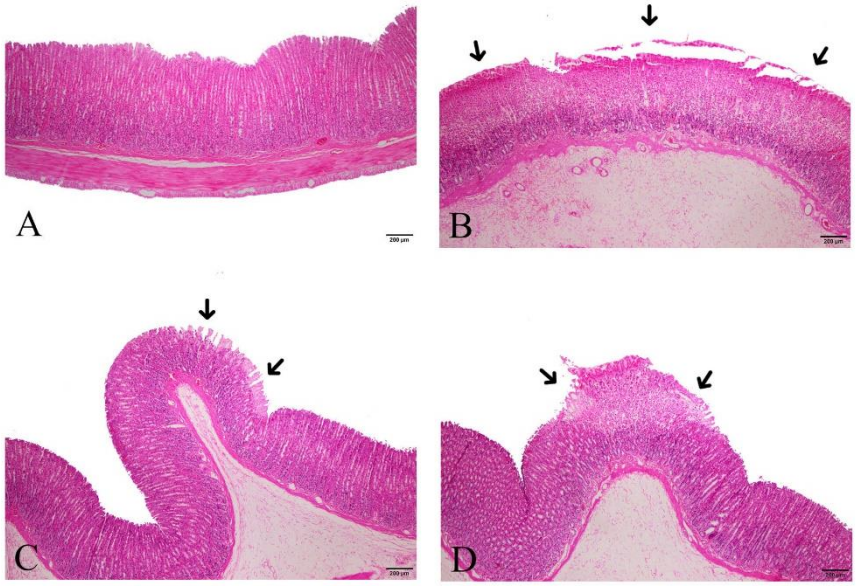


**Figure 2.** Microscopic images of gastric lesions in rats treated with saline (A), absolute ethanol (B) or L-CAR at a dose of 50 mg/kg (C) or 100 mg/kg (D) in combination with absolute EtOH. Arrows show the areas of gastric mucosal lesions

**Table 2.** Effects of L-carnitine treatment on gastric mucosal lesions induced by ethanol

| Group | Necrosis | Hemorrhage | Neutrophil infiltration | Submucosal edema |
|---|---|---|---|---|
| **CON** | $0.0 \pm 0.0^{a}$ | $0.0 \pm 0.0^{a}$ | $0.0 \pm 0.0^{a}$ | $0.0 \pm 0.0^{a}$ |
| **EtOH** | $2.63 \pm 0.18^{b}$ | $2.38 \pm 0.18^{b}$ | $1.63 \pm 0.18^{b}$ | $3.00 \pm 0.0^{b}$ |
| **EtOH + L-CAR50** | $1.50 \pm 0.18^{c}$ | $1.25 \pm 0.25^{c}$ | $0.75 \pm 0.16^{c}$ | $2.25 \pm 0.25^{c}$ |
| **EtOH + L-CAR100** | $1.88 \pm 0.12^{c}$ | $1.75 \pm 0.31^{bc}$ | $1.25 \pm 0.25^{bc}$ | $2.75 \pm 0.16^{bc}$ |
| **P value** | <0.001 | <0.001 | <0.001 | <0.001 |

CON: Control; EtOH: Ethanol; L-CAR: L-carnitine. $^{a-c}$ Means bearing different superscript within the same column differ significantly

### *Real-time PCR*

Figure 3 shows that EtOH administration increased the gastric mucosal *COX-2*, *iNOS*, *eNOS*, *TNF-α*, and *HSP70* mRNA expression while decreasing *TFF-2* levels in comparison with control group. The administration of 50 mg/kg or 100 mg/kg of L-CAR with EtOH significantly reduced gastric mucosal *iNOS* and *TNF-α* mRNA levels compared to EtOH group. There was no statistically significant difference in *iNOS* and *TNF-α* mRNA levels between L-CAR doses. In addition, 50 mg/kg L-CAR treatment significantly reduced gastric mucosal *COX-2* and *eNOS* mRNA levels compared to EtOH administration only. *HSP70* mRNA expression in the gastric mucosa was found to increase even after L-CAR administration at a dose of 50 mg/kg. L-CAR at 50 mg/kg was found to significantly increase the expression of gastric mucosal *TFF-2* mRNA compared to EtOH-treated group. Neither EtOH nor L-CAR had any effect on gastric mucosal *COX-1* expression.

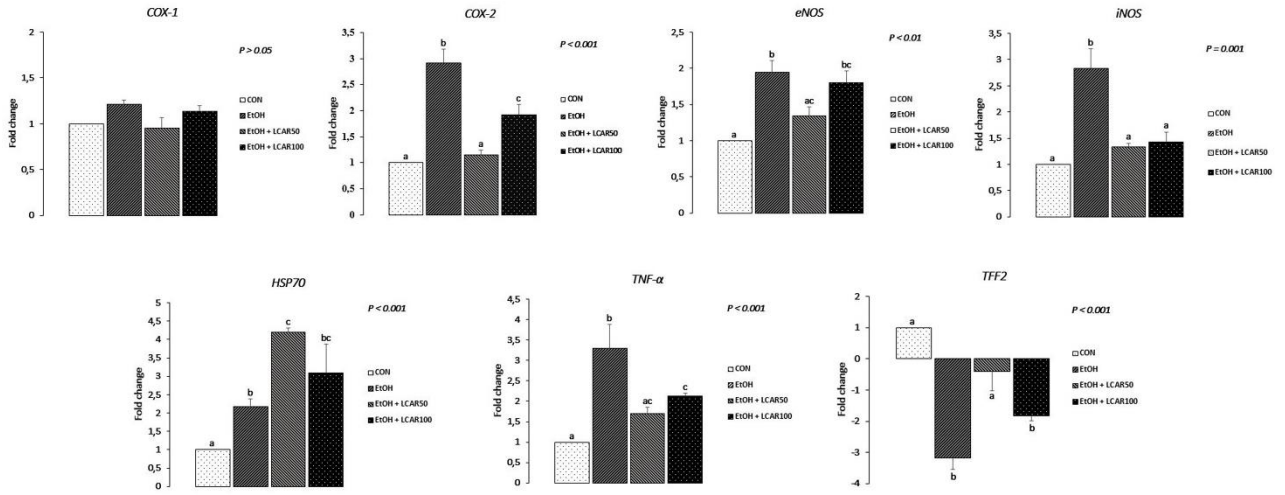


**Figure 3:** The effect of 50 mg/kg or 100 mg/kg L-CAR on gastric mucosal *COX-1*, *COX-2*, *iNOS*, *eNOS*, *TNF-α*, *HSP70*, and *TFF2* mRNA expression prior to EtOH administration. The data show fold change compared to control rats. [a-c] Bars with different superscripts differed significantly. *COX-1*: Cyclooxygenase 1; *COX-2*: Cyclooxygenase 2; *eNOS*: Endothelial nitric oxide synthase; *iNOS*: Inducible nitric oxide synthase; *HSP70*: Heat Shock Protein 70; *TNF-α*: Tumor Necrosis Factor-α; *TFF2*: Trefoil Factor 2.

## DISCUSSION

Consistent with previous findings (Dokmeci et al., 2005; Guth et al., 1984; Lacy and Ito, 1982), we determined that gastric hemorrhagic lesions formed quickly in rats given absolute EtOH intragastrically. Microscopic examination of these lesions revealed shedding of surface epithelium, necrosis and exfoliation of the glandular glands, intralesional hemorrhage, submucosal edema, vascular hyperemia, and thrombosis. Endothelial damage increased vascular permeability, and endothelial activation all play important roles in the pathophysiology of EtOH-induced gastric lesions. Gastric mucosal lesions caused by EtOH are most likely the result of gastric mucosal hypoxia caused by vascular lesions (Oates and Hakkinen, 1988; Szabo et al., 1985). Consistent with previous findings (Arafa and Sayed-Ahmed, 2003; Dokmeci et al., 2005; Madi and Al-Barr, 2014), we determined that administering L-CAR prior to EtOH reduced the severity and extent of EtOH-induced gastric mucosal lesions. Given that EtOH-induced gastric mucosal lesions are caused by microvascular lesions, it is possible that L-CAR has a gastroprotective effect by reducing endothelial cell damage.

Prostaglandins (PG), synthesized from arachidonic acid by the action of cyclooxygenases, strengthen the gastric mucosal barrier and exert a protective effect against acute gastric mucosal damage (Robert et al., 1979). Constitutional expression of COX-1 related PG is associated with gastric physiological functions. Unlike COX-1, COX-2-mediated PG induced in response to gastric mucosal damage protects against luminal irritants by increasing the regional blood flow, stimulating mucus and bicarbonate secretion, and by limiting the inflammatory response (Martin and Wallace, 2006). Our findings showed that EtOH administration increased *COX-2* expression while having no effect on gastric mucosal *COX-1* expression. Despite the fact that EtOH administration increases COX-2 expression, which helps to strengthen the gastric mucosal barrier, gastric mucosal PGE2 levels have been reported to decrease, contrary to expectations (Bakalarz et al., 2021; Magierowska et al., 2015; Zhao et al., 2009). Although COX-2 expression is induced, the decrease in gastric mucosal PGE2 level appears contradictory. This was explained by the fact that EtOH administration reduced PGE2 synthesis by inhibiting COX-2 enzyme activity (Zhao et al., 2009). The increase in *COX-2* mRNA expression after EtOH administration

is most likely due to decreased PGE-2 synthesis.

Experimental studies have shown that L-CAR administration protects the gastric mucosa from damage caused by various irritants (Arafa and Sayed-Ahmed, 2003; Dokmeci et al., 2005; Erkin et al., 2006; Izgüt-Uysal et al., 2007; Madi and Al-Barr, 2014). It has been established that intragastric L-CAR administration reduces EtOH-induced gastric mucosal damage and has a gastroprotective effect by reducing gastric lipid peroxidation with its antioxidant properties (Arafa and Sayed-Ahmed, 2003; Dokmeci et al., 2005). Madi and Al-Barr (2014) determined that administering 50 mg/kg of L-CAR for 7 days reduced EtOH-induced gastric mucosal damage by increasing gastric mucosal PGE2 expression. In our study, L-CAR treatment had no effect on gastric mucosal *COX-1* expression while decreasing *COX-2* expression. Concurrently, L-CAR administration reduced the severity of EtOH-induced gastric mucosal damage. These findings suggest that L-CAR downregulated *COX-2* expression by reducing EtOH-induced gastric mucosal damage with its protective effects.

Endothelium-derived relaxing (NO and prostacyclin) and vasoconstrictive (endothelin-1) factors regulate gastric microcirculation, which is directly related to gastric mucosal integrity (MacNaughton et al., 1989a). Vascular lesions have been shown in studies to play an important role in the pathogenesis of EtOH-induced gastric mucosal lesions (Oates and Hakkinen, 1988; Szabo et al., 1985). According to reports, these lesions form as a result of an imbalance between endothelium-derived factors (MacNaughton et al., 1989b). In response to EtOH-induced gastric mucosal damage, NO synthesized from L-arginine via a reaction catalyzed by the nitric oxide synthase enzyme (NOS) reduces the gastric lesions by increasing regional blood flow, decreasing leukocyte infiltration, and decreasing mast cell degranulation. NOS is divided into three isoforms: nNOS, eNOS, and iNOS. nNOS and eNOS are constitutionally expressed proteins found in enteric neurons and endothelium of the stomach, respectively. iNOS is a protein that is activated in the endothelium, epithelial cells, and immunocytes in response to gastric mucosal damage (Kubes and Wallace, 1995). We determined that EtOH administration increases the gastric mucosal *eNOS* and *iNOS* expression. Given its role in regulating gastric microcirculation, it was thought that the increased *eNOS* expression in response to EtOH administration occurred in order to regulate the impaired microcirculation. NO produced by iNOS is thought to have gastroprotective properties by reducing gastric leukocyte infiltration and preventing mast cell degranulation (Kubes and Wallace, 1995). Tepperman and Soper (1994) determined increased iNOS activity in the stomachs of rats given endotoxin and demonstrated that these rats were more resistant to EtOH-induced gastric mucosal damage. These findings confirms that iNOS expression is increased to protect the gastric mucosa. In our study, we discovered that EtOH administration increased gastric mucosal *iNOS* expression. Increased *iNOS* expression in rats exposed to EtOH suggested that this was a developing compensatory mechanism to reduce gastric mucosal damage. L-CAR treatment significantly reduced *eNOS* and *iNOS* levels in the gastric mucosa. Concurrently, it was determined that L-CAR administration reduced the severity of EtOH-induced gastric mucosal damage. The lower *eNOS* and *iNOS* expression in rats given L-CAR compared to those given EtOH suggest that rats given L-CAR had less gastric mucosal damage.

HSP family proteins are essential for normal cell growth and cell integrity during pathological conditions (Rokutan, 2000; Tsukimi and Okabe, 2001). In unstressed gastric epithelial cells,

HSP70 expression is either too low or undetectable. Experimental studies have shown that HSP70 expression is induced in the gastric epithelium in response to EtOH-induced gastric mucosal damage (Saika et al., 2000; Sun et al. 2022). It has been proposed that the HSP response is activated after EtOH administration to reduce the amount of unfolded and misfolded protein accumulated within the cell (Rokutan, 2000). In this study, we determined that EtOH administration significantly increased the gastric mucosal *HSP70* expression compared to the control group. Gastric mucosal HSP70 level was analyzed to determine whether the expression of HSP70 induced by EtOH administration was affected by L-CAR administration. In our study, L-CAR administration enhanced the HSP70 expression in rats exposed to EtOH. *In vitro* studies have shown that HSP70 overexpression induced by high temperature protects monolayers of guinea pig gastric mucous cells against EtOH-induced damage (Nakamura et al., 1991). Higher *HSP70* expression in rats treated with L-CAR in combination with EtOH compared to EtOH alone in this study suggests that L-CAR application made gastric mucosal cells more resistant to EtOH-induced damage by upregulating the *HSP7*0 expression.

An acute inflammatory response characterized by increased blood flow, plasma fluid exudation, and leukocyte infiltration is stimulated in response to gastric mucosal damage caused by exogenous and endogenous irritants to reduce tissue damage and facilitate regeneration of damaged tissue (Wallace, 2008). A number of mediators work together to coordinate the inflammatory process. Experiments have shown that the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) signal is important in initiating the inflammatory response associated with EtOH (Yu et al., 2020). Damage-associated molecular patterns released from damaged or dying cells in response to EtOH administration stimulate the degradation of inhibitor subunit of NF-κβ that traps NF-κβ in the cytosol, resulting in the translocation of NF-κβ to the nucleus. TNF-α mRNA expression is then induced by NF-κβ, which is one of the primarily mediators of the inflammatory process triggered by EtOH (Kim et al., 2020). In this study, EtOH administration was found to increase gastric mucosal TNF-α mRNA expression when compared to control rats. L-CAR treatment significantly reduced the expression of TNF-α mRNA in the gastric mucosa, which was elevated by EtOH administration. Based on these findings, it is possible to argue that L-CAR treatment protects against EtOH-induced gastric mucosal damage by regulating the inflammatory response.

TFFs are a type of peptide with a low molecular weight that is found primarily in the gastrointestinal tract. TFF2 released from mucous neck cells in the gastric corpus and antrum has been shown to play an important role in maintaining the gastric mucosal integrity (Hanby et al. 1993). TFF2, secreted by epithelial cells in response to acute gastric damage caused by a variety of irritants, promotes the epithelial restitution by stimulating the migration of healthy cells near the denuded area and inhibiting the apoptosis of migrating cells (Xue et al., 2011). In addition to these effects, TFF2 also increases the viscosity of gastric mucin and stabilizes the mucus gel network (Thim et al., 2002). Oral administration of recombinant TFFs has been shown in rodent studies to protect against gastric mucosal damage caused by various irritants (McKenzie et al., 2000; Poulsen et al., 1999). As previously reported (Aziz et al., 2019), the gastric mucosal *TFF2* expression decreased with EtOH administration in this study. This is the first study showing the role of TFF2 in gastroprotective effect of L-CAR against EtOH-induced gastric mucosal damage. L-CAR treatment increased the gastric mucosal *TFF2* levels, which decreased when EtOH was administered, by upregulating the *TFF2* expression. Given the effects of TFF2 on the gastric mucosal defense system, it can be opined

that L-CAR administration prior to EtOH reduces the EtOH-induced gastric mucosal damage by inducing re-epithelialization and stabilizing the mucous barrier.

## CONCLUSION

It was established that L-CAR treatment has a significant protective effect against EtOH-induced gastric mucosal damage. L-CAR treatment was regulated the expression of gastric mucosal cyclooxygenase and nitric oxide synthase systems, reduced the expression of inflammatory cytokines, activated the cellular stress response, and stimulated the expression of mucus secretion and gastric epithelium restitution factors. Therefore, it can be summarized that L-CAR treatment protects against EtOH-induced gastric mucosal damage by regulating the expression of factors important in maintaining the gastric mucosal integrity in addition to its previously suggested antioxidative effect.

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Conflict of interest: The authors declared that there is no conflict of interest.

Ethical statement: All the experimental procedures were approved by the local ethics committee of Aydın Adnan Menderes University (approval no. 64583101/2020/009).

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# Relationship between electrical conductivity and colostrum quality in farm level


## ABSTRACT

Good quality colostrum intake is essential component in calf health programs. There are different methods to determine the quality of colostrum. The aim of the present study is to investigate the relationship between Immunoglobulin G (IgG), which is used to determine colostrum quality, and electrical conductivity in farm level. Two groups were performed according to results of IgG analyses. Samples which had <50 mg/mL IgG concentration were assigned into group 1 (G1, n=27) and accepted as insufficient quality colostrum. Samples that had >50 mg/mL IgG concentration were accepted as good quality colostrum and assigned into group 2 (G2, n=68). IgG concentrations were measured by ELISA, then the electrical resistance (ER) and conductivity (EC) measured by Draminski Mastitis Detector (MDQ4, MDQ). MDQ and ER results were statistically higher in G2, and EC results were statistically higher in G1, but difference was not statistically significant (P>0.05) in G1, there was moderate positive correlation between IgG and ER, EC and MDQ (P<0.01). Presented study revealed strong correlation between EC and IgG concentration in low-quality colostrum. There are lots of variables that effect conductivity and resistance of colostrum, so to eliminate uncertainties of use of MDQ further research must be done. Moreover, MDQ readings show considerable potential for being useful tools in colostrum management systems to improve calf health in dairy farms.


**Keywords:** Colostrum, dairy cow, electrical conductivity, electrical resistance, IgG

## INTRODUCTION

During the last weeks of pregnancy and first days of post-partum period colostrum is the first secretion of mammary gland that is composed of different components including immunoglobulins (Baumrucker et al., 2022; Buczinski and Vandeweerd, 2016). Cows have an epitheliochorial placenta (Kara and Ceylan, 2021; Turini et al., 2020). Due to this placenta type, calves are born hypogammaglobulinemic (Kara and Ceylan, 2021). In bovine species, because of being born aggloblunemic, colostrum is crucial and critical. Though white blood cells and cytokines are important for calf immunity, IgG has critical role (Stelwagen et al., 2009). Sufficient and punctual supply of colostrum is vital for newborn calves (Immer et al., 2022). The transfer of immunoglobulins (Ig) from cow to fetus is prevented because placenta membranes have sparse permeability. Calves are born with low level of antibody on account of Ig cannot pass through placenta membranes (Ahmann et al., 2021). Intestinal permeability to IgG absorption rapidly decreases after birth (Hare et al., 2020) which makes delivering good quality colostrum as soon as possible after birth crucial. Therefore, good quality colostrum intake is essential component in calf health programs (Godden et al., 2019).

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**Research Article**

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Intestinal permeability to IgG absorption rapidly decreases after birth (Hare et al., 2020) which makes delivering good quality colostrum as soon as possible after birth crucial. Therefore, good quality colostrum intake is essential component in calf health programs (Godden et al., 2019). To prevent infectious diseases, getting colostrum right after birth with high immunoglobulin concentration is indispensable. If calves do not drink enough high-quality colostrum or quality of colostrum is not enough, failure of passive transfer (FPT) develops (Topal et al., 2018; Turini et al., 2020). So, to achieve good passive transfer, colostral IgG content is very important (Gelsinger et al., 2015).

Management of good colostrum leads to decrease in morbidity and mortality in the first days of life (Ahmann et al., 2021). Also, it effects further rearing, first calving age, further body weight and milk yield (Furman-Fratczak et al., 2011; Kessler et al., 2020). To complete successful passive transfer, colostrum should be given as soon as possible, in sufficient quantity and quality (Jaster, 2005). In cow colostrum, most important Igs are IgG, IgA and IgM. Main component of cattle colostrum is IgG which is up to 95% of total Ig concentration (Godden et al., 2008; Martin et al., 2021). The quality of colostrum could be measured by concentration of IgG (Crouch et al., 2000; Godden et al., 2019). Direct methods such as RID and ELISA represent gold standard for estimating the IgG concentration of colostrum (Ahmann et al., 2021). Generally, IgG concentration of colostrum is wanted to be higher than 50mg/mL (McGuirk and Collins, 2004). Calves need to receive 100-200 g IgG to have successful passive transfer immunity. Considering a newborn calf drink 3-4 liters colostrum withing first 6 hours to achieve success and based on these facts 50 g/L IgG concentration becomes arbitrary cut-point to define colostrum quality (Buczinski and Vandeweerd, 2016; McGuirk and Collins, 2004; Morrill et al., 2012). This threshold has been widely used to define the quality of colostrum by different researchers (Chigerwe and Hagey, 2014; Godden et al., 2019; Immer et al., 2022).

There are different methods to determine the quality of colostrum. Some methods measure Ig concentration which are accepted as direct methods. On the other hand there also indirect methods, whereas these methods give summary about the Ig concentration based on change in the physical and chemical properties of colostrum (Ahmann and et al., 2021). On farm level, measurement of colostrum quality should be easy to use, accurate and effective. Moreover, the costs should be kept minimal for feasible dairy cow industry (Bartens et al., 2016; Bielman et al., 2010). The aim of the present study is to investigate the relationship between IgG, which is used to determine colostrum quality, and electrical conductivity in farm level.

## MATERIALS AND METHODS

### *Animals and sampling*

The study was conducted in dairy farm of Holstein-Friesian breed. General condition and udder health of animals were evaluated by clinical examination. Udders of the cows were examined visually and by palpation for general mastitis changes (redness, pain, swelling, heat). Cows who had general signs of diseases (fever, loss of appetite, weight loss, lethargy) were not included in the study. The material of the study consisted of colostrum of 96 Holstein-Friesian cattle.

### *Groups*

Two groups were performed according to results of IgG analyses. Samples which had <50 mg/mL IgG concentration were assigned into group 1 (n=27) and accepted as insufficient quality colostrum. Samples that had >50 mg/mL IgG concentration were accepted as good quality colostrum and assigned into group 2 (n=68).

### *Samples*

Colostrum was taken from the cows by using single milking machines within the first hour after birth. All colostrum samples were labeled and stored in freezers at -20ºC until analyses were done.

### *Laboratory analysis*

IgG analyzes and electrical conductivity of samples were performed at same day. All frozen samples were thawed at room temperature and IgG concentrations were measured by ELISA kits (Biox, Belgium) then the electrical resistance was measured 4 times repeatedly using the Draminski mastitis detector (MD4Q-4896, Olsztyn, Poland). The MD4Q measures electrical resistance in the range of 10-1000 Ω. The electrical resistance (ER) value measured by MDQ was converted into electrical conductivity (EC) as stated before (Khatun et al., 2019).

ER (Ω) = unit read in MDQ / 1.944

EC (1000 mS) = EC (1 S) = 1 reciprocal ohm (1/Ω)

### *Statistical analyses*

Statistical analyses were performed using IBM SPSS Statistics software Version 23.0 and MedCalc 16 statistical software. Before performing the statistical analysis, data were examined for parametric test assumptions. Descriptive statistics for each variable were calculated and presented as "Mean ± Standard Error of Mean (SEM)". To test the differences in IgG, ER, EC and MDQ parameters between groups (Low-High IgG), student t test was used. Pearson correlation coefficient was performed to assess the correlation between IgG, ER, EC and MDQ. In addition, Pearson correlation coefficient was used to evaluate the correlation between IgG, ER, EC and MDQ for each group (low IgG and high IgG) separately. A receiver operating characteristics (ROC) analysis was performed to calculate the electrical conductivity diagnostic test characteristics (sensitivity, specificity, positive likelihood ratio and negative likelihood ratio) for evaluating low-high IgG. Areas under the ROC curves (AUC) were assessed to determine the discrimination ability of the IgG level. The statistical significance level was set at $P<0.05$.

## RESULTS

Immunoglobulin G levels were statistically higher in group 2 (G2) than group 1 (G1) and difference was statistically significant ($P<0.001$). MDQ and ER results were statistically higher in G2, and EC results were higher in G1, but difference was not statistically significant ($P>0.05$). Results of IgG, MDQ, ER and EC are presented in Table 1.

**Table 1.** Results of IgG, ER, EC and MDQ analyses

| Parameters | Groups (Mean ± SEM) | | P value |
|---|---|---|---|
| | G1 (n=27) | G2 (n=68) | |
| **IgG (mg/mL)** | 28.97±2.60 | 109.70±5.67 | <0.001 |
| **ER (ohm)** | 299.21±9.44 | 313.17±6.29 | 0.234 |
| **EC (S)** | 3.43±0.11 | 3.28±0.06 | 0.233 |
| **MDQ (Units)** | 581.67±18.35 | 608.80±12.23 | 0.234 |

IgG: Immunoglobulin G; ER: Electrical Resistance; EC: Electrical Conductivity; MDQ: Results of Draminski MD4Q

In G1, G2 and without groups, high positive correlation between ER, EC and MDQ were found ($P<0.001$). In addition, in G1, there was moderate positive correlation between IgG and ER, EC and MDQ ($P<0.01$). The cross correlations of IgG, ER, EC, and MDQ results within groups and without grouping are shown in Table 2, 3 and 4.

**Table 2.** Correlations between IgG, ER, EC and MDQ without groups (n=95)

| Parameters | IgG | ER | EC | MDQ |
|---|---|---|---|---|
| **IgG** | 1 | 0.180 | -0.186 | 0.180 |
| **ER** | | 1 | -0.981*** | 0.994*** |
| **EC** | | | 1 | -0.981*** |
| **MDQ** | | | | 1 |

***Correlation is significant at the 0.001 level (2-tailed). IgG: Immunoglobulin G; ER: Electrical Resistance; EC: Electrical Conductivity; MDQ: Results of Draminski MD4Q

**Table 3.** Correlations of IgG, ER, EC and MDQ in G1 (n=27)

| Parameters | IgG | ER | EC | MDQ |
|---|---|---|---|---|
| **IgG** | 1 | -0.514** | 0.500** | -0.514** |
| **ER** | | 1 | -0.982*** | 0.995*** |
| **EC** | | | 1 | -0.982*** |
| **MDQ** | | | | 1 |

**: Correlation is significant at the 0.01 level (2-tailed). ***: Correlation is significant at the 0.001 level (2-tailed). IgG: Immunoglobulin G; ER: Electrical Resistance; EC: Electrical Conductivity; MDQ: Results of Draminski MD4Q

**Table 4.** Correlations of IgG ER EC and MDQ in G2 (n=68)

| Parameters | IgG | ER | EC | MDQ |
|---|---|---|---|---|
| **IgG** | 1 | 0.208 | -0.223 | 0.208 |
| **ER** | | 1 | -0.981*** | 0.992*** |
| **EC** | | | 1 | -0.981*** |
| **MDQ** | | | | 1 |

***: Correlation is significant at the 0.001 level (2-tailed). IgG: Immunoglobulin G; ER: Electrical Resistance; EC: Electrical Conductivity; MDQ: Results of Draminski MD4Q

Three different ROC analysis were performed to determine thresholds for ER, EC and MDQ values to predict IgG values. The results of ROC curves analysis are presented in Table 5.

The ROC curves for thresholds between ER, EC, MDQ and IgG were shown in Figure 1.

**Table 5.** ROC curve analysis for cut-off and threshold values

| Variables | Threshold | Se | %95 Cl for Se | Sp | %95 Cl for Sp | AUC | + LR | - LR | p |
|---|---|---|---|---|---|---|---|---|---|
| **ER** | > 253.34 | 92.75 | 83.9- 97.6 | 25.93 | 11.2-46.3 | 0.574 | 1.25 | 0.28 | 0.242 |
| **EC** | ≤ 3.89 | 92.75 | 83.9-97.6 | 25.93 | 11.2-46.3 | 0.574 | 1.25 | 0.28 | 0.264 |
| **MDQ** | > 492.5 | 92.75 | 83.9-97.6 | 25.93 | 11.2-46.3 | 0.574 | 1.25 | 0.28 | 0.424 |

IgG: Immunoglobulin G; ER: Electrical Resistance; EC: Electrical Conductivity; MDQ: Results of Draminski MD4Q

## DISCUSSION

The objective of the presented study was the evaluation of electrical conductivity and resistance compared with ELISA assessment of IgG concentrations in frozen thawed colostrum. The hypothesis of the study assumed that there was a connection between amount of IgG which is determines the quality of bovine colostrum and electrical conductivity. The present study show that electrical conductivity and resistance might be useful indicators for determination of colostrum quality. The difference of IgG results between groups were statistically significant. The difference in IgG concentrations may have been due to calving season, nutrition, environment, parity, and timing of colostrum collection (Conneely et al., 2013; Gulliksen et al., 2008; Moore et al., 2005). Samples were collected in random order and sampling took place during whole year, so IgG concentrations may be affected from both season and nutrition. In this manner, composition of the herd could not be represented by the samples. All colostrum samples were analyzed frozen and thawed. Though one freeze thaw cycle had little or no effect on some farm-level devices such as hydrometer and refractometer (Morrill et al., 2015), so it can be concluded that results and correlations of the study were not affected by freeze thaw cycles.

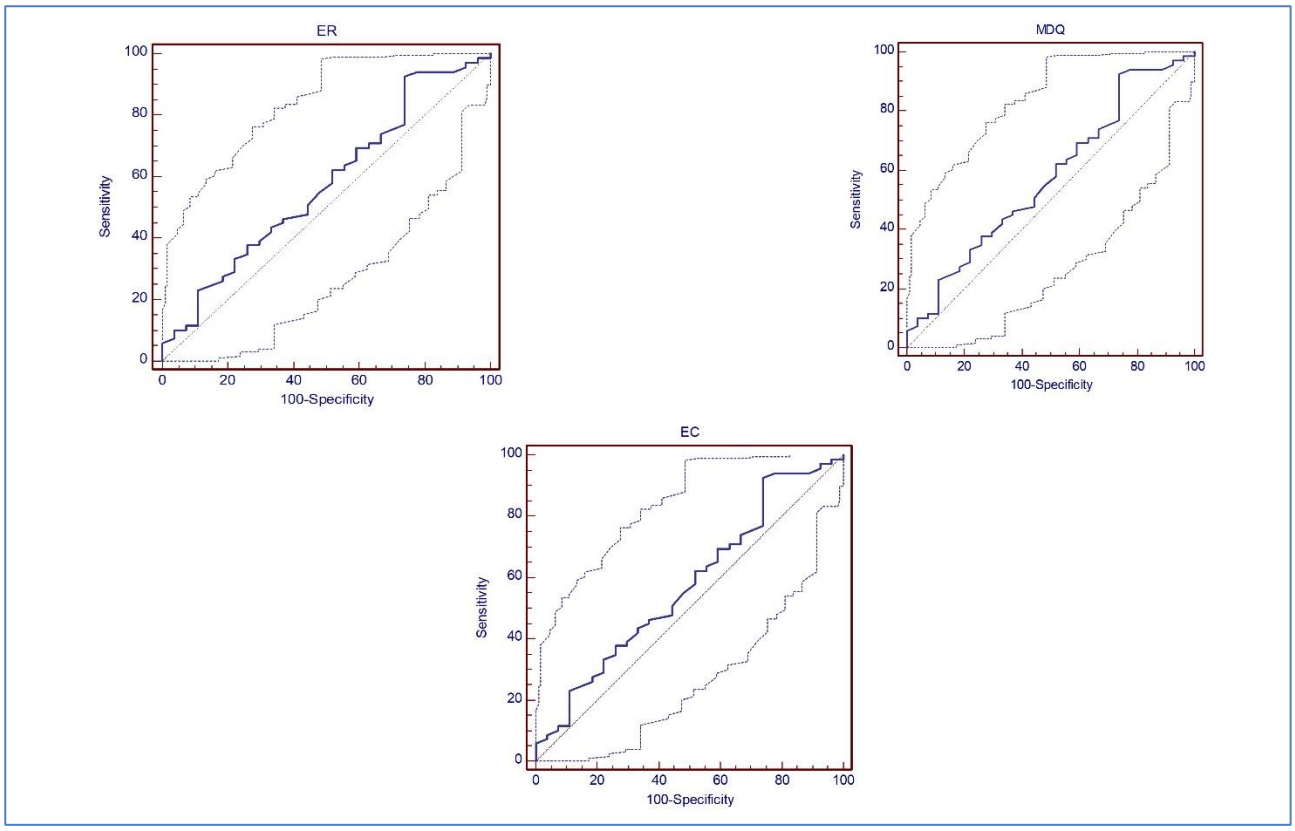


**Figure 1.** ROC curve graphs of ER, MDQ and EC. ER: Electrical Resistance; EC: Electrical Conductivity; MDQ: Results of Draminski MD4Q

Readings of MDQ between groups were not statistically significant. Since ER and EC are conversions of MDQ, difference of ER and EC between groups were not statistically significant as well. Average readings of MDQ for G1 and G2 were 581.67±18.35 and 608.80±12.23 respectively. Galfi et al. (2015) reported that at peak lactation (days 0-50), MDQ readings were between 260 and 700 (403±80.14). Our results were within their range but, higher than their average. MDQ readings are based on electrical conductivity. It was reported that healthy cow's milk conductivity is from 4.0 to 5.5 mS at 20ºC (Walstra, 1999). In addition, Kozheshkurt et al. (2021) indicated that electrical conductivity of whole colostrum was measured within the range of 0.37 and 0.43 S at 18±1ºC. Our results are not consistent with the previous studies. It was thought that there might be few reasons that conductivity of colostrum in both groups were lower than aforementioned studies. It is known that electrical conductivity is affected by temperature (Kozheshkurt et al., 2021). In the presented study, frozen colostrum samples were thawed at room temperature. Since average freezing point of cow milk is reported as -0.5ºC (Fox et al., 1998; Kuczaj et al., 2001; Navratilova et al., 2006), it shouldn't be assumed that after thawing process, temperature of samples was at room temperature. However, in the presented study temperature of samples were not analyzed, so it is not possible to determine exactly the effect of colostrum temperature on electrical conductivity. This is the most likely reason of the inconsistency with the reported studies and our results. Moreover, since electrical resistance is measured by formula from conductivity, it would be logically to expect similar results for ER.

Results of the study suggest that there is no relationship between EC, ER and IgG content of colostrum, but in low quality colostrum there were significant correlations between ER and IgG content. There were strong correlations between MDQ, ER and EC within groups and within all samples. Reason for those correlations

was ER and EC are calculated from MDQ readings. There was not statistically significant correlation between IgG and EC in all samples, but correlation between IgG and electrical resistance was found in group 1. Also, EC levels were found higher and ER levels were lower in G1 than G2. Reason of that difference might be because of IgG and protein levels of groups. Colostrum has elevated level of immunoglobulins (Smolenski et al., 2007) and total protein of colostrum is made up almost 80% by immunoglobulins (McGrath et al., 2016). It is reported that protein level of colostrum effects electrical conductivity. Removal of protein fractions from colostrum resulted in significant increase in electrical conductivity (Kozheshkurt et al., 2021).

Electrical resistance and conductivity are simple measurements of milk and widely used in detection of subclinical mastitis in dairy cows as marker (Fernando et al., 1982; Galfi et al., 2015; Norberg et al., 2004) and has not been evaluated in determination of colostrum quality. ER and EC could be indicators of low-quality colostrum in farm level. However, present study showed that ER, EC and MDQ had high Se (92.75) but low Sp (25.93) for IgG content of colostrum.

## CONCLUSION

In conclusion, Draminski Mastitis Detector tool have high sensitivity, but low specificity compared to one of the gold standard ELISA lab tests. However, present study revealed strong correlation between EC and IgG concentration in low-quality colostrum. There are lots of variables that effect conductivity and resistance of colostrum, so to eliminate uncertainties of use of MDQ further research must be done. Moreover, MDQ readings show considerable potential for being useful tools in colostrum management systems to improve calf health in dairy farms.

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# Koyunlarda sarkoptik uyuzun sağaltımında bitkisel kombinasyonların kısa süreli değerlendirilmesi: 4 vakadan oluşan bir seri

# Short-term evaluation of herbal combination for the treatment of sarcoptic mange in sheep: A series of 4 cases


## ÖZET

Koyunlarda sarkoptik uyuz klinik olarak belirtileri şiddetli seyreden bulaşıcı bir deri enfestasyonudur. Bu olgu sunumunun amacı sarkoptik uyuz tanısı konulan koyunlarda verilen nutrisyonel sağaltımın klinik etkinliğinin gözlemlenmesidir. Bu amaçla araştırmaya klinik bulguları gösteren ve deri kazıntılarında doğal *Sarcoptes* spp. uyuzu ile enfekte olduğu belirlenen 4 adet dişi koyun dahil edilmiştir. Sarkoptik uyuzlu her koyuna *Curcuma longa* (Turmeric, Zerdeçal) ve *Silybum marianum* (Milk Thistle, Deve Dikeni) ekstraktı sağaltım prosedürü 0. ve 5. günlerde 2 kez uygulanarak klinik indeks ve dışkı skorlaması belirlendi. Araştırmamızda bitkisel ürünlerden hazırlanan formülasyonların 0. ve 5. gün uygulamalarından sonra klinik skorun kısa süre içerisinde hızla gerilediği gözlemlendi. Sonuç olarak bitkisel kombinasyonun sağaltımda yardımcı olabileceği ancak daha fazla sayıda çalışmaya ihtiyaç olduğu düşünüldü.


**Anahtar Kelimeler:** Doğal bileşenler, fitoterapi, uyuz, zerdeçal


## ABSTRACT

Sarcoptic mange in sheep, is a highly contagious skin infestation with severe clinical symptoms. The aim of this study was to examine the clinical efficacy of nutritional therapy in sheep diagnosed with sarcoptic mange. For this purpose, four female sheep exhibiting clinical signs and confirmed to be infected with natural *Sarcoptes* spp. mite in skin scrapings were included in the study. The herbal extracts of *Curcuma longa* (Turmeric) and *Silybum marianum* (Milk Thistle) were administered twice on days 0 and 5 of the treatment procedure to each sheep with sarcoptic mange. Clinical index and fecal scoring were used to evaluate the treatment. In our study, it was observed that the clinical score rapidly decreased shortly after the application of formulations prepared from herbal products on days 0 and 5. In conclusion, herbal combined products can be safely used in the treatment of sarcoptic mange in sheep.


**Keywords:** Natural compound, phytotherapy, scabies, turmeric

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## GİRİŞ

Sarkoptik uyuz, epidermise yerleşerek aktif olarak stratum corneum'a nüfuz eden ve astigmat bir akar olan *Sarcoptes scabiei*'nin (Sarcoptiformes, Sarcoptinae) neden olduğu oldukça bulaşıcı bir deri hastalığıdır (Arlian ve Morgan, 2017).

Sarkoptik uyuz dünya çapında 150'den fazla konakçı türünü etkilemekte ve farklı konakçılar arasında bulaş özelliğiyle epidemiyolojik plastisite göstermektedir (Moroni vd., 2022). Enfekte hayvanlardaki semptomlar şiddetine göre değişkenlik göstermekle birlikte kaşıntılı papüller, eritem, kepeklenme, kabuklanma ve alopesi ile karakterizedir; kronik formlar hiperkeratoz ve/veya eksüdatif kabuk oluşumu ile ortaya çıkmaktadır (Cardells vd., 2021; Rahman vd., 2010).

*S. scabiei* enfeksiyonunun insan sağlığının yanı sıra hayvancılık üzerindeki ağır etkisi büyük ölçüde görülmektedir. Nitekim enfekte koyunlardaki verim kaybı, aşırı yem tüketimi, ölüm ve gençlerdeki büyüme geriliği hastalığın bilinen zararlarındandır. Amerika Birleşik Devletleri'nde Sarcoptoidea nedenli kayıpların her yıl takriben 3 milyar doları bulduğu bildirilmektedir (Larsen ve Storin, 1980; Tishenkova, 2005).

Organofosfatlar, makrosiklik laktonlar, formamidinler, piretroidler ve izoksazolinler gibi kimyasal akarisit ilaçlar, farklı hayvan türleri için sarkoptik uyuz tedavisinde veteriner kullanımı için ruhsatlandırılmıştır (Bernigaud vd., 2019). Bununla birlikte, bu ilaçların çiftlik hayvanlarında yoğun bir şekilde kullanılması, et ve diğer hayvansal ürünlerde kalıntıların varlığı ve ayrıca çevresel kontaminasyon tehdidini oluşturabilmektedir.

İnsanlarda bu enfeksiyonların tedavisi yakın zamanda gözden geçirilmiştir (Bernigaud vd., 2019; Shiven ve Kapoor, 2020). Permethrin, *in vitro* akar direnci bildirilmesine rağmen, en uygun topikal ilaç olarak kabul edilmektedir (Walton vd., 2000). Oral formülasyondaki ivermektin etkilidir, ancak gebelik sırasında ve vücut ağırlığı 15 kg'dan az olan hastalarda önerilmemektedir (Paasch vd., 2000). Ayrıca, ivermektine karşı direnç gözlenmiştir (Chandler ve Fuller, 2019; Mounsey vd., 2008). Diğer taraftan ifade edilen ajanların etken yumurtaları üzerinde etkileri olmadığı için tekrarlayan uygulama gerektirmektedirler (Bernigaud vd., 2019).

Bu nedenlerle, halk hekimliğinde yaygın olarak kullanılan şifalı bitkiler hem insanlarda hem de hayvanlarda bu rahatsızlıkların tedavisinde giderek daha fazla araştırılmakta ve değerlendirilmektedir. Aslında bu bileşikler, çevre üzerinde düşük bir etkiye sahip olan yeşil ürünler olarak kabul edilmektedir (Benelli vd., 2016; Pavela vd., 2018). Nitekim enfekte insanlarda bazı bitkisel müstahzarların çay ağacı yağı, Lippia yağı, neem yağı ve özleri, karanfil yağı, zerdeçal, kafur yağı vb. kullanımına değinilmektedir (Gopinath vd., 2018).

Bu çalışma ile sarkoptik uyuz tanısı konulan koyunlarda verilen nutrisyonel sağaltımın klinik etkinliğinin gözlemlenmesi amaçlanmıştır.

## OLGU SERİSİ

Çalışmamız çoğu geçim kaynağının tarım ve hayvancılık ile sürdürüldüğü ve aile tipi işletmelerin yer aldığı Işıklı Mahallesi, Efeler Aydın'daki bir aile tipi işletmede gerçekleştirildi. Işıklı Mahallesi yazları sıcak ve kurak, kışları ise ılık ve yağışlı olan Akdeniz iklimi hava koşullarında yıllık otalama 17-18°C sıcaklık ve yaklaşık %70 nem oranında sahip olup etkenin konakçı dışında yaşamasında çevresel şartları sağlamaktadır. Çalışmamızda yer alan koyunlar, yarı kapalı sundurma ağılda

yetiştirilmekte olup, arpa, süt yemi ve buğday otu ile beslenmekteydiler. İşletmenin önünde yer alan arazide meraya (denetimsiz) çıkmakta oldukları saptandı.

### *Tanı ve takip*

Hastalığın tanısı klinik bulguların yanı sıra hayvanlardan elde edilen deri kazıntılarında etken varlığı ile konuldu. Alınan örnekler fakültemiz laboratuvarına getirilerek mikroskobik incelemeleri yapıldı. *S. scabiei*'nin ortalama 0,3 mm boyutlu, kahverengi-şeffaf renkli, yuvarlak vücut şekline sahip olmasıyla ön bölgesinde keskin çeneleri, çengel şeklinde pençelerin varlığı ile arka çift bacaklarından daha kısa ön bacaklara sahip morfolojisiyle belirlendi (Şekil 1). Deri kazıntılarının toplanması 0. gün ve 5. günlerde 2 kez gerçekleştirildi. Çalışma sonrası 10. günde koyunlarda klinik iyileşmenin takibi yapıldı.

**Şekil 1.** Klinik belirtiler 1. koyun, 0. güne ait görüntüler

### *Bitki ekstraktının hazırlanması*

Sağaltım amacıyla hazırlanan karışım için 100 mg *Curcuma longa* (Turmeric, Zerdeçal) Ekstraktı, 100 mg *Silybum marianum* (Milk Thistle, Deve Dikeni) Ekstraktı ve 20 mg Beta-glukan kullanıldı. Ekstrakt 20 ml izotonik saline solusyonu ile dilüe edildi. Her uygulama da bitkisel karışım taze olarak hazırlandı ve uygulandı.

### *Çalışma prosedürü*

Çalışmamız koyun işletmesinde belirtilen hava şartlarının bulunduğu sezonda (Şubat-Mart) gerçekleştirildi. *Sarcoptes* spp. ile enfeste 4 adet koyuna 0. gün ve 5. günlerde aynı prosedür ile sağaltım uygulamaları yapıldı. İlk olarak sırayla her koyunun lezyonlu bölgelerinden derin deri kazıntısı hafif kan görülene kadar alınarak mikroskobik inceleme yapılmak üzere lama aktarıldı ve kazıntı bölgesi batikon ile temizlendi. Devamında lezyonlu bölgeler izotonik su ile ıslatıldı ve üzerine asetat bant yapıştırıldı. Kısa süre sonra bant çıkarıldı ve sürüntü mikroskobik analizi yapılması amacıyla lama aktarıldı. Uygulamanın akabinde lezyonların en çoğunluklu olduğu baş bölgesine önceden hazırlanan bitkisel içerikli karışım topikal

olarak uygulandı. Baş bölgesine yüksek miktarda uygulanan karışımın, masaj yapılarak deriye olabildiğince nüfuz etmesi sağlandı. Bunu takiben, koyunların dışkı skorlaması yapıldı ve 20 ml bitkisel karışım, sonda yardımıyla rektal yolla uygulandı. Tüm uygulamalar tamamlandıktan sonra 500 mg *Echinacea purpurea* (L.) Moench ekstraktı ve 135 mg *Pelargonium reniforme* (*Pelargonium sidoides)* ekstraktı içeriği oral yolla verildi. Hayvanların görüntüleri ve yapılan uygulamalar kayıt altına alındı.

Her prosedür gününde her koyun için görülen *S. scabiei* uyuz akarlarının sayısı, klinik ve lezyon skorlama indeksi (Fthenakis vd., 2001; Rodríguez-Cadenas vd., 2010) Tablo 1'e göre belirlendi. Tablo 2'de de sağaltım öncesi ve sonrası klinik indeks ve dışkı skorlaması belirtildi.

**Tablo 1.** Koyunlarda deri lezyonları için klinik indeks skorlarının tanımı (Fthenakis vd., 2001; Rodríguez-Cadenas vd., 2010'den uyarlanmıştır)

| Skor | Sınıflandırma | Tanımlama |
|---|---|---|
| **0** | Lezyon yok | Lezyon yok |
| **1** | < %10 lezyon | Burun üzerinde lokalize hafif deri hasarı ve fokal lezyonlar |
| **2** | %10-25 lezyon | Kulaklarda ve yüzün çeşitli bölgelerinde lezyonlar ve belirgin deri hasarı fakat kaşıntı yok |
| **3** | %25-50 lezyon | Yüzde orta şiddetli lezyonlar ile kaşıntı ve bazı sekonder bakteriyel enfeksiyon |
| **4** | %50-75 lezyon | Yüz ve kulaklarda şiddetli kontamine lezyonlar ve generalize kaşıntı ile birlikte vücudun diğer bölgelerinde lezyonlar bulunan |
| **5** | > % 75 lezyon | Kronik şiddetli generalize lezyonlar |

**Tablo 2.** Koyunlardaki klinik indeks ve dışkı skorları

| Olgular | Klinik indeks skoru | | Dışkı skoru | |
|---|---|---|---|---|
| | 0. gün | 5. gün | 0. gün | 5. gün |
| **Olgu 1** | 4 | 3 | 1 | 2 |
| **Olgu 2** | 3 | 2 | 1 | 2 |
| **Olgu 3** | 2 | 1 | 2 | 2 |
| **Olgu 4** | 3 | 2 | 1 | 1 |

Klinik indeks skoru Tablo 1'e göre verilmiştir. Dışkı skoru: 1 = Yumuşak; 2 = Normal

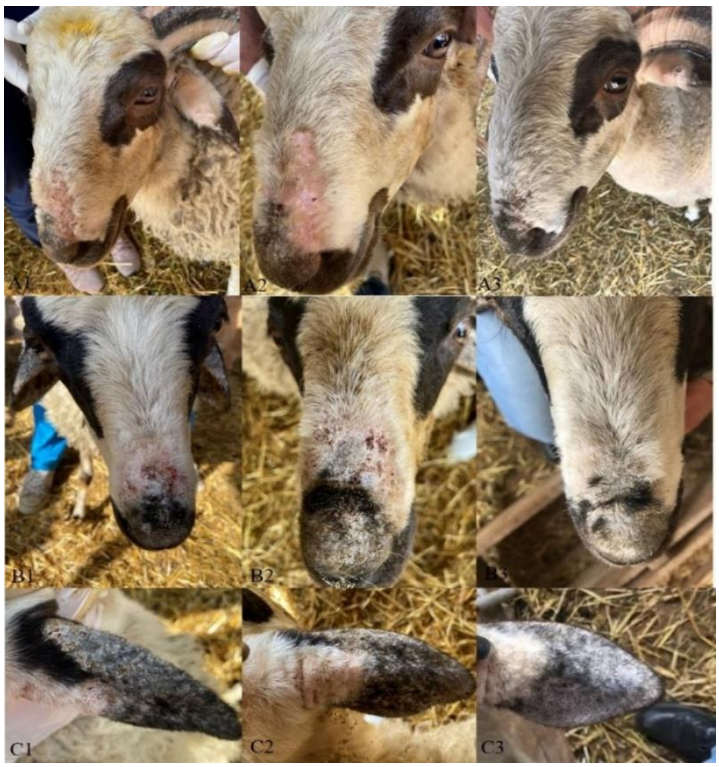
## OLGU-1

Birinci olgumuz 3 yaşlı, dişi, Sakız ırkı bir koyun olup olgunun *Sarcoptes* spp. ile enfekte olduğu yukarıdaki prosedürde tüm olgularda ortak şekilde belirlendi. Olgumuzun lezyonları şiddetli olup baş bölgesinde (periokuler, burun, kulak, ağız çevresi) eritem, alopesi, beyazımsı/kahverengi kabuklanma, ekskoriasyon, çatlama, şiddetli kaşıntı mevcuttu (Şekil 1). Yukarıda belirlenen tüm uygulama prosedürü uygulandıktan sonra 5. gün klinik indeks skorunda değişme olmadı ve eritem bulgusu haricinde diğer bulguları gözlemlendi. Dışkı skorlamasında 0. gün yumuşak dışkıdan 5. gün normal dışkıya dönüştüğü gözlemlendi. 10. günde olgu 1 sahibi tarafından satıldığı için klinik gözlem yapılamadı.

## OLGU-2

İkinci olgumuz 3,5 yaşlı, dişi, Sakız ırkı bir koyun olup olgunun *Sarcoptes* spp. ile enfekte olduğu yukarıdaki prosedürde tüm olgularda ortak şekilde belirlendi. Olgumuzun lezyonları baş bölgesinde olup eritem,

beyazımsı/kahverengi kabuklanma, ekskoriasyon ve kaşıntı mevcuttu (Şekil 2A). Yukarıda belirlenen tüm uygulama prosedürü uygulandıktan sonra 5. gün olgumuzda eritem ve kahverengi kabuklanma gözlenmedi ancak klinik skor indeksi değişmeyerek ekskoriasyon ve kaşıntı görüldü. Dışkı skorlamasında 0. gün belirtilen yumuşak dışkısı 5. gün normal dışkıya döndüğü bulundu. 10. günde olgumuzun tüm klinik belirtilerinde düzelme gözlemlendi.




**Şekil 2.** Koyunlarda 0. gün sol sütun, 5. gün orta sütun, 10. gün sağ sütun görüntüleri; A1-3 Olgu 2; B1-3 ve C1-3 Olgu 4

## OLGU-3

Üçüncü olgumuz 4 yaşlı, dişi, Sakız ırkı bir koyun olup olgunun *Sarcoptes* spp. ile enfekte olduğu yukarıdaki prosedürde tüm olgularda ortak şekilde belirlendi. Olgumuzun lezyonları baş bölgesinde olup alopesi, beyazımsı kabuklanma ve çatlama mevcuttu. Yukarıda belirlenen tüm uygulama prosedürü uygulandıktan sonra 5. gün olgumuzda klinik lezyonlarından derinin çatlaması görülmedi ancak yine klinik indeks skoru aynı seviyede devam ettiği gözlemlendi. 10. günde olgu 3 sahibi tarafından satıldığı için klinik gözlem yapılamadı.

## OLGU-4

Dördüncü olgumuz 3 yaşlı, dişi, Sakız ırkı bir koyun olup olgunun *Sarcoptes* spp. ile enfekte olduğu yukarıdaki prosedürde tüm olgularda ortak şekilde belirlendi. Olgumuzun lezyonları baş bölgesinde eritem, alopesi, beyazımsı/kahverengi kabuklanma, çatlama ve kaşıntı mevcuttu (Şekil 2B-C). Yukarıda belirlenen tüm uygulama prosedürü uygulandıktan sonra 5. gün olgumuzda klinik lezyonların çoğunda azalma görüldü. Olgumuzda 5. gün kabuklanma haricindeki eritem, alopesi, kahverengi kabuklanma ve çatlama bulguları ortadan kalkmıştır ve klinik indeks skorunun 3'den 2'ye düştüğü belirlenmiştir. Uygulama öncesi ve sonrası diğerlerinin aksine dışkı skoru değişmemiştir. 10. günde olgunun klinik belirtilerinde düzelme gözlemlendi.

## TARTIŞMA

Koyun uyuzu, bir akar istilası sonucu meydana gelen oldukça bulaşıcı bir deri hastalığıdır.

Endemik olduğu ülkelerde hayvancılık sektöründe büyük kayıplara sebebiyet vermektedir (Larsen ve Storin, 1980; Tishenkova, 2005).

Araştırmamızda lezyon dağılımlarının Irak' ta yapılan bir çalışmayla (Shamsa vd., 2008) benzer şekilde büyük oranda baş bölgesinde olduğu tespit edildi. Koyunlarda uyuzun klinik belirtileri arasında pullu, kabuklu sarımtırak lezyonlar, deri hasarı ve yapağı kaybı yer almaktadır (Mitra vd., 1993). Klinik bulguların da daha önce bildirildiği (Kettle, 1995; Solusby, 1968) gibi aşırı kaşıntı, sürtünme ve baş bölgesinde (periokuler, burun, kulak, ağız çevresi) alopesi ve kahverengi/beyazımsı kabuklanma şeklinde tespiti söz konusuydu. Enfekte koyunlardaki klinik bulgular mevcut akarların deri altında tüneller açmasıyla açıklanabilmektedir. Akarların tükürükleri, deri katmanlarını (yüzeyselden derine), eritebilen ve akarların yuvası olacak tünelleri oluşturan güçlü sindirim enzimlerinden oluşmaktadır (Al-Shebani vd., 2012). Ayrıca sarkoptik uyuz genellikle istila edilmiş konakçıda yumurtladıkları ve yaşam döngülerini sürdürdükleri deri tünelleri oluşturan oyuk akarları olarak adlandırılmaktadır (Jimenez vd., 2010; Niedringhaus vd., 2019). Bu süreç boyunca yaşayan sarkoptik uyuz sonucu etkenin dökülen deri, nimf ve yumurta kabukları ile ölü formları ile büyük miktarda antijenik uyaran açığa çıkarmakta ve söz konusu bu durum da akarlara karşı aşırı duyarlılığın artmasına neden olmaktadır (Morgan vd., 2016; Niedringhaus vd., 2019). Özellikle olgun dişiler tarafından yapılan deri altı tünelleri, epidermisin kalınlaşmasına ve kabuk oluşumuna neden olmaktadır (Teodoro vd., 2018) ve bu çalışmada gözlenen mevcut bulgular belirtilen etkenin yaşam şeklinden kaynaklanmaktadır.

Patojenlerden bazıları birden fazla çeşitli konak türünde bulaşmayı sürdürmekle birlikte, epidemiyolojik olarak önemli olan bu becerinin nasıl kazanıldığı bilinmemektedir. *S. scabiei*, memeli parazitleri içinde konakçıya özgü olmayan (generalist) ve en bulaşıcı ev dirençli uyuz etkenlerinden biridir. Elizabeth ve diğerleri (2022) sürekli bulaşmaya aracılık eden patojen ve konakçı özelliklerini sentezleyip üç bulaşma mekanizmasını da (direkt, indirekt ve kombine) gösteren vakalar sunmaktadır. *S. scabiei*' nin söz konusu başarısını açıklayan patojen özellikleri arasında bağışıklık yanıtı modülasyonu, konakçı üzerindeki hareket yeteneği, konak dışı arama davranışları ve çevresel kalıcılık yer almaktadır. Sosyallik ve konak yoğunluğu, direkt bulaşmanın baskın olduğu konaklarda önemliyken yalnız konaklarda ortak kullanım alanlarının varlığı indirekt bulaşma için önem arz etmektedir. Sosyal yaşam kuran türlerde direkt ve indirekt bulaşmanın birlikte görülmesi muhtemeldir. *S. scabiei*' nin konakçı türlerde endemik hale gelmesini sağlayan mekanizmalar nadir olarak ele alınmış ve çalışmalar daha çok salgınlara odaklanmıştır. Elizabeth ve diğerleri (2022), yaptıkları çalışmada parazitlerin konak türleri arasındaki bulaşmayı sürdürmek için geliştirdiği mekanizmaların adaptasyon süreçlerini açıklığa kavuşturmayı hedeflemiştir.

Geçmişten günümüze pek çok akarisidal ajan bitkilerden elde edilmiştir (Akram vd., 2020; Tooning vd., 1988). *S. scabiei*'ye karşı akarisidal aktiviteye sahip çeşitli tıbbi bitkilerin kullanımı birçok memeli türünde bildirilmiştir (Akram vd., 2020; Chen vd., 2019; Pasipanodya vd., 2021; Shiven vd., 2020). Genel olarak sarkoptik uyuzda kullanılan birçok akarasid etkinliğini, etkenin ekdizon büyüme hormon sentezi ve sitokrom P450 mono-oksijenaz detoksifikasyon mekanizmasını baskılayarak göstermektedir (Verma vd., 2011).

Koyunlarda sarkoptik uyuza karşı etkili olduğu en iyi bilinen bitkisel ürün neem yağıdır (Tabassam vd., 2008). 14 gün süreyle %20'lik formülasyonun topikal uygulanmasıyla %100

klinik iyileşme bildirilmiştir (Tabassam vd., 2008). Yine koyunlarda korunga bitkisinden elde edilen ekstraktın 14 gün süreyle uygulandığında klinik iyileşme sağladığı ifade edilmektedir (Shahatha, 2020). İçerisinde *C. longa*'nın da yer aldığı bitkisel merhem karışımının topikal uygulandığı sarkoptik uyuzlu buzağılarda 7-15 gün içerisinde iyileşmenin sağlandığı ve uygulaması sonrası 7-30. günlerde yapılan mikroskobik incelemelerde canlı akar ile karşılaşılmadığı görülmüştür (Naresh vd., 2005). Araştırmamızda da bitkisel ürünlerden hazırlanan formülasyonlar sonrası klinik skorun kısa süre içerisinde hızla gerilediği gözlemlendi.

Çalışmamızda oluşturulan bitkisel formülasyonlarda yer alan *C. longa* tavşanlarda standart uyuz sağaltımında yer alan ivermektin enjeksiyonu yanında rasyona adjuvant olarak eklendiğinde enfestasyona bağlı gelişen oksidatif yanıtı elemine ederek ivermektinin anti-paraziter etkinliğini arttırdığı, performansı arttırdığı ve immüniteyi desteklediği görülmüştür (Abu Hafsa vd., 2021). Bufalolarda (Naresh vd., 2005) ve develerde (Atal vd., 2023; Periasamy vd., 2018) yine zerdeçal içeren kombine topikal ürünlerin iyileşme sağladığı bildirilmiştir.

## SONUÇ

Sonuç olarak zerdeçal ve devedikeninden oluşan bitkisel kombinasyonun özellikle topikal başta olmak üzere yine rektal yolla da uygulanmasının sağaltımda yardımcı olabileceği ancak bu klinik iyileşmenin ve sağaltım etkinliğinin daha fazla sayıda hayvanın çalışmaya dahil edildiği, uzun süreli takipli araştırma ile desteklenmesi gerektiği düşünüldü.

## AÇIKLAMALAR

Etik beyan: Çalışmamızda yem katkı maddeleri ile doğal destek sağlandığından, deneysel bir çalışma kapsamında herhangi bir ilaç uygulamasında bulunulmamış, yine de hasta sahibinden bilgi onam formu alınmıştır.

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# Investigation of the effect of sodium selenite on metallothioneine expression in the liver and kidney in experimental cyclophosphamide toxicity

Research Article


## ABSTRACT

In this study, the effect of sodium selenite (SS) on metallothionine expression in liver and kidney tissues of rats administered cyclophosphamide (CP) at high dose intraperitoneally (i.p.) was investigated. In the study, a total of 24 Wistar Albino female rats with a weight of 200±10 g were used in 4 groups. Grouping in the study: Group I (Control group; daily 1 mL serum physiologique given as ip), Group II (CP group; 200 mg/kg/day CP administered as ip for 1 day), Group III (SS Group; 1 day ip 1 mg/kg/day SS was given as a treatment), Group IV (CP Group + SS Group; 200 mg/kg/day CP and 1 mg/kg/day SS was given as ip for 1 day) by forming 4 different groups. The effect of histopathological findings was found to be significantly lower in the group in which CP was given at high doses and SS was applied at the same time, compared to the group in which only CP was applied. When immunohistochemical findings related to MTs of liver and kidney tissues in this group were compared with histopathological findings, similar results were observed in terms of evaluation results. The study was concluded to be important in terms of animal and human health because SS is well tolerated in the organism and has no side effects at the appropriate dose.


**Keywords:** Cyclophosphamide, metallothionein, sodium selenite


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## INTRODUCTION

Cyclophosphamide (CP) is one of the alkylating antineoplastic agents widely used in oncology (Poll et al., 1988). It is a drug in the oxazophosphorine structure, which is in the alkylating class of chemotherapeutic agents (Bernacki et al., 1987). For CP treatment purposes, it is used effectively in pediatric solid tumors, acute lymphocytic leukemia in children (Limandal, 2013), non-Hodgkin lymphomas (Glode et al., 1981), soft tissue sarcomas, rhabdomyosarcomas, thrombocytopenic purpura, systemic lupus erythematosus (Bertram, 2012) and Behcet's disease (Ozyazgan et al., 1992). CP that is metabolized in the liver is excreted by the kidneys within 48 hours. 3-25% of the dose that was taken into the organism; is excreted in the form of the parent compound. The liver cytochrome P-450 enzymes allow CP to be activated to 4-hydroxy-cyclophosphamide and isomerized to aldophosphamide. Aldophosphamide is converted to phosphoramide mustard (FAM) and to acrolein (ACR), which have a cytotoxic effect in the urinary bladder (Kawabata et al., 1990; Bertram, 2012).


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Selenium (Se) was accepted as a toxic element until the 1930s. The protection of Se in liver tissue degeneration was first reported by Schwarz and Folz in 1957 (Schwarz & Foltz, 1957). The main source of Se is soil, but the way it is found in animals and plants is different. It is generally found in animal tissues in the form of selenocysteine and selenomethionine, while in plants it is found in the form of Se-methyl-selenomethionine, selenocysteine, selenocysteine and selenomethionine (Cousin & Cairney, 1961; Ullery, 1992; Aksoy, 2000). Selenoproteins enable Se to perform its biological activity in tissues. Se, located in the active site of the glutathione peroxidase enzyme in cells is in the form of selenocysteine. The glutathione peroxidase enzyme is very important for erythrocytes, it is a detoxifier of hydrogen peroxide (Akkus et al., 1991). Diseases such as nutritional myopathy, mad chick disease, exudative diathesis, pancreatic fibrosis, decreased egg production, and decreased brood productivity occur in sheep when Se is deficient (Bildik et al., 1996; Göger, 1997). The discovery of metallothioneines (MT) dates back to 1957. Margoshes and Vallee reported that MTs bind cadmium in mammalian kidney cells (Margoshes & Vallee, 1957). MTs exist in different forms in animals and plants, prokaryotes and eukaryotic microorganisms (Klaassen et al., 1999). MTs are important in the homeostasis of basic metals such as Zn and Cu and in detoxifying heavy metals such as Cu, Cd, Hg and Ag. MTs are proteins with a high cysteine content, the ability to bind metals, being soluble and temperature-stable, consist of 61-68 amino acids, and having a molecular weight of 6000-7000 Da (Viarengo & Nott, 1993). Metals and other physical factors such as hunger, pesticide-induced oxidative stress, salinity, heat-cold trauma, exercise, UV rays, chemicals such as carbon tetrachloride-paraquat, alkylating agents, drugs applied in tumor treatment, inflammation, interleukin I-6, and TNF induce MT expression. It can also be induced by hormones such as cytokines, bacterial infections, glucocorticoids, angiotensin-II, and glucagon (Sato & Bremner, 1993; Viarengo et al., 1999; Mosleh et al., 2005). It has been reported that MTs show cytotoxic resistance to the drugs used in malignant tumor treatments, especially in cases with poor prognosis (Cherian et al., 2003; Gomulkiewwicz et al., 2010). Antioxidants, alpha tocopherols and glutathione play an active and important role in the protection of liver cells. MTs, which are similar in structure to glutathione, are potential antioxidant proteins and have a very important place in the protection of liver cells (Zhou et al., 2002).

## MATERIALS AND METHODS

### *Antioxidant and drug used*

In the study, i.p. SS given as Sigma Aldrich (Germany), and as CP; Endoxan® (Mccaroll et al., 2008) containing 1069.9 mg of CP monohydrate, equivalent to 1g CP, was supplied from Eczacıbaşı Pharmaceutical Company (Türkiye).

### *Experimental animals used*

Experiment and study design were approved by Aydın Adnan Menderes University, Experimental Animals Ethics Committee with the decision numbered 64583101/2016/146.

A total of 24 adult female Wistar Albino rats weighing 200±10 grams were used in the study. Rats were kept in transparent polycarbonate cages throughout the study. The rats were given drinking water and standard feed ad libitum. They were kept in special cages in special rooms with a temperature of 22±2°C, a humidity of 50-55%, and a lighting of 12;12 hours light/dark.

### *Experiment design*

The rats used in the study were randomly determined and grouped into 4 different groups, with 6 rats in each group (Sabık & El-Rahman, 2009). Group I (Control group): During the study, 1 ml 0.9% saline daily i.p. given as). Group II (CP group): Each rat was given 1 day i.p. 200 mg/kg/day CP was given. Group III (SS

group): Each rat was given i.p. for 1 day. 1 mg/kg SS was given. Group IV (SS Group + CP Group): Each rat was administered i.p. for 1 day. As a result, 1 mg/kg SS and 200 mg/kg/day CP were given.

***Pathological examination***

After the necropsy procedures applied to rats, kidney and liver tissue samples were taken into formalin solution (10%) for 48 hours and tissue fixation was made. Afterwards, the fixed tissues were trimmed. Then, after the trimmed samples were taken into tissue tracking cassettes and washed under tap water, they were placed in the tissue tracking device (Leica TP1020, Germany), tissue tracking was performed and the samples were brought to the sectioning stage by blocking in paraffin. Block tissue samples in paraffin (Leica RM 2135, Germany) were cut with a microtome at a thickness of 4-6 microns and stained with hematoxylin-eosin for histopathological examinations and examined under a light microscope. Sections deemed necessary were stained with Masson's Trichrome and oil red O dye, respectively, and examined under a light microscope to examine connective tissue and hepatic lipidozis (Culling et al., 1985). Findings found as a result of macroscopic and microscopic examinations were evaluated by semiquantitative method in terms of parameters such as dilatation, hyperemia, degeneration, necrosis, hepatic steatosis, glomerular congestion, bleeding, separation of tubular basement membranes (0; no finding, 1; mild, 2; moderate and 3; severe) (Chmielewska et al., 2015).

***Immunohistochemical examination***

MT expression in tissues was evaluated immunohistochemically by streptavidin-biotin immunoperoxidase method. For this purpose, monoclonal anti-metallothionine clone E9 antibody (Thermo Fisher, U.S.A) was used (Chmielewska et al., 2015). Sections of 6 µm thickness cut from paraffin blocks were taken on slides coated with poly-L-lysine. Sections taken on slides were placed in an oven at 40°C, where they were incubated for 10 minutes and then serially exposed to xylol and alcohol, then washed with phosphate buffered solution (PBS; pH 7.2) for 3x5 minutes. Endogenase peroxidase activity in the tissues was removed by incubation for 30 minutes in absolute methanol containing 3% hydrogen peroxide ($H_2O_2$) at room temperature. Tissues on the slides were washed with phosphate buffered solution for 3x5 minutes and then kept in a 0.1% proteinase K solution at 37°C in a humid chamber for 10 minutes. In order to prevent non-specific antigenic binding, 1% bovine serum albumin was kept in a humid chamber for 20 minutes. Tissues were then coated with the primary antibody and incubated overnight at +4°C. Following this, the tissues were coated with biotinylated secondary antibody and incubated for 15 minutes at room temperature. The Histostain Plus IHC Kit (Thermo Fisher, U.S.A) was used for staining. Afterwards, after the sections were incubated with horseradish streptavidin peroxidase conjugate for 15 minutes at room temperature, 3,3'-diaminobenzidine tetrahydrochloride-$H_2O_2$ (DAB) (Invitrogen DAB-Plus Substrate Kit, U.S.A) substrate was applied. After using Harris hematoxylin for counterstaining, the sections were dehydrated in alcohol series. After the sections were cleared in xylol, they were mounted with adhesive (Entellan). After staining the sections under the same conditions and procedures, histopathological findings and MT expression intensities in tissues were evaluated semiquantitatively under light microscopy.

***Statistical analysis***

The data of the study were analyzed using SPSS 22 (Inc., Chicago, II, USA) software. The conformity of the data obtained in the study to the normal distribution was evaluated using the

Kolmogorov-Smirnov test. One-way analysis of variance was performed for the data that were suitable for normal distribution. Kruskal-Wallis test was used for the data not suitable for normal distribution. Post hoc multiple comparisons were performed using the Mann-Whitney U test with Bonferroni corrected. Within the scope of one-way analysis of variance, the homogeneity of the available data was determined by Levene's test. Statistical differences between groups according to the homogeneity of the obtained data were determined by Tamhane or Tukey test (Conover, 1980). Statistical differences between study groups were determined by the post-hoc Tukey test from GLM procedures. If the P value was less than 0.05, it was considered significant.

## RESULTS

### *Macroscopic and microscopic findings*

In the study, macroscopic findings were detected only in experimental animals in Group II. The cross-sectional surfaces of the liver and kidneys were edematous. Macroscopically, barely visible hyperemia and congestions were detected in both organs. The severity of hyperemias and congestions was slightly milder in Group IV. No macroscopic finding was observed in the other groups in the study. The severity and distribution of the microscopic findings of the liver and kidney tissues of Group II and Group IV are given in Table 1 and Table 2.

**Table 1.** Statistical evaluation of semiquantitative results of microscopic findings of livers of rats given high dose of cyclophosphamide (CP)

| **Liver** | | | | | |
|---|---|---|---|---|---|
| **Groups** | Histopathological Findings | | | | |
| | Sinusoidal dilation | Hyperemia | Degeneration | Necrosis | Steatosis |
| **Group I** | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| **Group II** | $1.75 \pm 0.39^{b}$ | $1.18 \pm 0.30^{b}$ | $3.00 \pm 0.00^{c}$ | $2.46 \pm 0.08^{c}$ | $0.08 \pm 0.04^{b}$ |
| **Group III** | $0.01 \pm 0.04^{a}$ | $0.10 \pm 0.06^{a}$ | $0.15 \pm 0.16^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| **Group IV** | $1.70 \pm 0.20^{b}$ | $1.08 \pm 0.17^{b}$ | $1.76 \pm 0.22^{b}$ | $1.40 \pm 0.25^{b}$ | $0.00 \pm 0.00^{a}$ |
| **P value** | <0.05 | | | | |

Group I (Control group), Group II (CP; 200 mg/kg/day), Group III (SS; 1mg/kg/day), Group IV (CP; 200 mg/kg/day + SS; 1mg/ kg/day). $^{a\text{-}c}$: statistical difference in the same column, SS: Sodium selenite

**Table 2.** Statistical evaluation of semi-quantitative results of microscopic findings of kidneys of rats given high dose of cyclophosphamide (CP)

| **Kidney** | | | | | | |
|---|---|---|---|---|---|---|
| **Groups** | Histopathological Findings | | | | | |
| | Dilatation of tubules | Glomerular congestion | Hemorrhage | Degeneration | Necrosis | Separation of tubular basement membranes |
| **Group I** | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| **Group II** | $0.35 \pm 0.50^{a}$ | $0.50 \pm 0.08^{c}$ | $0.56 \pm 0.45^{b}$ | $2.83 \pm 0.24^{c}$ | $2.75 \pm 0.29^{c}$ | $2.10 \pm 0.55^{c}$ |
| **Group III** | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| **Group IV** | $0.16 \pm 0.26^{a}$ | $0.30 \pm 0.10^{b}$ | $0.26 \pm 0.29^{ab}$ | $1.68 \pm 0.14^{b}$ | $1.81 \pm 0.38^{b}$ | $1.51 \pm 0.24^{b}$ |
| **P value** | <0.05 | | | | | |

Group I (Control group), Group II (CP; 200 mg/kg/day), Group III (SS; 1mg/kg/day), Group IV (CP; 200 mg/kg/day + SS; 1mg/ kg/day). $^{a\text{-}c}$: statistical difference in the same column, SS: Sodium selenite

The most common and severe microscopic findings in the livers of Group II and Group IV animals, in which CP was given at high doses, were found in Group II. Microscopic findings were milder in Group IV, in which CP was administered simultaneously with sodium selenite. The detected lesions were listed as dilatation, congestion, disruption in the arrangement of the remark cords, hyperemia, degeneration (Figure 1) and single cell necrosis in hepatocytes. Dilatations in the sinusoids were mostly detected in the centrilobular areas. Single cell necroses and degenerations showed an irregular distribution in contrast to the dilatations. Degeneration, necrosis, sinusoidal dilatation, hyperemia and adiposity levels were found to be statistically significantly higher in experimental animals in Group II, where only CP was given as high dose, compared to the control group and the group given only selenium ($P<0.05$). Although the dilatation and hyperemia levels in the sinusoids were higher than Group IV in which CP was applied simultaneously with selenium, the difference was not statistically significant. Again, degeneration, necrosis and steatosis levels seen in hepatocytes were found to be lower in Group IV compared to Group II ($P<0.05$). In Group II and Group IV, where CP was given at high doses, the most severe findings in the kidneys were seen in Group II. The most common microscopic findings in this group are; tubular epithelial degenerations and necrosis (Figure 2). The hemorrhages were located cortical and medullary. Although the rate of dilatation in the renal tubules was higher in the CP group alone, it was not statistically significant compared to the other groups. Degeneration and necrosis rates were found to be lower in the group in which CP was applied together with SS compared to the group in which only CP was applied, but significantly higher than the control and only SS groups ($P<0.05$).

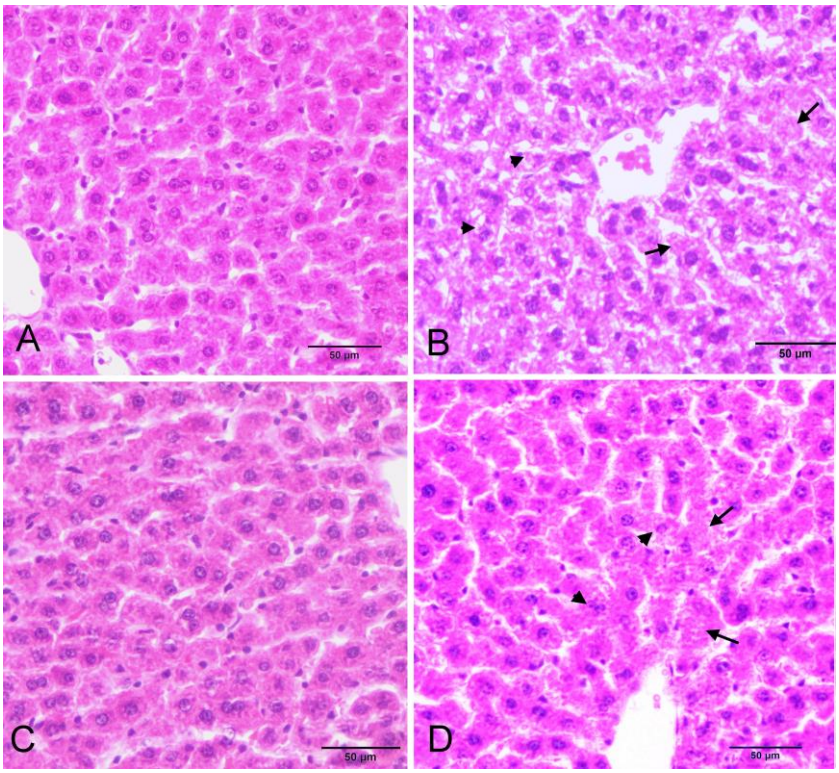


**Figure 1.** Liver. Degeneration (arrowheads) and necrosis (arrows) in hepatocytes. A: Group I (Control group), B: Group II (CP; 200 mg/kg/day), C: Group III (SS; 1 mg/kg/day), D: Group IV (CP; 200 mg/kg /day + SS; 1 mg/kg/day). HE. CP: Cyclophosphamide, SS: Sodium selenite

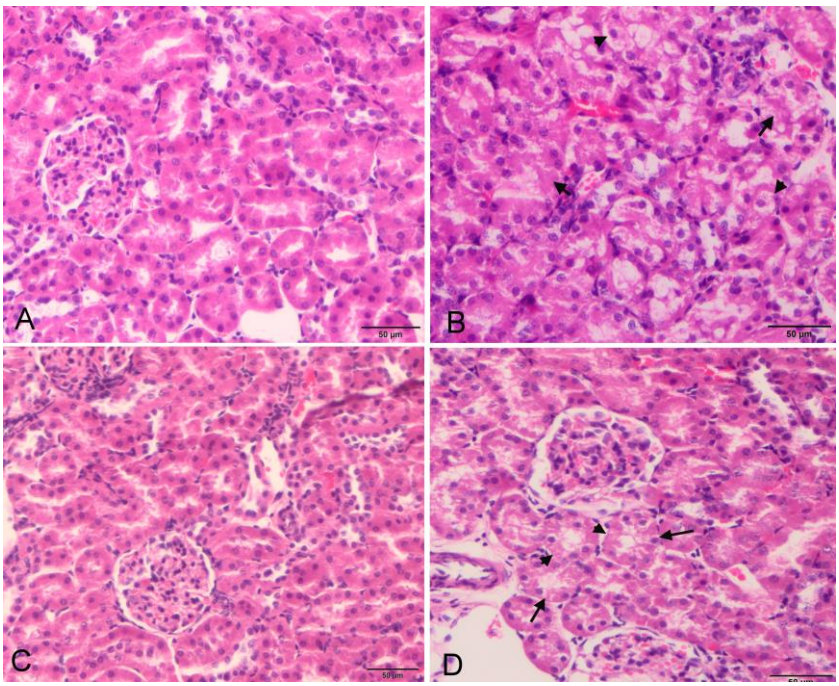


**Figure 2.** Kidney. Degeneration (arrows) and necrosis (arrowheads) in tubular epithelium. A: Group I (Control group), B: Group II (CP; 200 mg/kg/day), C: Group III (SS; 1 mg/kg/day), D: Group IV (CP; 200 mg/kg /day + SS; 1 mg/kg/day). HE. CP: Cyclophosphamide, SS: Sodium selenite

### *Immunohistochemical findings*

The semi-quantitative evaluation results of the immunoistochemical findings of MT detected in the liver and kidney tissues of these groups in Groups II and Group IV, where CP was administered at high doses, are summarized in Table 3.

**Table 3.** Statistical evaluation of semiquantitative results of immunohistochemical findings of metallothionein antibody in which CP was given at high doses

| Groups | Organs | |
|---|---|---|
| | Liver | Kidney |
| **Group I** | $4.3 \pm 0.2^{a}$ | $5.0 \pm 0.2^{a}$ |
| **Group II** | $84.1 \pm 3.2^{c}$ | $85.7 \pm 3.3^{c}$ |
| **Group III** | $5.5 \pm 0.3^{a}$ | $6.2 \pm 0.2^{a}$ |
| **Group IV** | $41.8 \pm 1.0^{b}$ | $42.7 \pm 0.9^{b}$ |
| **P value** | <0.05 | |

Group I (Control group), Group II (CP; 200 mg/kg/day), Group III (SS; 1 mg/kg/day), Group IV (CP; 200 mg/kg/day + SS; 1 mg/ kg/day). $^{a\text{-}c}$: statistical difference in the same column, CP: Cyclophosphamide, SS: Sodium selenite

In Group II and Group IV, where CP was administered at high doses, the most intense MT expression level in the liver (Figure 3) and kidneys (Figure 4) was seen only in Group II, where CP was administered. In Group IV, in which CP was applied together with SS, MT expression intensity was found to be statistically significantly low ($P<0.05$).

## DISCUSSION

In cases where tumors show resistance to antineoplastic agents or the application doses of these agents are insufficient in human and veterinary medicine, high doses of many agents applied in chemotherapy, especially CP, are required (Cavalletti et al., 1986; Kaya et al., 2007). The most important factor limiting the increase of good therapeutic efficacy of CP, one of these chemical agents used in chemotherapy, is the toxic effects of the drug on liver and kidney tissues (Pool et al., 1988; Droller et al., 1982; Fraser et al., 1991; Schimmel et al., 2004).

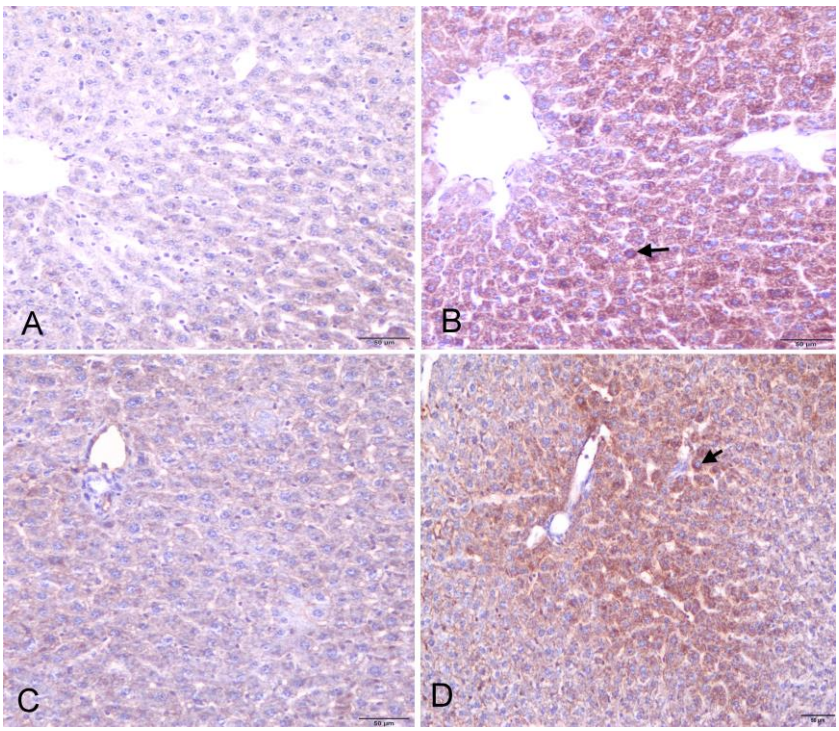


**Figure 3.** MT expressions in the livers of rats given high dose CP (arrows). A: Group I (Control group), B: Group II (CP; 200 mg/kg/day), C: Group III (SS; 1 mg/kg/day), D: Group IV (CP; 200 mg/kg /day + SS; 1 mg/kg/day). CP: Cyclophosphamide, SS: Sodium selenite

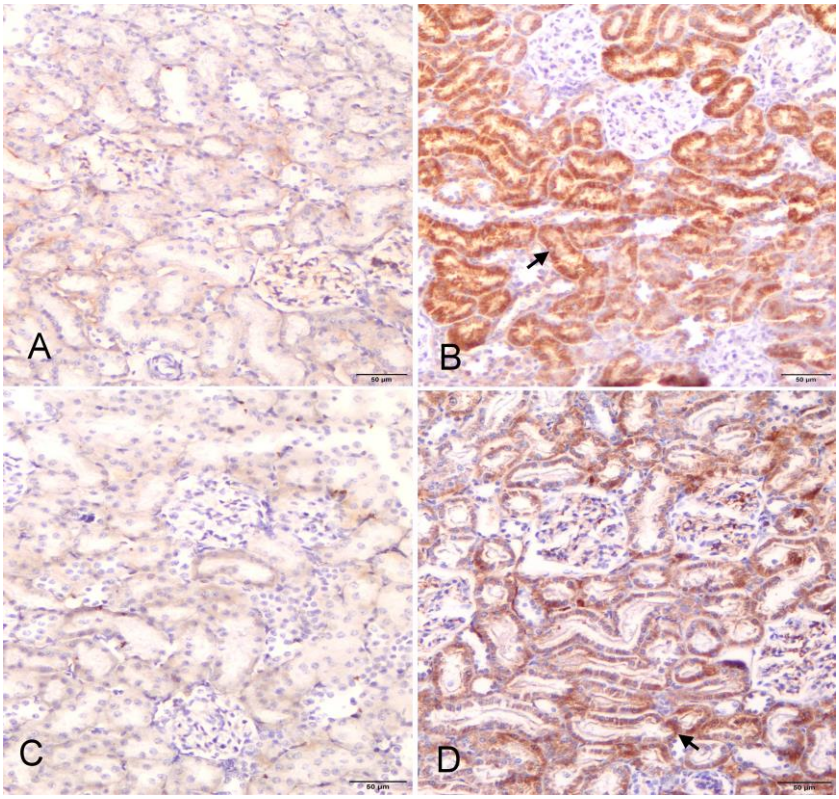


**Figure 4.** MT expressions in the kidneys of rats given high doses of CP (arrows). A: Group I (Control group), B: Group II (CP; 200 mg/kg/day), C: Group III (SS; 1 mg/kg/day), D: Group IV (CP; 200 mg/kg /day + SS; 1 mg/kg/day). CP: Cyclophosphamide, SS: Sodium selenite

The toxic effect of CP in tissues is related to its active metabolite, ACR. It has been stated that this toxic effect occurs by the destruction of the antioxidant defense systems of the organism by ACR, which is revealed by the metabolism of CP, and causes the formation of free radicals at high rates (Mccaroll et al., 2008). Although oxidative stress is effective in many pathological mechanisms in the organism, the excess free radicals that occur in these events are neutralized by the antioxidant system and a balance is provided in the organism. In case of disruption of this balance between the antioxidant system and free radicals, a number of pathological disorders occur (Bulkley, 1989; Valko et al., 2006). It has been reported that free radicals have a strong toxic effect and cause peroxidation and modification by oxidizing lipids, carbohydrates, proteins and DNA in cells (Hallıwell & Gutteridge, 1984). In the present study, it was observed that high dose CP administered to rats for 1 day caused hyperemia, degeneration, steatosis, necrosis and sinusoidal dilatation in hepatocytes in the liver, consistent with previous studies (Senthilkumar et al., 2006; Avcı et al., 2016). The histopathological findings we obtained in our study were associated with the toxic effect of CP caused by free radicals that started with FAM and emerged afterwards. In addition, high doses of CP were observed to cause glomerular congestion, hemorrhage, degeneration, separation and necrosis in the basal membranes of the tubules, in accordance with previous studies (Senthilkumar et al., 2006; Abraham et al., 2007). The histopathological findings in our study confirmed the knowledge that ACR, which is the metabolite of the toxic effect of CP, causes a high rate of free radical formation by destroying the antioxidant defense systems (Mccaroll et al., 2008). In this context, in our study, the use of SS, which is a strong antioxidant, was preferred in order to allow the use of CP at high doses. Se exerts its biological functions through the selenoproteins in the tissues. Selenoproteins modulate inflammation, eicozonoid synthesis, and prevent further progression of oxidative damage to biomolecules such as lipid, lipoprotein, and DNA (Rayman, 2000). Se is a necessary part of the glutathione peroxidase enzyme system; Glutathione is the coenzyme of glutathione peroxidase and plays an important role in scavenging free oxygen radicals. It also provides regeneration of antioxidant systems, reduction of nucleotides in DNA synthesis and intracellular redox state, which is important for cell proliferation. It protects endothelial cells against damage caused by peroxynitrite (Acar, 2015). There are studies showing that Se has a protective effect on the cell membrane by suppressing lipid peroxidation, as well as reducing the side effects of cytotoxic drugs such as cisplatin (Ilio et al., 1987; Yang et al., 2000). Regarding the protective efficacy of Se, in a study on CP-induced hepatotoxicity (Bhattacharjee et al., 2014), the protective efficacy of Se against CP-induced toxicity was confirmed histopathologically and reported. In another study (Acar, 2015) it was reported that Se given together with CP reduced the severity of histopathological findings such as liver degeneration and necrosis, and thus showed a protective effect against oxidative damage in the liver tissue. In the present study, it was observed that the severity of histopathological findings in the liver tissue in Group IV, which was administered high-dose CP+SS, was statistically significantly reduced compared to Group II, which was administered only high-dose CP. Again, this result was supported by the MT expression level in liver tissue. It was suggested that the decrease in the severity and distribution of histopathological findings in liver tissues may be related to the reduction of oxidative stress damage by sodium selenite. When the kidney tissues of rats belonging to all groups were examined, the most common and severe histopathological findings were observed only in Group II, where high-dose CP was given. These findings were similar to previous studies with CP in rats (Senthilkumar et al. 2006). In the study, the severity of the histopathological changes

observed in Group IV, in which CP+SS was applied, was found to be lower than in Group II, where only high-dose CP was applied. This result revealed that sodium selenite has a protective feature on kidney tissues at the histomorphological level in cases where CP is given at high doses. In addition, the low level of MT expression immunohistochemically, which confirmed the study, supported this view. Metallothionines are stress proteins. While they provide homeostasis of basic metals such as Zn and Cu, they also provide detoxification of heavy metals such as Cd and Cu (Viarengo & Nott, 1993). It has been stated that they participate in the process as natural anti-oxidative proteins, as they prevent cell damage against the oxidative effects of oxygen free radicals and hydroxyl radicals that arise for different reasons (Zhou et al., 2002). MTs have direct effects on cell proliferation by forming complexes with metals such as Cu and Zn. MTs have important effects in the prevention and treatment of inflammatory diseases in organs such as liver, kidney, pancreas, intestine and brain. Therefore, it is reported that the use of MTs for therapeutic purposes will be beneficial in maintaining the normal physiological activities of the organism (Simsek & Alabay, 2007). In this context, it was observed that sodium selenite, especially given together with high doses of CP, significantly reduced the severity of histopathological findings in liver and kidney tissue, and also significantly decreased the MT expression levels immunohistochemically in both tissues (Chmielewska et al., 2015). This result made us think that MTs can also be used as a stress protein marker in the liver and kidney when CP is given at high doses (Zambenedetti et al., 1998; Candan et al., 2017). In the present study, it was observed that sodium selenite was effective in terms of MT expression level against the toxic effects of CP in the liver and kidney tissues in cases requiring the use of CP in high doses. Therefore, it was concluded that sodium selenite can be used as an alternative supplement in cases where CP will be used in high doses.

## CONCLUSION

In recent years, research on the development of methods that prevent the toxic effects of many drugs, especially CP, which is one of the chemotherapeutic drugs, and allow these drugs to be used at low doses for a long time or at high doses, continues. When these drugs are used in high doses and frequently, they cause toxic side effects as well as their intended therapeutic effects. In the study, the most severe histopathological findings were observed in Group II, in which only high-dose CP was used, and we concluded that CP had a highly toxic effect on liver and kidney tissues. The high level of MT expression in liver and kidney tissues immunohistochemically supported this view. The low severity of histopathological findings and immunohistochemical MT expression level in the liver and kidney tissues in Group IV, in which SS was applied together with CP, compared to Group II, which was given only CP, suggest that SS can be used in the treatment in cases that require the use of CP at high doses. conclusion was reached.

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Conflict of interest: The authors declared that there is no conflict of interest.

Ethical statement: This study was approved by Aydın Adnan Menderes University Veterinary Faculty Experimental Animals Ethics Committee with the decision numbered 64583101/2016/146.

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# The effect of resveratrol on live weight, serum biochemistry and tissue antioxidant enzymes in rats


## ABSTRACT

The aim of this study is to determine the effects of resveratrol used in adding it to drinking water. The study was carried out with 24 Wistar albino male 20 days rats for 40 days. The study design was determined as one control and two trial groups (each group with 2 parallels and each parallel with 4 rats). The first group was given a basal ration and drinking water. The second group was given the basal ration and drinking water with 10 mg/kg resveratrol; and the third group was given the basal ration and drinking water 20 mg/kg resveratrol. In serum biochemistry parameters, significant decreases were observed in serum Aspartate aminotransferase (AST) enzyme activity, blood urea nitrogen (BUN), albumin, total protein and globulin concentrations and increase in albumin/globulin ratio in groups given resveratrol ($p<0.05$). Regardless of the dose, an increase in the concentration of glutathione (GSH) from the antioxidant parameters was observed in the liver; In the kidney, an increase was observed in the Res10 group. In addition, a decrease in muscle superoxide dismutase (SOD) activity and an increase in muscle and kidney catalase (CAT) activity were observed. A decrease was observed in malondialdehyde (MDA) concentrations, which is a parameter of lipid peroxidation in the kidney. As a result, it was determined in the present study that resveratrol has antioxidant effects regardless of the way of use, and it was concluded that this additive can be used for various purposes.


**Keywords:** Antioxidant, rat, resveratrol, serum biochemistry, water

## INTRODUCTION

Resveratrol (3,5,4'-trihydroxytrans-stilbene) is a polyphenolic compound found in 72 plants, mainly grapes and peanuts (Joe et al., 2002). This compound exists in two isomeric forms. Most of the transform is found in the peel of grapefruit. The synthesis of this form can be stimulated by ultraviolet (UV) light, puncture and fungal infection. The cis form is produced from the transform. It is usually absent or barely detectable in grapes. However, it was reported that it occurs during wine production (Moreno et al., 2008). Harada et al. (2011) found the average concentration of resveratrol in red wine as 4.7 mg/L.

Resveratrol was reported to have numerous pharmacological effects, including antioxidant, preventing cancer, anti-coagulation and inflammation, anti-aging, hypoglycemic and hypolipidemic effects (Nosá et al., 2014; Singh et al., 2015; Xie et al., 2013). It also shows antiestrogenic activity by inhibiting platelet aggregation (Stivala et al., 2001). Adrenaline induced by resveratrol has been reported to have a lipolytic effect (Szkudelska et al., 2009). Resveratrol is also reported to provide a decrease in inflammation through inhibition of prostaglandins and cyclooxygenase-2 (COX 2) (Shankar et al., 2007).

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**Research Article**

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In mouse modeling studies, the addition of resveratrol was reported to increase the concentrations of SOD and glutathione peroxidase (GPx), while reducing the concentration of MDA (Liu et al. 2012). Similarly, there are studies that report resveratrol increasing the concentration of antioxidant enzymes such as CAT, SOD and GSH-Px (Khan et al., 2013; Şahin et al., 2012). The antioxidant activity of resveratrol can also inhibit the oxidation of low-density lipoproteins (LDL). Therefore, it was also reported to prevent endothelial damage associated with cardiovascular disease (Frankel, 1993; Nigdikar et al., 1998). It was also reported that resveratrol increases the phosphorylation levels of proteins involved in the insulin signaling pathway in the liver in obese mice (Hong et al., 2014).

This study, unlike other studies, was designed on the hypothesis that Resveratrol would have positive effects by adding it to water instead of feed. In this context, the effects of Resveratrol on live weight change, feed consumption, serum biochemistry and antioxidant parameters in animals that consumed Resveratrol and those in the control groups will be examined.

## MATERIALS AND METHODS

### *Animal and treatment groups*

The study was performed with 24 Wistar albino male post weaned 20-day-old rats for 40 days. Resveratrol in the study (molecular formula C14H12O3, Cas no.: 501-36-0, purity: 99.13%, Chem-Impex International Company, USA) was provided from the market. The study design was determined as one control and two trial groups (each group with 2 parallels and each parallel with 4 rats). *Ad libitum* feed and water were given to the control and experimental groups. The first group (control) was given a basal ration and drinking water with 0 mg/kg resveratrol. The second group was given the basal ration and drinking water with 10 mg/kg resveratrol; and the third group was given the basal ration and drinking water 20 mg/kg resveratrol. The study was conducted in accordance with the dosage recommended by the OECD (1995). The rations used in the experiment were formulated according to the recommendations of NRC (1994). At the end of the study, the animals were weighed at beginning and the end to determine their live weight changes.

### *Biochemical parameters analysis*

At the end of the study, blood taken from animals (eight per group, total of 24) was separated from serum with a centrifuge device (Hettich - Eba 200, Germany) at 3000 rpm for 10 minutes. Glucose, total protein, albumin, globulin, creatinine, BUN, total bilirubin, total cholesterol, AST, gamma glutamyl transferase (GGT) levels in blood serum were determined by analyzing in a biochemistry device (Mindray 200, China) with an auto analyzer device.

### *Antioxidant parameter analysis*

At the end of the study, the blood serum, liver and muscle tissue homogenates were prepared. CAT, SOD, GSH and MDA for lipid peroxidation enzyme levels among the supernatant antioxidant parameters obtained from serum and tissue homogenates were determined by enzyme-linked immunosorbent assay (ELISA) testing device using commercial kits.

### *Statistical analysis*

In the study, arithmetic mean was used as descriptive statistics for groups and standard error was used as a measure of prevalence. Conformity of data to normal distribution was determined by Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) was used to determine the significance of differences in body weight changes, biochemical parameters and antioxidant parameters between groups, and Tukey and Tamhane's T2 multiple comparison test was used to determine differences between groups.

Analysis results were decided according to the significance level of $p<0.05$. The data were analyzed using the IBM SPSS Statistic V 21.0 package program.

## RESULTS

There was no statistical difference between the groups depending on the effect of resveratrol on the parameters of live weight and feed utilization rate at the beginning and end of the study ($p>0.05$) (Table 1).

**Table 1.** The effect of resveratrol on body weight and daily body weight gain in rats.

| Parameters | Control | Res10 | Res20 | p value |
|---|---|---|---|---|
| **Initial live weight, g** | 52.50±0.93 | 51.25±0.75 | 52.50±1.31 | 0.610 |
| **Finish live weight, g** | 146.38±1.16 | 139.75±4.90 | 145.25±4.60 | 0.460 |
| **Daily live weight gain, g** | 2.68±0.05 | 2.53±0.14 | 2.65±0.13 | 0.620 |

There was a significant reduction in parameters of BUN, AST, albumin, total protein, globulin and albumin/globulin ratio among serum biochemistry parameters depending on resveratrol ($p<0.05$). There was no statistical difference between the experimental groups in terms of creatinine, total cholesterol, GGT, total bilirubin, glucose parameters ($p>0.05$) (Table 2).

**Table 2.** The effect of resveratrol on serum biochemical parameters in rats.

| Analyzes | Control | Res10 | Res20 | p value |
|---|---|---|---|---|
| **Creatinine, mg/dL** | 0.50±0.03 | 0.51±0.06 | 0.37±0.06 | 0.140 |
| **BUN, mg/dL** | 28.38±1.77$^{a}$ | 26.25±1.31$^{ab}$ | 20.75±2.28$^{b}$ | 0.020 |
| **Total cholestrol, mg/dL** | 72.09±3.51 | 59.54±4.48 | 47.65±5.31 | 0.070 |
| **AST, U/L** | 154.80±4.45$^{a}$ | 130.71±5.17$^{b}$ | 93.00±7.99$^{c}$ | 0.001 |
| **GGT, U/L** | 1.00±0.01 | 1.25±0.25 | 1.50±0.29 | 0.530 |
| **Total Bilirubin, mg/dL** | 0.10±0.02 | 0.08±0.01 | 0.08±0.02 | 0.670 |
| **Albumin, g/dL** | 3.30±0.06$^{a}$ | 3.23±0.13$^{ab}$ | 2.18±0.35$^{b}$ | 0.002 |
| **Total Protein, g/dL** | 6.23±0.11$^{a}$ | 5.84±0.25$^{a}$ | 4.14±0.76$^{b}$ | 0.010 |
| **Globulin, g/dL** | 2.93±0.07$^{a}$ | 2.61±0.12$^{ab}$ | 1.79±0.40$^{b}$ | 0.010 |
| **Albumin/globulin, g/dL** | 1.13±0.02$^{b}$ | 1.24±0.03$^{ab}$ | 1.58±0.21$^{a}$ | 0.050 |
| **Glucose, mg/dL** | 144.38±7.20 | 146.25±5.88 | 128.60±7.43 | 0.220 |

BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase. $^{a-c}$: The difference between values with different letters on the same line is significant

Among the experimental groups within the scope of antioxidant parameters, GSH enzyme in liver tissue; GSH, MDA and CAT enzymes in kidney tissue; SOD and CAT enzyme concentrations in muscle tissue; statistically significant differences were found. ($p<0.05$). There was no statistical difference in serum GSH, MDA, SOD and CAT enzyme concentrations ($p>0.05$) (Table 3).

## DISCUSSION

Resveratrol was first used in Chinese and Japanese traditional medicine for the treatment of inflammation, allergies and hypertension diseases (Smoliga et al., 2013). Unlike previous studies, the current study tries to determine the effects of application in the form of adding resveratrol to water in varying dosages instead of dietary supplements.

There are many studies on the effect of resveratrol on live weight and feed consumption (Carbo' et al., 1999; Dal-Pan et al., 2010; He et al., 2019; Juan et al., 2002; Pallouf et al., 2019; Sridhar et al., 2015; Turner et al., 1999; Zhang et al., 2014, 2017). In this study; Juan et al. (2002)

found that 2g/kg of resveratrol has no negative effect on growth in rats; Turner et al. (1999) and Carbo' et al. (1999) reported that oral administration of 20 mg/kg of resveratrol for 28 days did not affect body weight or growth rate. In a resveratrol-administered study, no change in feed consumption was determined in mice fed with a diet high in fat and sugar for 12 months (C57BL/6) (Pallouf et al., 2019). A study conducted on different species reported that the addition of 0.5% and 1.0% resveratrol to the diets of broilers exposed to aflatoxin caused a decrease in body weight and feed consumption during a 5-week period but did not affect the feed conversion rate (Sridhar et al., 2015). It was reported that the addition of resveratrol to broiler rations at different rates (200, 400 and 800 mg/kg) does not affect the feed performance parameters (Zhang et al., 2014). In the current study, a similar effect to those of previous studies was found (Carbo' et al., 1999; Sridhar et al., 2015). However, He et al., (2019) reported that the addition of resveratrol at different levels (200, 350, 500 mg/kg) in broiler chickens under heat stress affects the average daily feed consumption and increases body weight. Zhang et al., (2017) reported that the average daily feed consumption, average live weight and feed utilization rate increased with the addition of resveratrol to broiler diets under heat stress. There are also studies reporting that dietary resveratrol supplementation (in the resveratrol groups of 100 and 400 mg/kg) caused a reduction in feed consumption, and claiming that this effect may be due to the softness of resveratrol (Dal-Pan et al., 2010).

**Table 3.** The effect of resveratrol supplementation on antioxidant and peroxidant enzyme concentration in liver, kidney, muscle and serum tissues of rats.

| Samples | Enzymes | Control | Res10 | Res20 | p value |
|---|---|---|---|---|---|
| Liver | GSH U/mL | 39.26±9.38$^{c}$ | 148.48±16.94$^{b}$ | 340.48±17.94$^{a}$ | 0.001 |
| | SOD U/mL | 65.17±3.23 | 66.08±1.23 | 65.97±2.45 | 0.960 |
| | MDA nmol/mL | 4.69±0.44 | 6.33±0.89 | 4.92±0.76 | 0.280 |
| | CAT U/mL | 0.04±0.01 | 0.07±0.02 | 0.05±0.01 | 0.330 |
| Kidney | GSH U/mL | 72.81±4.80$^{ab}$ | 82.78±5.59$^{a}$ | 55.72±2.22$^{b}$ | 0.020 |
| | SOD U/mL | 75.38±2.23 | 71.65±1.58 | 74.16±1.56 | 0.360 |
| | MDA nmol/mL | 15.18±1.49$^{a}$ | 13.94±0.59$^{a}$ | 5.44±0.33$^{b}$ | 0.001 |
| | CAT U/mL | 0.05±0.01$^{b}$ | 0.14±0.01$^{a}$ | 0.15±0.01$^{a}$ | 0.001 |
| Muscle | GSH U/mL | 80.00±6.57 | 53.37±2.60 | 51.11±4.96 | 0.300 |
| | SOD U/mL | 86.53±0.62$^{a}$ | 81.43±1.02$^{b}$ | 80.15±1.98$^{ab}$ | 0.020 |
| | MDA nmol/mL | 11.23±0.87 | 8.89±0.38 | 9.76±1.40 | 0.340 |
| | CAT U/mL | 0.04±0.01$^{c}$ | 0.11±0.01$^{b}$ | 0.51±0.07$^{a}$ | 0.001 |
| Serum | GSH U/mL | 49.45±1.61 | 46.67±3.01 | 60.94±5.56 | 0.110 |
| | SOD U/mL | 86.27±1.69 | 89.16±0.22 | 89.38±0.20 | 0.070 |
| | MDA nmol/mL | 3.10±0.28 | 3.85±0.48 | 4.20±0.30 | 0.100 |
| | CAT U/mL | 0.04±0.01 | 0.03±0.01 | 0.06±0.01 | 0.310 |

GSH: Glutation peroxidase, SOD: Super oxide dis mutase. MDA: Malondialdehyde, CAT: Catalase, $^{a-c}$: The difference between values with different letters on the same line is significant.

Routine blood parameters are widely used to study the effects of various factors on the body (Chand et al., 2018). Serum glucose, triglyceride and total protein parameters, which are generally used for this purpose, reflect sugar, fat and protein metabolism (Ghasemi and Nari, 2020; Hu et al., 2021). Especially serum triglyceride and cholesterol parameters are accepted as key

factors of lipid metabolism balance (Helkin et al., 2016). In a study conducted in quails, it was stated that 100, 200 and 400 mg/kg of resveratrol supplementation in the ration did not affect glucose, triglyceride and total protein levels in serum (Ölmez et al., 2020). In another study, it was stated that 400 mg/kg resveratrol added to the broiler ration did not affect glycogen, lactate and lactate dehydrogenase (LDH) values in breast meat (Zhang et al., 2018). Again, in a study conducted in sheep, it was reported that 450 and 900 mg/kg doses of curcumin added to the diet did not affect serum glucose, triglyceride and total cholesterol levels (Jiang et al., 2019). The serum biochemical profile provides valuable information about the health and immune status of animals, and serum BUN, creatine and creatine kinase levels are very important for kidney function (Comba et al., 2016). Increased blood urea level and creatinine may result from increased protein catabolism and/or adequate conversion of ammonia to urea (Badgujar et al., 2015). In a study, it was suggested that increased serum creatinine and urea levels as a result of fipronil intoxication reflect the toxicity, as well as seriously jeopardizing the kidney's capacity to filter wastes from the blood, and this may be an indicator of kidney damage (Mossa et al., 2015). In the current study, the fact that resveratrol added to water statistically lowers serum BUN, creatinine, albumin, total protein and globulin concentration may mean that it has no negative effects on the kidneys.

There are many biochemical studies on the use of resveratrol in animal trial studies (Poulsen et al., 2013; Wilson et al., 1996). In a study conducted in rats, it was reported that the injection of resveratrol in two doses of 20 and 40 mg/kg for 21 days did not change the ratio of cholesterol due to high-density lipoprotein (HDL) or LDL (Juan et al., 2002). Ghanim et al. (2010) reported in a study conducted on sick and healthy people that resveratrol supplementation of 40 mg for 6 weeks did not change the cholesterol (total, LDL and HDL) and triglycerides levels in the trial groups compared to the control group. When hypercholesterolemic rabbits were given resveratrol, no differences in lipoprotein levels were found (Wilson et al., 1996). Zhang et al. (2017) reported that the addition of resveratrol to broiler rations affected triglyceride and total protein concentrations and decreased glucose concentration. He et al. (2019) reported that resveratrol supplementation reduced serum glucose and total protein concentrations, while triglyceride concentrations did not change. Unlike previous studies, the current study found that Resveratrol supplementation reduces the level of total cholesterol, but that it also reduces the level of total protein similar to previous studies. The data in the current study are supported by studies which report that resveratrol also inhibits the oxidation of LDL and prevents cardiovascular diseases associated with endothelial damage (Frankel et al., 1993; Nigdikar et al., 1998).

There are many studies which report that resveratrol has antidiabetic effects (Bhatt et al., 2012; Hausenblas et al., 2014). Poulsen et al. (2013) reported that resveratrol supplementation prevents diseases such as diabetes, cancer and fatty liver, and also reduces insulin resistance (Szkudelski and Szkudelska, 2015). Although it was not statistically significant, the current study determined that the concentration of glucose decreased in direct proportion to the resveratrol ratio. It was reported to facilitate glycemic index control in diabetic animals given a diet with Resveratrol, to decrease serum LDL and triglyceride levels significantly, and to increase high-density lipoprotein (HDL) levels. In the same study, it was stated that animals given resveratrol was improved tolerance to glucose, but did not produce changes in liver and kidney function parameters (Raskovic et al., 2019).

In the evaluation of liver enzymes, Alema´n et al. (1998) reported that the AST concentration in rats given resveratrol was significantly higher than in the control group, but that the alanine aminotransferase (ALT) concentration did not

change. Another study showed that resveratrol (20 mg/kg daily for 4 weeks) inhibited dimethylnitrosamine-induced elevation of serum alanine transaminase, aspartate transaminase, alkaline phosphatase, and bilirubin (Lee et al., 2010). However, Upadhyay et al. (2008) reported that serum ALT and AST concentrations decreased significantly in a study conducted on Swiss albino mice with 10 mg/kg of resveratrol for 1-4 weeks. Likewise, the current study observed that the serum AST concentration decreased, in contrast to some previous studies. It is believed that this effect occurs similar to the positive effects of resveratrol on triglycerides, cholesterol and glycemic index.

The use of antioxidants in diet and skin care products has increased its popularity in the last few years. Intensive research has been conducted on resveratrol in recent years. Resveratrol is considered a very powerful antioxidant, which makes it a unique product. Research has shown that the polyphenols found in wines are some of the most powerful antioxidants, several times more powerful than vitamins A, C and E. Idebenone, the ubiquinone analogue, is considered the strongest topical antioxidant. However, recent studies have shown that resveratrol has about 17 times higher potency than Idebenone (Baxter, 2008). It was reported that the addition of Resveratrol to broiler rations helps them increase their antioxidant activity, and also contributes to the development of protein and total antioxidant capacity in plasma (Sridhar et al., 2015). Khan et al. (2013) and Şahin et al. (2012) reported that resveratrol increases the regulation of antioxidant enzymes such as CAT, SOD and GSH-Px, reducing oxidative stress and inflammation. There are studies, which report that resveratrol can increase the expression of various antioxidants, but it reduces the enzyme level and MDA content (Xia et al., 2017; Zhang et al., 2017). Şahin et al. (2010) reported that the serum MDA level was not affected in oviparous quails after the addition of resveratrol. In contrast to this study, Liu et al. (2014) stated that the addition of 400 mg/kg of Resveratrol to chicklet rations increased antioxidant capacity and reduced MDA content. In the presented study observed increases in GSH (liver), and CAT (muscle and kidney) concentrations. In general, a slight increase in serum MDA concentration, which is a lipid peroxidation parameter, was observed. A decrease was observed GSH and MDA concentration in muscle tissue, while it did not change SOD concentration in kidney tissues.

## CONCLUSION

As a result, in this study, it was aimed to determine the effects of resveratrol, which is used in various ways, by adding it to drinking water. There was no change in terms of live weight and daily live weight gain parameters as a result of resveratrol. In serum biochemistry parameters, however, a significant decrease was observed in urea, BUN, albumin, total protein, globulin and albumin/globulin ratios. It is thought that these decreases may occur due to the fact that resveratrol is structurally condensed with protein structures. There was also a significant decrease in the liver enzyme AST. A general increase in GSH levels among antioxidant parameters in the liver was observed, regardless of the dosage. Additionally, a very slight decrease was observed in the muscle tissue in SOD parameter. In general, a decrease was observed in MDA concentrations in the kidney tissue, a parameter of lipid peroxidation. It is believed that the increase in antioxidant enzyme levels and the decrease in MDA concentration are due to the antioxidant aspect of resveratrol. As a result, it was determined in the current research that resveratrol has antioxidant effects without depending on the usage patterns; and it was concluded that this additive can be used for various purposes.

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Conflict of interest: The authors declared that there is no conflict of interest.

Ethical statement: This study was carried out per Cumhuriyet University's approved ethical rules (protocol no. 2021/422 date: 20/04/2021).

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# Idebenone protects against ethanol toxicity in HT-22 cells through strengthening neuroimmune response

Research Article


## ABSTRACT

Idebenone, an analogue of coenzyme Q10, may function as a neuroprotective agent with its antioxidant and anti-inflammatory properties. The current report was designed to examine the beneficial effects of idebenone on ethanol-related neurotoxicity in hippocampal neuronal HT-22 cells in vitro and annotate the neuroprotective mechanism of idebenone. 75 mM ethanol was applied to the cells for 24h to develop ethanol toxicity. Then, different concentrations of idebenone (final concentration in the well to be 1, 2.5, and 5 µM) were applied to HT-22 cells for 24 h to explore the protective impact against ethanol-induced hippocampal damage. Cell viability was evaluated with MTT test. MDA, SOD, and GSH concentrations were examined to interpret oxidative damage. Moreover, the effects of idebenone on IL-1β, IL-6, and IL-23 neuroimmune-related genes expression levels were assigned by the RT-PCR analysis. In our study, 75 mM ethanol decreased neuronal cell viability by approximately 61%. All concentrations of idebenone were not toxic to neurons. In addition, idebenone increased cell viability by reducing the damage caused by alcohol. Idebenone reversed the reduction in antioxidant capacity caused by ethanol through decreasing MDA and increasing SOD and GSH levels. In addition, idebenone attenuated ethanol-induced impairment in neuroimmune and neuroinflammatory responses by reducing IL-1β, IL-6, and IL-23 mRNA expression levels. Treatment with idebenone increased antioxidant capacity and a significant improvement was achieved in neuroimmune and neuroinflammatory parameters. Possible mechanisms underlying these beneficial effects cover the down-regulation of IL-1β, IL-6, and IL-23 receptors, and antioxidant restoration of idebenone.


**Keywords:** Ethanol toxicity, HT-22, idebenone, neuroimmunity, oxidative damage


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## INTRODUCTION

Alcohol dependence, or alcoholism, is a chronic and severe condition affecting 140 million individuals globally. In accordance with the World Health Organization, 5.3% of all global loss of life and 5.1% of the global burden of illness and injury can be based on alcohol use disorder (AshaRani et al., 2022).

Growing evidence demonstrates that ethanol exposure can lead to acute and long-term cognitive impairment including memory dysfunction, resulting in considerable disability and cost to society (Belhorma et al., 2021).

The hippocampus is one of the most-investigated brain regions which contributes to cognitive functions as well as memory, and learning (Meier et al., 2022). Substantial reports propose that one of the principal regions of influence of alcohol toxicity is the hippocampus; indeed the alcoholic population indicates neuronal forfeit and a decline of hippocampal total volume as demonstrated by magnetic resonance imaging (Meier et al., 2022; Mira et al., 2019).

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The underlying mechanisms of alcohol-induced hippocampal impairment still remain elusive. Thanks to liposoluble feature of alcohol, it can easily cross the blood-brain barrier. Acetaldehyde, a metabolized product of alcohol, is highly toxic to nerve cells as it promotes oxidative stress (Gorky and Schwaber, 2016; Peana et al., 2017). Alcohol dependence, or alcoholism, is a chronic and severe condition affecting 140 million individuals globally. In accordance with the World Health Organization, 5.3% of all global loss of life and 5.1% of the global burden of illness and injury can be based on alcohol use disorder (AshaRani et al., 2022). Growing evidence demonstrates that ethanol exposure can lead to acute and long-term cognitive impairment including memory dysfunction, resulting in considerable disability and cost to society (Belhorma et al., 2021).

The hippocampus is one of the most-investigated brain regions which contributes to cognitive functions as well as memory, and learning (Meier et al., 2022). Substantial reports propose that one of the principal regions of influence of alcohol toxicity is the hippocampus; indeed the alcoholic population indicates neuronal forfeit and a decline of hippocampal total volume as demonstrated by magnetic resonance imaging (Meier et al., 2022; Mira et al., 2019).

The underlying mechanisms of alcohol-induced hippocampal impairment still remain elusive. Thanks to liposoluble feature of alcohol, it can easily cross the blood-brain barrier. Acetaldehyde, a metabolized product of alcohol, is highly toxic to nerve cells as it promotes oxidative stress (Gorky and Schwaber, 2016; Peana et al., 2017). Alcohol-mediated oxidative stress includes various mechanisms such as lipid peroxidation, impairment of antioxidants inclusive of superoxide dismutase (SOD) and glutathione (GSH) levels, and DNA strand breaks (Tsermpini et al., 2022). In response to oxidative damage, glial cells including microglia, astrocytes, and also neurons intervene with neuroimmune reactions via the synthesis of neuroimmune factors, and pro-inflammatory cytokines (Crews et al., 2015). The enhanced immune activation leads to feed-forward to perpetuate inflammation (Erickson et al., 2019; Kelley and Dantzer, 2011).

Interestingly, interleukin-1 beta (IL-1β), one of the important proinflammatory cytokines, implicates in pursuing of immune responses through elevated expression from microglia and contributing to the severity of alcohol-related hippocampal neurodegeneration (Coleman et al., 2018). Mainly, IL-1β regulates the generation of IL-6 which exerts a role in immune/inflammatory responses in immunomodulation (Szelényi, 2001). IL-6 in combination with IL-23 promotes IL-17 production from memory CD4+ T cells (Nitsch et al., 2021). IL-23, a proinflammatory cytokine, release from astrocytes and infiltrating macrophages and mediating the development of neuroinflammation diseases (Lowe et al., 2018). Alcoholism changes the neuroimmune system and especially impacts the cytokines inclusive of IL-1β, IL-6, and IL-23 milieu of the brain (Kelley et al., 2011; Lowe et al., 2018). In our study, we wanted to focus on the usage of drugs currently in clinical employ for novel indications. Because the safety of these remedies is already well known and this significantly alleviates the risk of unexpected side impacts.

Idebenone, a synthetic analogue of coenzyme Q10, is a considerable endogenous antioxidant and a fundamental component of the ATP-generating mitochondrial electron transport chain and endogenous antioxidant (Suárez-Rivero et al., 2021). Although idebenone contains the identical quinone piece as CoQ, idebenone has higher solubility and bioavailability than CoQ due to its shorter hydrophobic tail (Gueven et al., 2015; Suárez-Rivero et al., 2021). Idebenone crosses the blood-brain barrier easily and provides neuroprotection in vitro and in vivo models of neuronal harm, inclusive of oxidative stress

(Jaber et al., 2020). Also, it could attenuate the expression of the proinflammatory cytokine through the overexpression of enzymes related to ameliorating lipid peroxidation such as NAD(P)H dehydrogenase quinone 1/SOD (Shastri et al., 2020).

In this context, in the current study, we aimed to explore the protective effects of idebenone in ethanol-induced hippocampal neuronal toxicity in vitro model using mouse hippocampal neuronal cell line HT-22, with a focal point on the furthermore, on the relationship of idebenone with oxidative stress and neuroimmune reactions.

## MATERIALS AND METHODS

### *Ethanol toxicity and idebenone treatment*

The mouse hippocampal neuronal HT-22 cells were kindly provided by Asst. Prof. Caner Gunaydin (Samsun University, Samsun). Cells were seeded into 96-well plates at density of $5 \times 10^4$ cells/well and left to attach overnight. As a stressor, 75 mM ethanol was added to the wells for 30 min., and ethanol toxicity was created. Then, different concentrations of idebenone (1, 2.5, and 5 µM) were applied to HT-22 cells and left for 24 hours of incubation, and the effect of the active ingredient against ethanol toxicity was evaluated. After application, it was incubated for 24 hours (5% $CO_2$, 95% humidity, and 37°C).

### *MTT tetrazolium assay concept*

The MTT analysis was actualized with a commercially obtainable kit (Sigma Aldrich, USA). Succinctly, 10 µL MTT solution was put on each well and then incubated for 4 hours (5% $CO_2$; 37°C). Then, the medium was suspended and dimethylsulfoxide (DMSO; 100 µL) (Sigma Aldrich, USA) was suffixed to each well for dissolving formazan crystals. Cell viability (%) was quantified by optical density (OD) determined at 570 nm with the Multiskan ™ GO Microplate Spectrophotometer (Thermo Scientific, Canada, USA) (Okkay et al., 2021).

### *Oxidative stress parameters*

Malondialdehyde (MDA), SOD, and GSH were found with a commercial kit (Elabscience, USA) with respect to producer directions as reported before Okkay et al., 2021). OD was evaluated at the 450 nm wavelength.

### *Measurement of relative gene expression for real-time PCR analysis*

Total RNA was collected from hippocampal neuronal cells with TRIzol® reagent (Thermo Scientific, USA). Total RNA was employed for synthesizing cDNA using cDNA reverse transcription kit (Thermo Scientific, USA). The expression of IL-1β, IL-1α, and IL-23 mRNA was determined with Rotor-Gene Q (QIAGEN). Taq Man Gene Expression Master Mix kit was employed for PCR amplification and quantification. Findings were given as relative-fold in comparison to the control. We normalized IL-1β, IL-6, and IL-23 gene expressions to β-actin using the $2^{-\Delta\Delta Ct}$ method (Cicek et al., 2023). Information about the primary sequences of the genes is given in Table 1.

**Table 1.** Primers used for real-time PCR analysis

| Genes | Forward Sequence (5′-3′) | Reverse Sequence (3′-5′) |
|---|---|---|
| **IL-1β** | TGGACCTTCCAGGATGAGGACA | GTTCATCTCGGAGCCTGTAGTG |
| **IL-6** | TACCACTTCACAAGTCGGAGGC | CTGCAAGTGCATCATCGTTGTTC |
| **IL-23** | CATGCTAGCCTGGAACGCACAT | ACTGGCTGTTGTCCTTGAGTCC |

### *Statistical analysis*

Findings were calculated with IBM SPSS Statistics (Version 22.0, IBM Co., Chicago, IL, USA) software. All calculations were carried out by one-way analysis of variance (ANOVA) with post hoc Tukey's test. $p<0.05$ was appraised as statistically significant. Results were given as mean ± SD.

## RESULTS

### *Effect of idebenone on ethanol-induced reduction of cell viability*

As shown in Figure 1 cell viability was reduced by approximately 61% in hippocampal neuronal cells treated with 75 mM ethanol (p=0.0003). Our results also revealed that idebenone (1–5 µM) did not exhibit any toxic effect on the neurons and protect cell viability (p>0.05). As shown in Figure 1, treatment with 1, 2.5, and 5 µM idebenone for 24h induced a significant increase in cell viability by p=0.0013, p=0.0003 and p=0.0001, respectively compared with ethanol group.

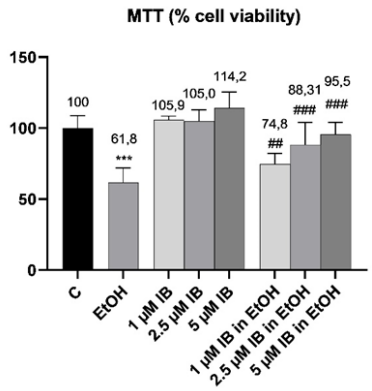


**Figure 1.** Cell viability in hippocampal HT-22 cell culture exposed to IB (alone) or in combination with 75 mM EtOH. 1, 2.5, and 5 µM concentrations of IB was added 30 min after to EtOH exposure and maintained in contact with neurons during EtOH exposure for 24 h. Control cultures were not exposed to EtOH. Data are expressed as the means ± SD. $^{***}p<0.001$ vs. control group, $\#p<0.05$ vs. EtOH group, $^{\#\#}p<0.01$ vs. EtOH group, $^{\#\#\#}p<0.001$ vs. EtOH group. C: Control; EtOH: Ethanol; IB: Idebenone.

### *Effect of idebenone on ethanol-induced oxidative damage*

As shown in Figure 2, the highest MDA level was observed in the ethanol group ($p<0.001$). Same time the increase of MDA level caused by ethanol decreased in all concentrations of idebenone treatment groups. The reduction in the MDA levels in the 1, 2.5, and 5 µM idebenone groups were statistically significant compared ethanol group (p=0.0227, p=0.0047, and p=0.0024; respectively). As shown in Figure 2, the SOD and GSH levels was the lowest in ethanol group ($p<0.001$). In addition, the decrease in SOD and GSH levels caused by ethanol increased as a result of the application of all concentrations of idebenone.

### *Effect of idebenone on ethanol-induced neuroimmune disorders*

The mRNA expression of IL-1β (p=0.0002), IL-6 ($p<0.001$) and IL-23 (p=0.0003) were markedly up-regulated in the ethanol group compared with the control group. Transcription levels of IL-1β, IL-6 and IL-23 at all concentrations of idebenone were significantly decreased in neuronal culture compared with that of ethanol control group (Figure 3).

## DISCUSSION

Alcohol is the most legal addictive drug worldwide and its excessive consumption is the third leading cause of death in the world. Excessive alcohol use is a health problem that causes brain intoxication, dementia, and cognitive disorders, and eventually to death as a result of depressive effects on the central nervous system (AshaRani et al.,2022; Belhorma et al., 2021; Meier et al., 2022; Mira et al., 2019). In addition to being a drug used for many congenital abnormalities, we think that idebenone may be a neuroprotective agent which can be improve alcohol neurotoxicity. However, there are no studies in the literature regarding the effectiveness and mechanism of action of idebenone on alcohol toxicity in hippocampal neuron culture. In this study, the effects of idebenone on neurodegeneration in the model of alcohol toxicity induced in hippocampal neuronal cells were investigated in vitro for the first time and important preclinical data on the therapeutic potential of idebenone in the treatment of alcohol toxicity were brought to the literature.

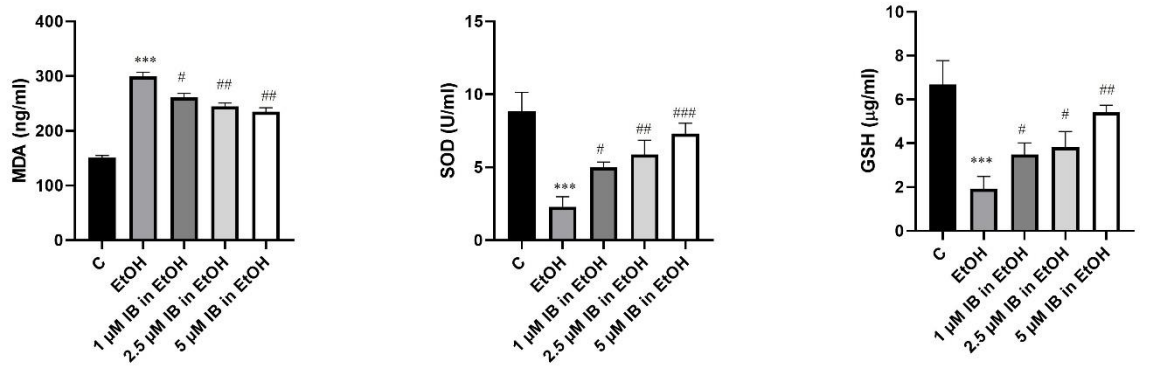


**Figure 2.** Effects of IB in combination with 75 mM EtOH on the oxidative stress parameters (MDA, SOD, and GSH) in hippocampal HT-22 cell culture. 1, 2.5, and 5 µM concentrations of IB was added 30 min after to EtOH exposure and maintained in contact with neurons during EtOH exposure for 24 h. Control cultures were not exposed to EtOH. Data are expressed as the means ± SD. ***p<0.001 vs. control group, #p< 0.05 vs. EtOH group, ##p<0.01 vs. EtOH group, ###p<0.001 vs. EtOH group. C: Control; EtOH: Ethanol; IB: Idebenone; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutathione.

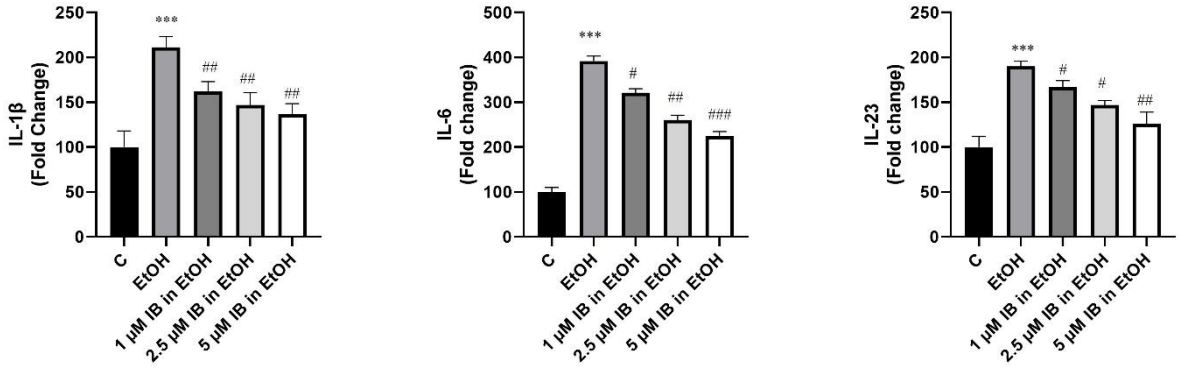


**Figure 3.** Effects of IB in combination with 75 mM EtOH on the neuroimmune markers (IL-1β, IL-6 and IL-23) in hippocampal HT-22 cell culture. 1, 2.5, and 5 µM concentrations of IB was added 30 min after to EtOH exposure and maintained in contact with neurons during EtOH exposure for 24 h. Control cultures were not exposed to EtOH. Data are expressed as the means ± SD. ***p<0.001 vs. control group, #p<0.05 vs. EtOH group, ##p<0.01 vs. EtOH group, ###p<0.001 vs. EtOH group. C: Control; EtOH: Ethanol; IB: Idebenone; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; IL-23: Interleukin 23.

The toxic effects of alcohol on neurons have been demonstrated in different preclinical models, including in vitro hippocampal, cortex cultures, etc. (Bailey et al., 2022; Bhowmick et al., 2022). Wang et al. demonstrated that hippocampal neuron injury was simulated by 200 mM ethanol in vitro and reduced cell viability by up to 70% (Wang et al., 2017). In our study, a cell model of alcohol toxicity model was first established using 75 mM ethanol, which reduced cell viability approximately 61%. Alcohol concentrations applied to hippocampal neuron cultures vary in the literature (Bailey et al., 2022; Bhowmick et al., 2022; Wang et al., 2017). However, we brought forward that these contradictions may be related to differences in cultural conditions including the continuum of cultures and the medium. Our finding demonstrated that 75 mM dose of alcohol exposure in vitro compromises the survival of cultured hippocampal neurons. Treatment with idebenone markedly protected in a in the HT-22 hippocampal neuron culture against ethanol-induced neurotoxicity and increased the cell viability. Muscolia et al. (2002) reported that liposomally entrapped idebenone reduced ethanol-induced injury of astrocytes by increasing of cellular viability. On the other hand, alcohol-related reduction of neuron viability was associated with a significant augmentation of

oxidative processes in cell cultures. Quintanilla et al. (2020) demonstrated that ethanol exposure in hippocampal neuron culture lead to enhancement of intracellular reactive oxygen species. Also, another study clearly demonstrated an increase in ethanol-induced ROS production and a decrease in GSH level and SOD and CAT activity involved in antioxidant defence (Song et al., 2015). In this report, we found that ethanol remarkably elevated MDA level and decreased SOD and GSH levels in HT-22 neuronal cells. Our results are in line with previous data demonstrating that treatment with ethanol produces oxidative stress and decreased efficiency of the cellular antioxidant mechanisms. Therefore, idebenone reversed neuronal damage in the ethanol-induced model by decreasing hippocampal neuronal oxidative stress. It is probably neuroprotective characteristics of idebenone by virtue of its antioxidant activity because it has been declared that antioxidants including α-tocopherol prevented the neurotoxicity (Lee et al., 2022).

It is renowned that oxidative stress can induce neuroimmune response and neuroinflammation (Dukay et al., 2021; Simpson and Oliver, 2020). Several studies have reported increases in the expression of genes encoding components of the IL-1β signaling pathways in neuron with a genetic predisposition to alcohol consumption (Patel et al., 2019; Varodayan et al., 2023). Alfonso-Loeches et al. (2016) findings show that ethanol triggers NLRP3 inflammasome activation in microglial cells and cultured microglia to cause the release of IL-1β which could amplify alcohol-induced neuroinflammation. Interestingly, the impaired neuroimmune response by chronic ethanol exposure is also associated with an elevation of IL-6 levels (Gano et al., 2017). It was also demonstrated that elevation of IL-6 in the hippocampus after ethanol intoxication is associated with the neuroimmune effects of ethanol (Gano et al., 2019). Our finding indicating that ethanol stimulated IL-6 expression in hippocampal cultures, is directly in line with this report. IL-23, a messenger of the immune system, is primarily secreted by microglia and infiltrating macrophages under inflammatory conditions (Nitsch et al., 2021). Also Lowe et al. (2018) demonstrated that alcohol treatment significantly increased the expression of IL-6 and IL-23 pro-inflammatory cytokine in the brain which was in line with our results. The data we obtained are consistent with the effects of ethanol: influences the neuroimmune system, specifically eliciting an increase in IL-1β, IL-6 and IL-23 proinflammatory cytokines. However, idebenone reversed the increased IL-1β, IL-6 and IL-23 induced by ethanol. These results indicate that restoration of neuroimmune response is a probable neuroprotective action of idebenone following ethanol exposure. The study of the effects of IL-1β, IL-6 and IL-23 on neuroimmune and neuroinflammation on the ethanol toxicity of idebenone for the first time in our study constitutes the specificity of our study.

## CONCLUSION

Our results indicated that exposure of hippocampal rat neuron culture to ethanol caused functional changes which are related to oxidative stress, neuroimmune response and neuroinflammation. These results are also confirmed by the evidence that the protective effects of idebenone on ethanol-induced toxicity might be associated with antioxidative and anti-inflammatory effects as well as the modulation of neuroimmune response via IL-1β, IL-6 and IL-23 signaling pathway. This may demonstrate a new and potentially useful approach to idebenone in the treatment of ethanol-induced neurodegenerative disorders.

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Not applicable

Ethical declaration: There are no ethical issues regarding the publication of this study.

Conflict of interest: There is no conflict of interest between the authors.

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# MS-222, propofol ve karanfil yağının *Daphnia magna*'larda anestezik etkisinin kalp atım oranlarına göre değerlendirilmesi

# Evaluation of the anesthetic effect of MS-222, propofol and clove oil in Daphnia magna according to heart rate

**Research Article**

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## ÖZET

Günümüzde Avrupa Birliği (EU), Amerikan Test ve Malzeme Kurumu (ASTM), Uluslararası Standardizasyon Teşkilatı (ISO), Ekonomik Kalkınma ve İş birliği Örgütü (OECD) gibi pek çok kuruluş; bazı kimyasal maddelerin, ilaçların gerek çevreye gerekse canlılara olan etkilerini invivo koşullarda araştırılması amacıyla *Daphnia magna* adı verilen sucul omurgasız canlıdan yararlanılmaktadır. Su ürünleri yetiştiriciliğinde karanfil yağı, MS 222 ve propofol yaygın bir şekilde kullanılan anestezik maddelerdendir. Ancak bu anestezik maddelerin tatlı su ortamında bulunan kabuklu canlılar için etkileri hakkında çok fazla bilgi yoktur. Yapılan bu çalışmada, anestezik etkisi olan, MS-222, propofol ve karanfil yağının su piresi olarak bilinen *Daphnia magna*'larda kısa süredeki kalp atım sayısında oluşturduğu farklar ve hayatta kalma etkisi araştırılmıştır. Anestezik maddeler 0.1-0.01-0.001 ml/L olarak uygulanmış ve çalışmanın 1., 3., ve 5. saatlerinde su pirelerinin kalp atım sayıları ve ölüm oranları değerlendirilmiştir. Deneme süresince elde edilen verilere göre kullanılan üç farklı anestezik maddenin kalp atım sayıları ve ölüm oranları üzerindeki etkileri bakımından anlamlı bir fark olduğu görülmüştür ($p<0.05$). Çalışmanın sonucu olarak düşük oranlarda bile bu üç farklı anestezik maddenin tatlı su ortamlarında bulunan omurgasız canlılar için toksik bir etki yaratabileceği ve ekolojik dengeyi etkileyebileceği görülmüştür.

**Anahtar Kelimeler:** Anesteziyoloji, karanfil yağı, MS 222, propofol, su piresi, toksikoloji

## ABSTRACT

Today, many organizations such as the European Union (EU), American Testing and Materials Agency (ASTM), International Organization for Standardization (ISO), Economic Development and Cooperation Organization (OECD); An aquatic invertebrate called Daphnia magna is used to investigate the effects of certain chemicals and drugs on both the environment and living things in vivo. Clove oil, MS 222 and propofol are commonly used anesthetics in aquaculture. However, there is not much information about the effects of these anesthetic agents for crustaceans in the freshwater environment. In this study, the effects of MS-222, propofol, and clove oil, which have anesthetic effects, in the short-term heart rate and survival effect of Daphnia magna, known as the water flea, were investigated. Anesthetic agents were administered as 0.1-0.01-0.001 ml/L, and heart rate and death rates of water fleas were evaluated at the 1st, 3rd, and 5th hours of the study. According to the data obtained during the trial, there was a significant difference in the effects of three different anesthetic agents on heart rate and death rates ($p<0.05$). As a result of the study, it has been seen that these three different anesthetic substances can create a toxic effect for invertebrates in freshwater environments and affect the ecological balance, even at low rates.

**Keywords:** Anesthesiology, clove oil, daphnia, MS 222, propofol, toxicology

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## GİRİŞ

*Phyllopoda* sınıfından planktonik bir organizma olan *Daphnia magna*; antibiyotikler, antidepresanlar, antiinflamatuar ilaçlar, beta-blokerler, lipid düzenleyiciler ve gibi birçok ilacın toksikolojik etkilerini belirlemek için kullanılmaktadır (Ebert, 2005; Tkaczyk vd., 2021). *Daphnia magna*; Avrupa Birliği, OECD, ASTM ve ISO gibi önemli organizasyonlar tarafından da sıklıkla toksisite testleri için tercih edilen organizmalardır (Ferraz, 2021).

Çeşitli ilaç çalışmalarında kullanılan *Daphnia magna* tatlısu kaynaklarında yaşar ve "su piresi" olarak da bilinmektedir. Oldukça küçüktürler ve 0.2-0.3 mm arasında değişen ebatlara sahiptir. Bu canlılar, akvaryum balıklarının beslenmesinde tercih edilmektedirler. Ayrıca gübreli toprak havuzlarda özel olarak yetiştirilen mersin balığı, sazan balığının da başlıca yemini oluşturmaktadır (Bat vd., 2008).

Son yıllarda, modern su ürünleri yetiştiriciliğinde anti-stres amaçlı olarak balıkların yakalanması, taşınması, suni üremesi, ameliyat prosedürlerine yardımcı olmak için farklı türde anestezikler kullanılmaktadır (Roubach vd., 2005). Anestezikler, merkezi sinir sistemi üzerinde, balığın solungaçlardan emilen ve atardamarlara giren anestezik bir solüsyona eklenmesi ile meydana gelir. Anestezikler ve metabolitler balığın tatlı suya geri bırakılması ile solungaçlar yardımıyla vücuttan atılması sayesinde, bu anestezik etki ortadan kalkar (Ross ve Ross, 1999).

Birkaç anestezik madde, avantaj ve dezavantajları ile balıkların anestezisinde etkili olduğunu kanıtlamıştır (Velíšek vd., 2006). Şimdiye kadar, ABD ve Birleşik Krallık'ta yemlik balıklarda kullanılmak üzere yalnızca MS-222 (trikain metansülfonat) tescil edilmiştir (Coyle vd., 2004).

Sedatif ve anestezik etkili olan Propofol (2,6 diizopropil fenol), ultra kısa etkili bir sedatif ajandır (Kay ve Stephenson 1980). Propofolün, yeşil iguanalar (Knotkova vd., 2005) gibi sürüngenlerde ve *Acipencer oxyrinchus De soti* (Fleming vd., 2003) gibi bazı balık türlerinde ve benekli bambu köpekbalıklarında (*Chiloscyllium plagiosum*) anestezi oluşturmak için kullanıldığı bildirilmiştir (Miller vd., 2005). Trikain metan-sülfonat (MS-222), 1967'de piyasaya sürülmesinden bu yana dünya çapında poikilotermler içinde en yaygın kullanılan anestezik ajanlardan biri olmuştur. Başlangıçta kokaine yerel bir analjezik alternatifi olarak üretilmiştir ve bu şekilde insanlarda kullanılmıştır. MS-222, suda yaşayan hayvanlar için de bir anestetik madde olarak kullanılabileceği kısa sürede anlaşılmıştır. MS-222, kristalimsi beyaz bir toz halindedir. Tatlı suda ve deniz suyunda kolaylıkla çözünür (Brown, 1993; Coyle vd., 2004; Daniel, 2009; EMEA, 1999; Maricchiolo ve Genovese, 2011; Treves-Brown, 2000; Ortuno vd., 2002).

*Eugenia caryophylatta* bitkisinin gövde, yaprak ve tomurcuklarından damıtma yoluyla elde edilen karanfil yağının etken maddesi yaklaşık %85-95 eugenol (4-ally- methoxyphenol $C_{10}H_{12}O_2$), %5-15 isoeugenol ve methyleugenol'dur (FDA, 2002; Kanyılmaz vd., 2007). Eugenol'ün prostaglandin H sentezini bloke etmesi, ileti engelleyici nörotransmitter olan GABA'ya (gama aminobütirik asit) agonist ve iletimde rol oynayan glutamat'a (N-metil-D- aspartat) antogonist etkisi ile, merkezi sinir sistemininde uyarıların kesintiye girmesi ile karanfil yağının anestezik etkisi meydana gelir (Yang vd., 2003; Pongprayon vd., 1991).

Yapılan bu çalışmada, anestezik etkisi olan, MS-222, propofol ve karanfil yağının su piresi olarak bilinen *Daphnia magna*'larda kısa süredeki kalp atım sayısında oluşturduğu farklar ve hayatta kalma etkisi araştırılmıştır.

## MATERYAL VE METHOD

Bu çalışmada sucul omurgasız su piresi olarak bilinen *Daphnia magna* kullanılmıştır. *D. magna* 2020 yılından beri su ürünleri laboratuvarımızda kültüre alınan ve üretimi yapılan canlı olup, su koşulları olarak ortalama 24°C su sıcaklığı ve 16 saat aydınlık, 8 saat karanlık ışık periyodunda tutulmaktadırlar.

### *Deneysel organizmalar ve kimyasallar*

Çalışma için seçilen canlılar ana kültür tanklarından alınıp 40x40x20 cm büyüklüğünde plastik malzemeden yapılmış tanka yerleştirilerek 3 gün boyunca *Chlorella sorokiniana* mikroalgi ile günde iki defa beslemeleri yapılmıştır. Çalışma da su ürünleri sektöründe kullanılan üç farklı anestezik madde MS 222 (Finquel, Argent Laboratuvarı), Propofol (BBraun, Almanya) ve Karanfil yağı denenmiş ve bunların *D. Magna*'lar için olumsuz etkileri saptanmaya çalışılmıştır. Bu anestezik maddeler üç farklı doz uygulanmış (0,1-0.01-0.001 ml/L) ve bunların *D. magna* üzerindeki kısa süreli etkileri değerlendirilmek için 1-3-5 saat aralıklarındaki kalp atım sayıları ölçülmüştür.

Her bir 20*10*20 cm büyüklüğündeki plastik kaplara 1litre su konulmuştur. Su sıcaklığı ortalama 24 ± 1°C tutulmuştur. Kullanılan anestezik ürünlerin suya herhangi bir pH değişikliği oluşturmamış ortalama 8.7 pH elde edilmiştir. Kullanılan karanfil yağı yerel bir aktardan alınarak 1:9 oranında %99.8 lik etanol ile seyreltilerek kullanılmıştır. MS 222 dozu, Western Chemical, Inc.'nin Pet ve Tropikal su canlılarına göre kullanımı esas alınarak hesaplanmıştır. Su pireleri canlı mikroalg kültürü Chlorella sorokiniana ile beslenmesi yapılmıştır.

### *Deneysel kurulum*

Uygulanan çalışma düzeneğinde 20*10*20 cm büyüklüğünde plastik malzemeden yapılmış kutular kullanılmıştır. Çalışma 3 farklı anestezik madde kullanılarak, 3 tekerrür olarak dizayn edilmiştir. Her bir grup da 24 adet canlı olup toplam 72 adet *Daphnia magna* kullanılmıştır. Her bir aneztezik madde için 0.1–0.01 ve 0.001 ml/L olarak dozlar uygulanmış olup her bir anestezik madde için canlıların boy ve en ölçümleri mikroskop yardımıyla oküler metre ile yapılmıştır. Ayrıca uygulanan dozların canlı üzerinde etkilerinin belirlenmesi amacıyla dakikadaki kalp atım sayıları belirlenmiştir.

### *Kalp atım oranı*

Bu uygulama için başlangıçta 1. saatte, 3. saatte ve 5. saatteki kalp atım sayıları SOİF marka mikroskop yardımıyla Sony marka fotoğraf makinası ile 6 sn süre ile görüntü kaydı alınmış ve görüntüler Windows Media Player ile yavaşlatılarak kalp atım sayıları belirlenmiştir (Şekil 1).

**Şekil 1.** *Daphnia magna* mikroskop görüntüsü. Kırmızı dairede kalbi görülmekte.

### *İstatistik analizler*

Verilerin istatistik analizinde IBM SPSS Statistics 25.0 paket programı kullanılmış olup, bağımsız grupların karşılaştırmasında Tek yönlü ANOVA testi ve gruplar arasındaki farklılığın tespiti amacıyla karşılaştırmalar Scheffe testi ile yapılmıştır ($p<0.05$). Tekrarlı ölçümlerin analizinde Genel Lineer Model üzerinden Tekrarlı Ölçümler için ANOVA testi yapılmıştır ($p<0.05$). Uygulanan farklı anestezik maddelerin oluşturduğu ölüm oranları ölçüm için belirlenen saatlerde ortamdan uzaklaştırılmıştır. Hayatta kalma oranları "Microsoft excel" programı ile tablo oluşturularak belirtilmiştir.

## BULGULAR

Tablo 1'e göre, 0.1 ml/L dozda 1. saatte karanfil yağı grubunda kalp atım oranı, MS-222 ve propofol grupları ile kıyaslandığında yaklaşık iki kat fazla olduğu görüldü. Aynı dozda 5. saatte karanfil yağının etkisinin 3. saate göre yaklaşık 2.5 kat fazla olduğu dikkat çekmiştir. Ancak Propofol ve MS-222 gruplarında 5. saatteki etkisinin giderek arttığı, kalp atım oranlarındaki azalış ile anlaşılmıştır. Çalışmamızda 0.01 ml/L dozda denenen anestezik maddelerde, en büyük değişim 5. saatte Propofol grubunda görülmüştür. Propofol, diğer anestezik maddelere göre kalp atım oranında bariz düşüş meydana getirmiştir. Karanfil yağı grubunda ise çalışmanın 0. saati ile 5. saati arasındaki fark giderek azalmıştır.

**Tablo 1.** *Daphnia magna* üzerinde farklı dozlarda uygulanan bitkisel ve kimyasal kökenli anestezik maddelerin kalp ritmi üzerindeki etkilerinin karşılaştırılması

| Doz mL/L | Kalp Atımı Ölçüm Saatleri | Anestezik Madde | | | | | | p value |
|---|---|---|---|---|---|---|---|---|
| | | Karanfil Yağı | | MS-222 | | Propofol | | |
| | | n | $\bar{x}$± SEM | n | $\bar{x}$± SEM | n | $\bar{x}$± SEM | |
| **0.1** | 0. saat | 24 | 321.29±2.64[a] | 24 | 321.67±2.60[a] | 24 | 322.08±2.82[a] | 0.979 |
| | 1. saat | 24 | 46.46±1.96[a] | 18 | 20.56±1.89[b] | 18 | 26.67±2.29[b] | <0.001 |
| | 3. saat | 20 | 25.50±1.85[a] | 13 | 13.85±1.40[b] | 15 | 19.13±1.19[b] | <0.001 |
| | 5. saat | 18 | 67.22±3.11[a] | 9 | 10.00±0.00[b] | 13 | 15.38±1.44[b] | <0.001 |
| **0.01** | 0. saat | 24 | 325.83±3.18[a] | 24 | 321.67±3.50[a] | 24 | 317.08±3.16[a] | 0.176 |
| | 1. saat | 24 | 161.67±3.17[a] | 21 | 53.57±2.27[b] | 22 | 44.55±2.25[b] | <0.001 |
| | 3. saat | 24 | 196.25±2.99[a] | 19 | 41.05±2.75[b] | 20 | 24.50±1.70[b] | <0.001 |
| | 5. saat | 24 | 245.21±5.23[a] | 17 | 30.88±1.73[b] | 19 | 24.21±1.92[b] | <0.001 |
| **0.001** | 0. saat | 24 | 321.67±2.23[a] | 24 | 322.08±2.82[a] | 24 | 320.83±3.51[a] | 0.953 |
| | 1. saat | 24 | 266.25±4.92[a] | 24 | 113.75±2.61[b] | 24 | 135.00±2.49[c] | <0.001 |
| | 3. saat | 24 | 302.92±2.52[a] | 24 | 99.17±2.33[b] | 24 | 122.92±2.04[c] | <0.001 |
| | 5. saat | 24 | 326.25±2.68[a] | 24 | 91.25±1.93[b] | 24 | 110.83±2.09[c] | <0.001 |

[a-c]: Aynı satırda farklı harflerle kodlanan değerler arasındaki fark istatistik olarak anlamlıdır.

**Tablo 2.** Tekrarlı ölçümlerde çoklu karşılaştırma sonuçları

| Anestezik Madde | | Ortalama Fark (i-j) | $S_X$ | p value[a] | Fark için %95 güven aralığı[b] | |
|---|---|---|---|---|---|---|
| i | j | | | | Alt sınır | Üst sınır |
| **Karanfil Yağı** | MS-222 | 97.205 | 1.321 | <0.001 | 94.008 | 100.403 |
| | Propofol | 93.776 | 1.155 | <0.001 | 90.982 | 96.571 |
| **MS-222** | Karanfil Yağı | 97.205 | 1.321 | <0.001 | -100.403 | -94.008 |
| | Propofol | -3.429 | 1.373 | 0.041 | -6.751 | -0.107 |

[a]: Ortalama fark 0.05 düzeyinde anlamlıdır. [b]: Çoklu karşılaştırmalar için Bonferroni düzeltmesi

Çalışmamızda 0.001 ml/L dozda ise Propofol grubunda diğerlerine göre daha etkili olduğu anlaşılmıştır. Karanfil yağı grubunda ise neredeyse önemli bir değişiklik oluşmamıştır. Tekrarlı ölçüm sonuçlarına göre (Tablo 2) tüm deneme süresi dikkate alındığında kullanılan anestezik maddelerin kalp atım sayıları üzerindeki etkileri bakımından gruplar arasında anlamlı farklılık oluşmuştur ($p<0.05$). Karanfil yağı ile MS-222 ve Propofol arasında oluşan fark, MS-222 ile Propofol arasındakinden daha fazladır.

**Tablo 3.** *Daphnia magna* farklı dozlarda uygulanan karanfil yağı, MS-222 ve propofolün kalp ritmi değişkenliği üzerindeki temel etkiler

| Etki | KO | sd | F | p value | $\eta^2$ |
|---|---|---|---|---|---|
| **Anestezik Madde** | 167496.28 | 2 | 4327.32 | <0.001 | 0.982 |
| **Doz** | 144751.30 | 2 | 3739.70 | <0.001 | 0.979 |
| **Anestezik Madde x Doz** | 22647.513 | 4 | 585.11 | <0.001 | 0.936 |
| **Hata** | 38.71 | 160 | - | - | - |

p: Tekrarlı ölçümlerde ANOVA; p<0.05

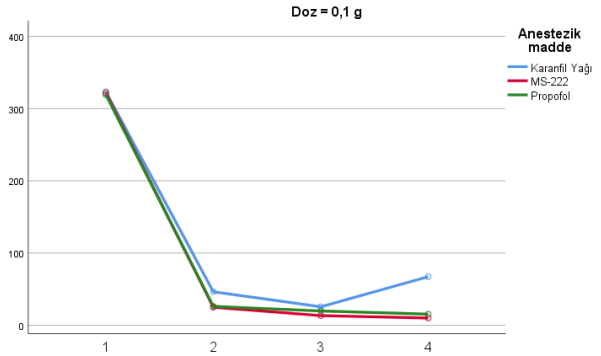
Kalp atımı üzerinde anestezik madde, doz ve bunların etkileşimlerinin (Tablo 3) temel etkilerinin istatistik anlamda önemli olduğu tespit edilmiştir (p<0.05). Bağımlı değişkendeki varyansın ne kadarının belirli bir bağımsız değişkenle açıklandığını gösteren değer olan eta kare ($\eta^2$) değerlerinin yüksek çıkması da dikkat çekicidir. Zaman, anestezik madde, doz ve bunların etkileşimleri (Tablo 4) istatistik olarak anlamlı bulunmuştur (p<0.05).

**Tablo 4.** *Daphnia magna* farklı dozlarda uygulanan karanfil yağı, MS-222 ve propofolün kalp ritmi değişkenliği üzerinde etkisi olan faktörler arasındaki etkileşimler

| Etki | KO | sd | F | p | $\eta^2$ | Mauchly's Test of Sphericity |
|---|---|---|---|---|---|---|
| **Zaman** | 1756455.69 | 3 | 9427.58 | <0.001 | 0.983 | 0.180 |
| **Zaman x Anestezik Madde** | 864432.66 | 6 | 463.92 | <0.001 | 0.853 | |
| **Zaman x Doz** | 65057.68 | 6 | 349.19 | <0.001 | 0.814 | |
| **ZamanxDozxAnestezik Madde** | 11979.29 | 12 | 64.30 | <0.001 | 0.616 | |
| **Hata** | 186.31 | 480 | - | - | - | |

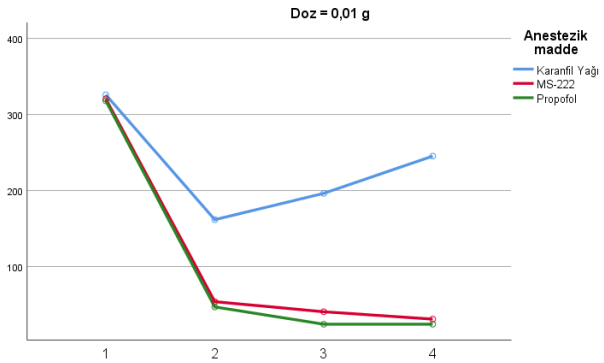
p: Tekrarlı ölçümlerde ANOVA; p<0.05

*Daphnia magna*'da gözlenen kalp atım sayılarındaki zaman göre seyir profili farklı dozlarda değişkenlik göstermiştir (Şekil 2, 3, 4). Yüksek dozda anestezik madde etkilerinin benzer olduğu gözlenirken, düşük doza inildikçe Karanfil yağının belirgin şekilde MS-222 ve Propofol'den farklılaştığı tespit edilmiştir.

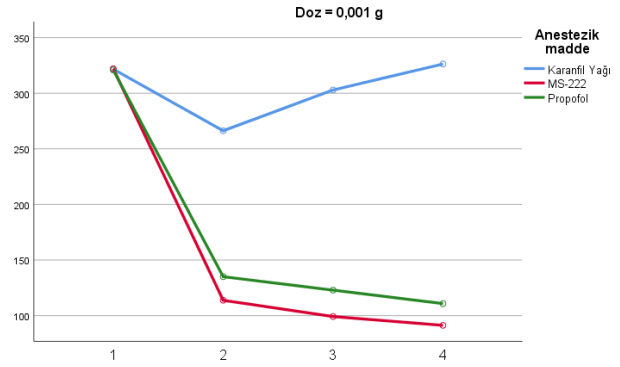



**Şekil 2.** Zamana bağlı kalp atım sayıları

Araştırmada ölüm oranı en fazla MS-222 grubunda en az ise Karanfil yağı grubunda gözlenmiştir (Şekil 5). Uygulanan farklı anestezik maddelerin oluşturduğu ölüm oranları ölçüm için belirlenen saatlerde ortamdan uzaklaştırılmıştır. Ölüm oranları bakımından gruplar arasında MS-222 grubunda en fazla, Karanfil yağı grubunda ise en az kayıp gözlenmiştir.



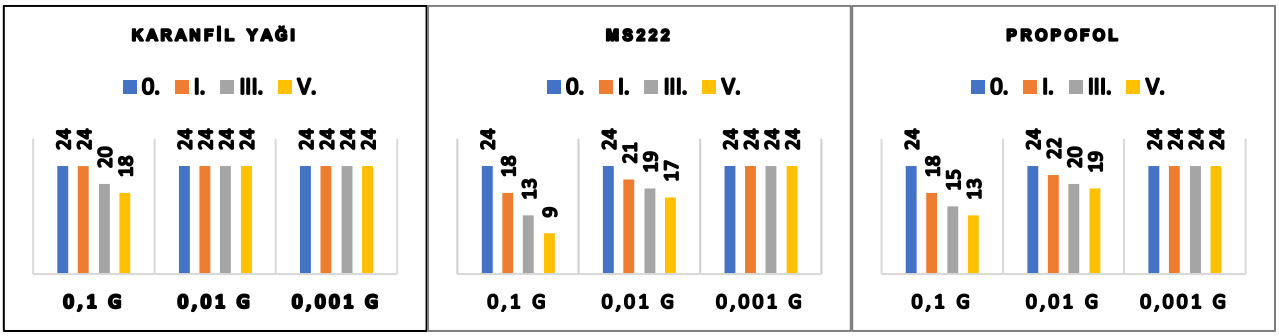

**Şekil 3.** Zamana bağlı kalp atım sayıları




**Şekil 4.** Zamana bağlı kalp atım sayıları

## TARTIŞMA

Daphnia'nın şeffaf olan kabuğu sayesinde basit bir ışık mikroskobu ile kalbi rahat görünebilmekte ve kalp atım sayısı belirlenebilmektedir (Tkaczyk vd., 2021). Anestezik maddeler uygulanan dozlar bazında ve her ölçüm dönemi ayrı olarak ele alınarak karşılaştırıldığında (Tablo 1), araştırma başlangıcında benzer olan kalp atım sayılarının 0,1 ve 0,01 ml/L dozunda karanfil yağının MS-222 ve Propofol'den farklılaştığı; 0,001 ml/L dozunda ise tüm grupların farklı etkilerinin olduğu gözlenmiştir. Özellikle karanfil yağının zamana bağlı olarak etkisinin azalmasının yağda çözülerek aktif hale getirilmiş olması, araştırma süresi içinde suyun değiştirilmemiş olması, oksijen verilmemesi ve uçucu olmasından kaynaklandığı düşünülmektedir.




**Şekil 5.** Hayatta kalma oranları

Tekrarlı ölçümler dikkate alınarak yapılan analizlerde (Tablo 2, 3, 4), zamanın, uygulanan dozun ve uygulanan anestezik maddenin kalp atımı üzerinde ayrı ayrı ve interaksiyonları ile etkili olduklarını göstermiştir. Yani her bir anestezik maddenin diğerinden ayrı etkisi olduğu, uygulanan dozlara göre farklı sonuçların gözlendiği ve süreye bağlı olarak anestezi etkisinde farklılıklar oluştuğu tespit edilmiştir. Özellikle yüksek dozda etkinin daha fazla olması nedeniyle MS-222 ve Propofol gruplarında kalp atımında doğrusal bir azalış ortaya çıkması beklenen bir sonuç olmakla beraber karanfil yağının uzun süreli etki göstermesi için suyun belli aralıklarla yenilenmesi gerektiği söylenebilir.

Bownik (2015)'e göre balık anestezisinde kullanılan karanfil yağının daha düşük dozları *Daphnia magna* için kalp atım sayılarında düşüşe neden olabilmektedir.

Ölüm oranları bakımından gruplar arasında MS-222 grubunda en fazla, Karanfil yağı grubunda ise en az kayıp gözlenmiştir. Félix vd. (2018) ifade ettiği üzere zebra balıkları embriyolarında yapılan çalışmada MS 222 anestezik maddenin 20 dakikalık kısa süreli etkisinde embriyoların mortalite oranında artış olduğunu vurgulamıştır. Aynı şekilde Jiang vd. (2023)'ne göre yaptıkları çalışmada intravenöz anesteziklerden olan propofolün sucul organizma olan zebra balıklarında potansiyel bir risk oluşturduğu görülmüştür. Doz esas alındığında 0.001 ml/L dozunda hiçbir grupta ölüm gözlenmemiş, 0.01 ml/L dozunda MS-222 ve Propofol gruplarında benzer sayıda ölüm gözlenirken, Karanfil yağı grubunda hiç ölüm görülmemiştir. 0.1 ml/L grubunda ise tüm gruplarda ölüm gözlenmiştir.

## SONUÇ

Çalışmada kullanılan karanfil yağı, etanol ile 1:9 oranında seyreltilerek kullanılmıştır. Etanol, uçucu bir etkiye sahiptir. Çalışmanın 1-3 ve 5.

saatlerindeki etkisi etanolün buharlaşması ve suyun değiştirilmemesi, oksijen kapasitesinin arttırılmaması ile karanfil yağının canlı üzerindeki anestezik etkisinin azalmasına sebep olduğu düşünülmüştür. MS-222 ise denekler üzerinde güçlü bir anestezik özelliğe sahip olmuştur. Propofol, diğer maddelere kıyasla orta seviyede etki gösterdiği belirlenmiştir. Böylelikle sucul organizmalar için MS-222 ve propofolün kullanımı potansiyel bir risk oluşturabileceği, karanfil yağının ise gösterdiği etkinin diğer kimyasal anesteziklere göre daha az olduğu görülmüştür.

## AÇIKLAMALAR

Çıkar çatışması: Yazarlar arasında herhangi bir çıkar çatışması bulunmamaktadır.

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# Iğdır yöresindeki koyunlarda *Schmallenberg virus* enfeksiyonunun belirlenmesi

# Detection of *Schmallenberg virus* infection in sheep in the Iğdır region


## ÖZET

Bu çalışmada, Iğdır ilinde yetiştirilen Morkaraman ırkı, atık yapmış ve *Brucella* spp. yönünden negatif koyunlarda, *Schmallenberg virus* (~SBV) enfeksiyonu yaygınlığının ortaya konulması amaçlanmıştır. Bu amaçla; Iğdır ilinde yetiştirilen Morkaraman ırkı, abort yapmış 2-6 yaş arasındaki toplam 180 adet koyundan alınan kan örneği ELISA yöntemi ile değerlendirildi. Yapılan çalışmada; incelenen 180 koyunun 7 (%3.88)'si SBV şüpheli ve 8 (%4.4)'i ise SBV seropozitif olarak tespit edildi. Sonuç olarak; Iğdır yöresinde ilk veri olma özelliği taşıyan çalışmamızın ayrıca Türkiye ortalamasına göre daha yüksek olması da dikkat çekicidir. Ülkemizde SBV enfeksiyonunun yaygınlığının ortaya konabilmesi için daha fazla materyal ile yürütülecek epidemiyolojik çalışmaların gerekli olduğu ve SBV enfeksiyonunun tüm risk faktörleri ile birlikte bu çalışmaların, hastalığın yayılması, bölgesel/ülkesel endemilerin önlenmesi, yavru kayıpları ile seyreden bu hastalıktan oluşabilecek ekonomik zararlarla mücadele de yüksek önem arz etmektedir.


**Anahtar Kelimeler:** Iğdır, koyun, *Schmallenberg virus*


## ABSTRACT

In this study, it was aimed to determine the prevalence of *Schmallenberg virus* (SBV) infection in Morkaraman breed, aborted and negative for *Brucella* spp. sheep reared in Iğdır province. For this purpose, blood samples taken from a total of 180 aborted Morkaraman breed sheep between 2-6 years of age were analyzed by ELISA method. In the study, 7 (3.88%) of 180 sheep were found to be SBV suspected and 8 (4.4%) were found to be SBV seropositive. In conclusion, it is noteworthy that our study, which is the first data in Iğdır region, is also higher than the average of Turkey. In order to determine the prevalence of SBV infection in our country, epidemiological studies to be carried out with more material are necessary and these studies, together with all risk factors of SBV infection, are of high importance in the spread of the disease, prevention of regional/national endemics, and combating economic damages that may arise from this disease, which is characterized by offspring losses.


**Keywords:** Iğdır, sheep, *Schmallenberg virus*

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## GİRİŞ

*Schmallenberg virus* (SBV) enfeksiyonu, çoğunlukla *Culicoides* türü sokucu ve kan emici sinekler ile taşınan enfeksiyöz bir hastalıktır (Conraths vd., 2013; Pawaiya ve Gupta, 2013; Yanase vd., 2013; Wernike vd., 2014). Adını Almanya'nın "Schmallenberg" kasabasından alan SBV enfeksiyonu; koyun ve keçilerde genellikle asemptomatik seyretmesine rağmen sığırlarda ateş, iştahsızlık, kondisyon kaybı, atık ve *arthrogryposis hydranencephaly sendromlu* yavru doğumlarıyla karakterizedir. Koyunlarda SBV enfeksiyonunda, kongenital malformasyonlara bağlı ölü doğumlar ve abortlar görülmektedir (Ducomble vd., 2012; Pawaiya ve Gupta, 2013; Wernike vd., 2013).

*Schmallenberg virus*, *Peribunyaviridae* familyasında, *Orthobunyavirus* genusunda Simbu serogrubundan zarlı, tek iplikçikli, segmentli bir RNA virustur (Doceul vd., 2013; Pawaiya ve Gupta, 2013; Tuncer ve Yeşilbağ, 2012; Wernike vd., 2013; Yanase vd., 2013). SBV; Almanya, Hollanda, Fransa, İtalya, Belçika, Büyük Britanya, Lüksemburg, İspanya ve Türkiye'de varlığı belirlenmiştir (Azkur vd., 2013; Bilk vd., 2012; Meroc vd., 2014). Bulaşmanın ruminantlarda sokucu ve kan emici sinekleri ile olmasının yanı sıra koyun ve keçilerde transplasental yolla da bulaş olduğu bildirilmiştir (Meroc vd., 2014; Wernike vd., 2014). Bulaşma riski altında bulunan diğer canlılardan köpeklerde antikor tespit edilirken, temaslı insanlardan alınan örneklerde antikora rastlanmadığı farklı araştırmacılar tarafından rapor edilmiştir (Ducomble vd., 2012; Reusken vd., 2012, Sailleau vd., 2013; Wensman vd., 2013).

Bu çalışmada, Iğdır ilinde yetiştirilen Morkaraman ırkı, atık yapmış, *Brucella sp.* yönünden negatif olan koyunlarda SBV enfeksiyonunun ELISA yöntemi ile varlığının ilk defa ortaya konulması amaçlanmıştır. SBV ülkemiz için nispeten yeni bir viral etken olup, Türkiye'deki varlığı/yaygınlığı ile ilgili oldukça sınırlı sayıda çalışma bulunmaktadır. Sunulan bu çalışma ile daha önce Iğdır bölgesinde varlığı bilinmeyen SBV'nin, atık yapmış koyunlarda varlığının belirlenmesi ve epidemiyolojik açıdan yeni araştırmalar için veri oluşumuna katkı sağlanması amaçlanmıştır.

## MATERYAL VE METHOD

### *Hayvan materyali*

Çalışma materyali olarak; Iğdır ilindeki 32 farklı morkaraman ırkı sürüsünde yetiştirilen, abort yapmış, 2-6 yaş arasındaki toplam 180 adet koyundan alınan kan örneği, 2021 Mayıs-Temmuz ayları arasında toplandı. Bu çalışma, Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 28.04.2021 tarihli ve 2021/077 numaralı etik kurul kararı ile yapılmıştır.

### *Kan örneklerinin toplanması*

Tüm hayvanların *Vena jugularis*'inden 10 mL'lik serum tüplerine kan örnekleri alındı. Alınan kan örnekleri 3000 devirde 10 dk santrifüj edilerek serumları ayrıldı, *Brucella sp.* için *rose bengal play test* ile negatifler belirlendi ve ELISA analizleri yapılıncaya kadar -20ºC'de dondurucuda muhafaza edildi. *SBV* spesifik antikorlar ticari bir ELISA (IDEXX Schmallenberg Ab Test®, IDEXX, Switzerland) kiti değerlendirildi. Sonuçlar üretici firmanın belirlediği [%S/P = 100 x (örnek OD – negatif kontrol OD)/(pozitif kontrol OD – negatif kontrol OD)] formülüne göre hesaplandı. Formüle göre hesaplanan sonuçlar %S/P <%30 ise negatif, ≥%30 ile <%40 arasında ise şüpheli, ≥%40 ise pozitif olarak değerlendirildi.

### *İstatistik analiz*

Çalışmada hayvan materyali aynı ırk, aynı yaş aralığı ve cinsiyete olduğu için kategorik değişkenler yüzde (%) olarak ifade edildi.

## BULGULAR

Çalışmaya dahil edilen, atık yapmış, *Brucella* spp. yönünden negatif 180 dişi koyundan

örneklenen serumların yalnızca 8 tanesinde (%4.4) *Schmallenberg virus* spesifik antikor varlığı pozitif tespit edildi. Testin uygulandığı koyunlardan 7 tanesinde (%3.88) ise test sonucu olarak şüpheli antikor pozitiflik belirlendi. Bunlardan seropozitif olanların 7 tanesi ve şüphelilerin 5 tanesinin ortalama 1200 m rakımda, diğer seropozitif 1 koyun ve şüpheli seropozitif 2 koyunun ise 1500 m'den yüksek rakımlarda barındırılmıştır (Tablo 1).

**Tablo 1.** Rakıma göre *Schmallenberg virus* özgül antikor pozitif ve şüpheli hayvan sayısı

| **Rakıma göre *Schmallenberg virus* örnek sayısı** | **Ortalama 1200 metre** | | **Ortalama 1500 metre ve daha yüksek** | |
|---|---|---|---|---|
| | Seropozitif | Şüpheli | Seropozitif | Şüpheli |
| | 7 | 5 | 1 | 2 |

## TARTIŞMA

Yeni bir *Orthobunyavirüs* olan SBV ilk olarak 2011 yılında izole edilmiştir (Hoffmann vd., 2012; Hoffmann vd., 2013; Pawaiya ve Gupta, 2013). SBV'un varlığı sığır, koyun, keçi, bizon, karaca ve alageyiklerde çeşitli araştırmalarda ortaya konulmuştur. SBV merkezi sinir sistemini de enfekte edebilir ve klinik olarak ateş, ishal, süt veriminde düşüş, konjenital malformasyonlar ve ölü doğumlara sıklıkla neden olabilir (Doceul vd., 2013; Pawaiya ve Gupta, 2013; Tuncer ve Yeşilbağ, 2012).

*Schmallenberg virus* kan emici sinekler aracılığı veya transplasental yol ile bulaşabilmektedir. Bununla birlikte keneler ve benzeri kan emici artropodların virüsü taşıdıklarını bildiren çalışmalara rastlanmamıştır (Doceul vd., 2013; Pawaiya ve Gupta, 2013; Tuncer ve Yeşilbağ, 2012). SBV'yi bulaştırabilen kan emicilerin biyolojik yaşam alanları içerisinde bu hastalığın görülebileceği, cinsiyetin ve yaşın hastalığın görülmesinde bir faktör olamayabileceği daha önceki çalışmalarda bildirilmiştir (Dik 1997; Meroc vd., 2014; Rossi vd., 2017; Van den Brom vd., 2012;)

*Schmallenberg virus* enfeksiyonunun inkübasyon periyodu 1-4 gün, viremi dönemi ise 1-6 gün olarak bildirilirken, hastalığın patogenezi hakkındaki veriler halen yeterli değildir. Enfeksiyonun, gebe hayvanlarda başta plasenta, fötusta ise dalak, serebrum, spinal kord olmak üzere çeşitli doku ve organlar üzerine etkili olduğu belirtilmektedir (Bilk vd., 2012; Doğan ve Dağalp, 2017).

*Schmallenberg virüs* enfeksiyonunu klinik olarak ilk defa Kuzeybatı Almanya ve Hollanda'nın doğu bölgesindeki yetişkin sığırlarda bildirilmiştir (Hoffmann vd., 2012). Fransa'daki sığır ve koyunlarda yapılan çalışmalarda seropozitiflik oranının %36-100 arasında değiştiği rapor edilmiştir (Doceul vd., 2013). Avusturya'da 2012-2013 yılları arasında yapılan bir çalışmada vahşi ruminatlarda SBV'nin seropozitifliği düşük oranda tespit edilirken bu oranın sığırlarda %98, koyunlarda ise %58.3-95.6 arasında olduğu ortaya konulmuştur (Steinrigl vd., 2014). Belçika'da 2011-2012 yıllarında yapılan çalışmalarda ise SBV seropozitifliğinin sığırlarda %91, koyunlarda ise %98,03 olduğu tespit edilmiştir (Garigliany vd., 2012; Meroc vd., 2014). Avrupa kıtasının dışında, Güneydoğu Afrika ülkesi olan Mozambik'te Blomström vd., 2014 yılında yaptıkları bir çalışmaya göre, SBV'nin seropozitifliğin sığırlarda %100, koyunlarda %43-97 ve keçilerde ise %72-100 oranlarında değiştiği rapor edilmiştir.

İsviçre'de SBV'ye karşı oluşan antikor varlığının süt örneklerinden değerlendirildiği ve hastalığın yaygınlığındaki mevsimsel farklılık üzerine yapılan bir çalışmada, temmuz ayında değerlendirilen 224 süt ineğinde %19.7 oranında seropozitiflik tespit edilirken Aralık

ayında değerlendirilen 211 süt ineğinde %99.5 oranında seropozitiflik olduğu araştırmacılar tarafından belirlenmiştir (Balmer vd., 2014). Benzer şekilde İrlanda'da SBV'nin 72 süt tankı örneğinde 9 pozitif 1 şüpheli seropozitif olarak rapor etmişler (Johnson vd., 2014).

Türkiye'de yapılan çalışmalarda, sığırlarda SBV'nin genom varlığı belirlenmiş (Yılmaz vd., 2014) ve seroprevalansın sığırlarda %39.8, keçilerde %2.5, koyunlarda %1.6 ve mandalarda %1.5 oranında olduğu bildirmiştir (Azkur vd., 2013). Afyonkarahisar ilinde ineklerde %13.51 oranında seropozitiflik belirlenmiştir (Bıyıklı vd., 2017). Macun vd., (2017) yılında Kırıkkale'deki koyunlarda yaptığı çalışmada SBV'nin seroprevalansını %0.38, Elmas vd., (2018) Sivas ilindeki Akkaraman ırkı koyunlarda yürüttüğü farklı bir çalışmada ise SBV'nin seroprevalansının %0.27 oranında olduğunu tespit edilmişlerdir.

Sunulan bu çalışmada elde ettiğimiz ve Türkiye'de daha önce yapılan çalışma verilerine göre nispeten yüksek olarak tespit ettiğimiz seroprevalans oranının, Iğdır ilinin mikroklima iklim özelliklerine sahip olması, vektör özellikte olan *Culicoides sp.* türü kan emen sokucu sinek yoğunluğunun, diğer araştırmaların yapıldığı illere göre fazla olma ihtimalinden kaynaklandığı düşünülmektedir.

Doğal enfekte yetişkin sığırlarda SBV enfeksiyonunu takiben 12-14 günlük sürede spesifik antikorun oluştuğu araştırmacılar tarafından bildirilmektedir (Conraths vd., 2013; Elbers vd., 2014). Sığırlarda oluşan bu spesifik antikorun 2 yıldan fazla sürede boyunca varlığının devam etmesine karşılık koyunlarda SBV ile doğal enfeksiyonda antikorların oluşumu veya varlığının devamı ile ilgili literatüre rastlanılmamaktadır. Bu çalışmada elde ettiğimiz seropozitifliğin varlığı, ülkemizde diğer bölgelerde yapılan çalışmaları desteklediği gibi, SBV pozitif seroprevalansın Türkiye'de yapılanlara göre yüksek verilere göre olması ve bu net değerin "şüpheli seropozitif örnek" oranına yakın olması, koyunlarda doğal enfeksiyon sonucu ortaya çıkan mevcut antikorların titresinde zaman içerisinde oluşabilecek bir azalma eğilimi ile açıklanabileceği kanaatine varıldı.

Bulaşmada önemli rol oynayan *Culicoides*'lerin yaşam aktiviteleri ışık, sıcaklık, rüzgar, nem ve rakım gibi birçok faktörden etkilenmektedir ancak etken Antarktika ve Yeni Zelanda hariç dünyanın her bölgesinde ve 4000 metre rakıma kadar görülebilen, 1368 üyesi olan, geniş yayılımlı bir genustur (Dik, 1997; Duan vd., 2019). Rossi vd., (2017) yabani ruminantlarda yaptıkları bir çalışmada 800 m'ye kadar olan rakımlarda SBV'nin seropozitifliğinde daha yüksek oranların belirlenebileceğine dikkat çekmişlerdir. Macun vd., (2017) ise ülkemizde yaptıkları çalışmada seropozitifliğin belli bir rakımda yoğunlaşmadığı görüşünü ileri sürmüşlerdir. Çalışma materyalimizi oluşturan koyunlar, Iğdır ilinde göçer hayvancılık yapılması nedeni ile çalışmanın yapıldığı aylarda yayla seviyesine yakın olan 1200 metre ve/veya daha üzeri rakımlarda bulunmaktadırlar. Koyunlardan seropozitif olanların 7 tanesi ve şüphelilerin 5 tanesinin yaklaşık 1200 m yükseklikte barındırıldıkları, diğer seropozitif 1 koyun ve şüpheli seropozitif 2 koyunun ise 1500 m'den yüksek rakımlarda barındırılıyor olması Macun vd., (2017)'nın bu konuda bildirdikleri ile daha uyumlu görülmüştür. Bununla birlikte koyunların barınma yüksekliği artıkça seropozitiflik veya şüpheli seropozitifliğin nispi ancak belirgin şekilde azalmış olduğu da çalışmamızdan elde ettiğimiz sonuçlar arasında dikkat çekmektedir.

## SONUÇ

Sonuç olarak, koyunlarda SBV seroprevalansının Iğdır'da ilk kez değerlendirildiği bu çalışmayla, virusun il genelinde ve bu coğrafyadaki varlığı serolojik olarak ortaya konulmuştur. Iğdır ilinde, morkaraman ırkı koyunlarda SBV

enfeksiyonunun, Türkiye'deki koyunlarda az sayıda yapılan çalışmalara göre önemli oranda yüksek seroprevalans değerine sahip olduğu belirlenmiştir. Çalışmamızdan elde ettiğimiz sonuçlar, Iğdır ilinde SBV enfeksiyonunun yaygınlığının ortaya konabilmesi için daha fazla materyal ile yürütülecek epidemiyololojik çalışmaların gerekli olduğu ve enfeksiyonunun tüm risk faktörlerin ile birlikte, başta *Culicoides* ve olası vektör sivrisineklerin araştırılmasının, hastalığın yayılması, bölgesel veya ülkesel endemilerin önlenmesi, yavru kayıpları ile seyreden bu hastalıktan oluşabilecek ekonomik zararlarla mücadele de yüksek önem arz ettiği kanaatindeyiz.

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# Aksaray Malaklı köpeklerinde tibia ve interkondiler genişlik indeksi üzerine morfometrik bir çalışma

# A morphometric study on tibia and intercondylar width index in Aksaray Malaklı dogs


## ÖZET

Bu çalışma Aksaray Malaklı köpeklerinde tibia ve interkondiler genişlik indeksinin belirlenmesi amacıyla yapılmıştır. Çalışmada 6 erkek ve 6 dişi olmak üzere toplamda 12 adet erişkin Aksaray Malaklı ırkı köpeğin toplam 14 adet tibia kemiği kullanılmıştır. Caudal yüzü kasete dönük olacak şekilde ve condylus lateralis ile condylus medialis'in uçları kasete temas edecek biçimde radyografisi çekilen tibia kemiklerinin ölçümü aynı cihaz üzerinde yapıldı (Fujifilm Fcr Prima T2 FVS-1000). İnterkondiler genişlik ölçümü için eminentia intercondylaris'i oluşturan iki çıkıntı üzerine doğrusal iki çizgi çizilerek bu iki çizgi arası, tibial plato ölçümü için condylus medialis ve condylus lateralis'in en uç kısımları arasında doğrusal bir çizgi çekilerek oluşan bu çizgi uzunluğu baz alındı. İntercondiler Genişlik İndeksi (ICWI), "interkondiler genişlik*tibial plato genişliği / 100'' formülü kullanılarak hesaplandı. Çalışmada kullanılan materyallerin tamamının ICWI indeksi ortalaması 20.09±2.48 cm, tibia uzunluğu 25.10±2.36 cm, gövde genişliği 2.12±0.41 cm, tibial plato genişliği 5.20±0.51 cm ve interkondiler genişlik ortalaması 1.06±0.19 cm olarak ölçüldü. Cinsiyetler arasındaki farkı anlayabilmek için Mann-Whitney U testi uygulaması yapılmış ancak istatiksel olarak anlamlı bir fark (p=0.798) bulunamamıştır. Yapılan çalışmada cinsiyetler arasında istatistiksel bir fark olmamakla birlikte; dişilerin ICWI indeksi ortalamasının (19.98±2.50 cm) erkeklere (20.19±2.65 cm) nazaran daha düşük olduğu tespit edilmiştir. Sonuç olarak; yapılan bu çalışmada, İntercondiler Genişlik İndeksi (ICWI)'nin CrCL ruptur insidansını etkilediği düşünülmektedir.


**Anahtar Kelimeler:** Aksaray Malaklı köpeği, intercondiler genişlik indeksi, tibia


## ABSTRACT

This study was carried out to determine the Tibia and Tibia Intercondylar Width Index in Aksaray Malaklı Dogs. A total of 14 tibia bones of 12 adult Aksaray Malaklı dogs, 6 male and 6 female, were used in the study. The measurement of the tibia bones, which were radiographed with the caudal side facing the cassette and the ends of the condylus lateralis and condylus medialis in contact with the cassette, were measured on the same device (Fujifilm Fcr Prima T2 FVS-1000). For the measurement of intercondylar width, two linear lines were drawn on the two projections forming the eminentia intercondylaris, and this line length was taken as a basis by drawing a linear line between these two lines, for the measurement of the tibial plateau, between the extreme parts of the condylus medialis and condylus lateralis. Intercondylar Width Index (ICWI) was calculated using the formula "intercondylar width*tibial plateau width / 100". The mean ICWI index of all the materials used in the study was 20.09±2.48 cm, tibia length was 25.10±2.36 cm, body width was 2.12±0.41 cm, tibial plateau width was 5.20±0.51 cm, and The mean intercondylar width was measured as 1.06±0.19 cm. Mann-Whitney U test was applied to understand the difference between the genders, but no statistically significant difference (p=0.798) was found. Although there is no statistical difference between the genders in the study; it was determined that the mean ICWI index of females (19.98±2.50 cm) was lower than that of males (20.19±2.65 cm). In conclusion, in this study, it is thought that the Intercondylar Width Index (ICWI) affects the incidence of CrCL rupture.


**Keywords:** Aksaray Malaklı dog, intercondylar width index, tibia

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**Research Article**

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## GİRİŞ

Articulatio genus (diz eklemi), femur, tibia ve patella kemiklerinin bir araya gelerek oluşturdukları bir eklemdir (König vd., 2007). Bu eklem, femur'la tibia arasındaki art. femorotibialis ve femur'la patella arasındaki art. femoropatellaris'ten oluşur (Dursun, 2006). Articulatio femorotibialis, femur'un extremitas distalis'i ile tibia'nın extremitas proximalis'i arasında oluşur (Done vd., 2009).

Femorotibial eklemi oluşturan unsurlardan ligamentum cruciata genus, eklemin gerilmesini önleyen, kemiklerin birbiri üzerinde kaymasını engelleyen "X" şeklindeki ligamentum cruciatum craniale ve ligamentum cruciatum caudale'dir (Dursun, 2005; Lopez vd., 2003; Tashman vd., 2004).

Ligamentum cruciatum craniale (CrCL) intra-articuler olarak femur'un condylus lateralis'inden orijin alıp, tibial plato üzerinde bulunan area intercondylaris cranialis'e yapışan oblik bir bağdır. (Tanegashima vd., 2019). Kraniyal çapraz bağ rupturu, köpeklerde önemli ekonomik ve klinik sonuçlar doğuran pelvik ekstremite topallığının en sık nedenlerinden biridir (Johnson vd., 1994, Wilke vd., 2005).

Köpeklerde ön çapraz bağ kopuğu ile ilgili birçok çalışmada (Guerrero 2007; Hayashi vd., 2004; Zeltzman 2005) nedeni tam olarak açıklanamamakla birlikte travmatik, dejeneratif nedenler, genetik faktörler, ırk predispozisyonu, cinsiyet, vücut ağırlığı faktörlerin rol oynadığı bildirilmiştir. Diz ekleminin aşırı gerilmesi, yüksekten düşme, atlama, zıplama sonucu, hayvanın birdenbire topallaması dikkat çekicidir (Buote vd., 2009; Cabrera vd., 2008; Doom vd. 2008).

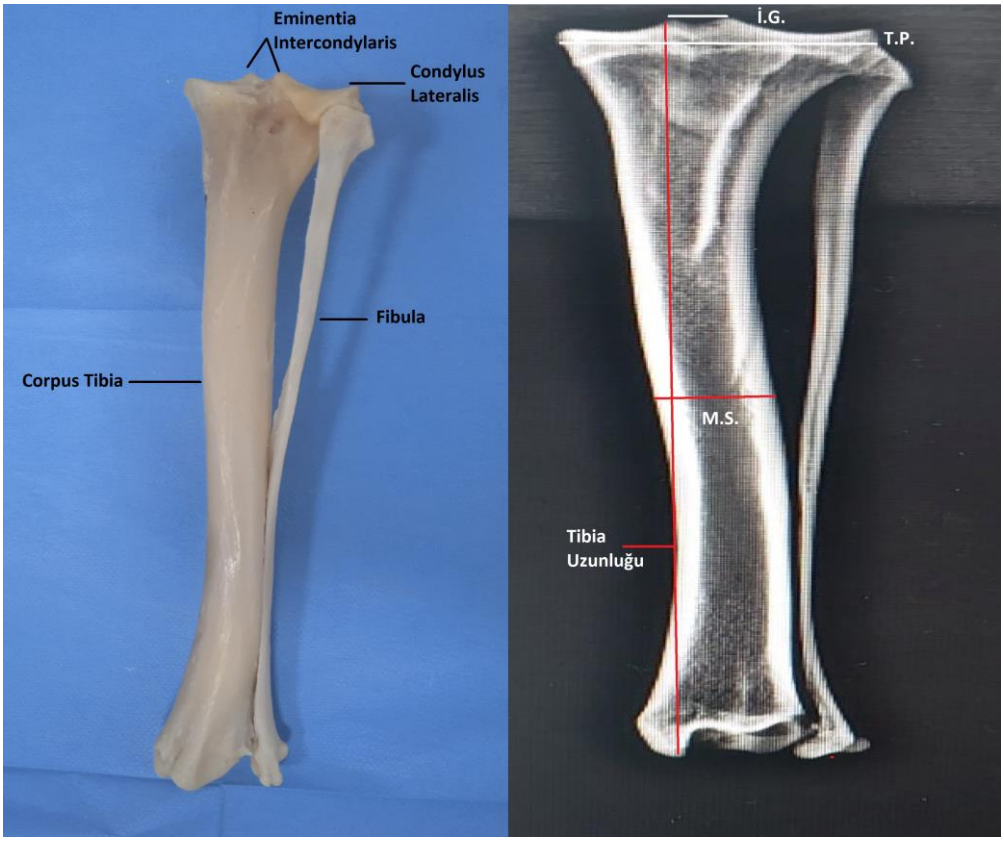
Ayrıca ön çapraz bağ kopuğu ile ilgili olarak femur'un trochlea ossis femoris'i ve tibial-plato eğimi gibi anatomik faktörlerin bu konuda etkili olabileceği vurgulanmıştır (Sabanci, 2022; Sabanci ve Öcal, 2014). Bu çalışmalar köpeklerde CrCL kopması tedavi stratejilerini ve potansiyel risk faktörlerini belirleme açısından önemli rol oynamaktadır. Bunlara ek olarak Sabancı (2022), CrCL boyutunun da CrCL rupturları konusunda anatomik yatkınlık açısından etkili olabileceğini bildirmiştir.

Anatomik faktörler göz önüne alındığında ligamentum cruciatum craniale boyunun, tibia'nın eminentia intercondylaris genişliğiyle yaklaşık olarak aynı olduğu var sayımı ile insanlarda "Eminens Genişlik İndeksi" (TEWI) geliştirilmiştir (Uhorchak vd., 2003). Bu indeks, tibia'nın eminentia intercondylaris genişliğinin tibial-plato genişliğine bölünmesiyle elde edilir. Bu veriler erkek ve kadınlarda non-travmatik CrCL rupturlarında olgu bulunan ve bulunmayan gruplar arasındaki farkı belirlemek amacıyla kullanılır (Uhorchak vd., 2003). Sabancı (2022), intercondiler terimini kullanmanın eminens teriminden daha uygun olduğunu ifade ederek "İntercondiler Genişlik İndeksi" (ICWI) ifadesini kullanmış ve bilindiği kadarıyla köpeklerdeki ilk ICWI ölçümünü yapmıştır. Bu çalışmada da "İnterkondiler Genişlik İndeksi" terimi "ICWI" (Intercondylar Width Index) kısaltması ile kullanılacak ve Aksaray Malaklı ırkı köpeği üzerine yapılan ölçümler sonucunda ICWI ölçümü yapılan köpek ırkları sayısının nicelik olarak artırılmasına katkı sağlanmış olacaktır.

## MATERYAL VE METHOD

Altı erkek ve altı dişi olmak üzere toplamda 12 adet erişkin Aksaray Malaklı ırkı köpeğin toplam 14 adet tibia kemiği kullanılmıştır. Kemikler, Aksaray Üniversitesi Veteriner Fakültesi Teşhis ve Analiz Laboratuvarı'na nekropsi amacıyla getirilen çeşitli sebeplerden ölmüş 12 adet yetişkin Aksaray Malaklı ırkı köpeği kadavrasından diseksiyon ve maserasyon işlemleri yapılarak ölçüme hazır hale getirildi. Hazırlanan tibia kemiklerinin craniocaudal radyografi görüntüleri, condylus lateralis ve condylus medialis uçları kasete temas edecek biçimde, kemiklerin caudal yüzleri kasete dönük olacak şekilde çekildi (Fujifilm Fcr Prima T2 FVS-1000) Radyografisi

çekilen tibia kemiklerinin ölçümü aynı cihaz üzerinde yapıldı. İnterkondiler genişlik ölçümü için eminentia intercondylaris'i oluşturan iki çıkıntı üzerine doğrusal iki çizgi çizilerek bu iki çizgi arası, tibial plato ölçümü için condylus medialis ve condylus lateralis'in en uç kısımları arasında doğrusal bir çizgi çekilerek oluşan bu çizgi uzunluğu baz alındı (Şekil 1).




**Şekil 1.** Aksaray Malaklı köpeği tibia'sının craniocaudal (normal ve radyografik) görüntüsü. İ.G.= İnterkondiler genişlik, T.P.= Tibial plato genişliği, M.S.=Mid-shaft genişliği

ICWI, "interkondiler genişlik*tibial plato genişliği / 100" formülü kullanılarak hesaplandı. 2 farklı kadavraya ait 4 tibia ölçümü sonucunda aynı kadavraya ait sağ ve sol tibia ölçümleri arasında anlamlı bir farklılık görülmediği tespit edildi. Ölçümü yapılan 10 tibia ise farklı kadavralardan elde edildi. Yapılan ölçümlere ait sonuçlar Tablo 1'de gösterildi. Dişi ve erkek kadavralara ait ICWI sonuçları arasında anlamlı bir fark olup olmadığını kontrol etmek amacıyla Mann-Whitney U testi uygulandı. İstatistiksel analiz için SPSS paket programı kullanılmıştır.

## BULGULAR

Altı erkek 6 dişi olmak üzere 12 adet Aksaray Malaklı ırkı köpeğin tibia kemiği incelendi. Bütün kemikler erişkin köpeklerden elde edildi. Bir erkek bir dişi kadavranın sağ ve sol tibia kemikleri birlikte incelendi. Anlamlı bir fark bulunmadığı üzere geri kalan tibia kemikleri unilateral olarak incelendi. Erkek Aksaray Malaklı ırkı köpeklerin kadavralarından diseke edilen tibia kemiklerinin ICWI indeksi ortalaması 20.19±2.65 cm olarak hesaplandı. Aynı gruba ait tibia uzunluğu ortalaması 26.98±1.48 cm, gövde genişliği ortalaması 2.24±0.44 cm, sırasıyla tibial plato genişliği ve interkondiler genişlik ortalamaları ise 5.23±0.39 cm ve 1.08±0.17 cm olarak ölçüldü. Dişi Aksaray Malaklı ırkı köpeklerin kadavralarından elde edilen tibia kemiklerinin ICWI indeksi ortalaması 19.98±2.50 cm olarak ölçüldü. Aynı gruba ait tibia uzunluğu ortalaması 23.23±1.30 cm, gövde genişliği ortalaması 2.00±0.38 cm,

sırasıyla tibial plato genişliği ve interkondiler genişlik ortalamaları ise 5.16±0.63 cm ve 1.04±0.23 cm olarak ölçüldü. ICWI indeksi ortalaması tüm köpekler için 20.09±2.48 cm bulundu. Tüm köpekler için tibia uzunluğu ortalaması 25.10±2.36 cm, gövde genişliği ortalaması 2.12±0.41 cm, tibial plato genişliği ortalaması 5.20±0.51 cm ve interkondiler genişlik ortalaması ise 1.06±0.19 cm olarak ölçüldü. Erkek ve dişi kadavralara ait Mann-Whitney U testi sonucuna göre erkek ve dişi kadavralara ait ICWI indeksi (p=0.85), Tibial plato genişliği (p=0.81), gövde genişliği (p=0.1) ve interkondiler genişlik (p=0.80) ölçüm değerleri arasında anlamlı bir fark bulunmadığı tespit edildi. Tibia uzunluğu ölçümleri arasında ise anlamlı bir farklılık olduğu sonucuna ulaşıldı (p=0.002, Tablo 1).

**Tablo 1.** Aksaray Malaklı ırkı köpeklerin tibia kemiğine ait ölçümler (cm)

| Cinsiyet | Tibia | İnterkondiler genişlik | Tibial plato genişliği | ICWI oranı (İ.G/T.P*100) | Gövde genişliği | Tibia uzunluğu |
|---|---|---|---|---|---|---|
| Erkek | N1 (sağ) | 1.22 | 5.08 | 24.01 | 1.95 | 25.87 |
| | N1* (sol) | 1.18 | 5.09 | 23.23 | 1.81 | 25.87 |
| | N2 (sol) | 1.01 | 5.21 | 19.38 | 1.99 | 24.98 |
| | N3 (sağ) | 0.81 | 5.00 | 16.20 | 2.03 | 26.88 |
| | N4 (sağ) | 1.08 | 5.59 | 19.32 | 2.23 | 28.17 |
| | N5 (sağ) | 1.28 | 5.89 | 19.15 | 3.01 | 29.01 |
| | N6 (sol) | 0.95 | 4.74 | 20.04 | 2.65 | 28,05 |
| | Ortalama±SD | 1.08±0.17 | 5.23±0.39 | 20.19±2.65 | 2.24±0.44 | 26.98±1.48 |
| Dişi | N7 (sol) | 1.32 | 5.78 | 22.84 | 2.53 | 24.63 |
| | N7* (sağ) | 1.32 | 5.90 | 22.37 | 2.58 | 24.89 |
| | N8 (sol) | 0.99 | 4.88 | 20.28 | 1.76 | 21.75 |
| | N9 (sağ) | 0.74 | 4.27 | 17.33 | 1.72 | 22.47 |
| | N10 (sağ) | 1.12 | 5.08 | 22.04 | 1.76 | 22.01 |
| | N11 (sol) | 0.81 | 4.57 | 17.72 | 1.75 | 22,72 |
| | N12 (sağ) | 0.98 | 5.66 | 17.31 | 1.95 | 24.16 |
| | Ortalama±SD | 1.04±0.23 | 5.16±0.63 | 19.98±2.50 | 2.00±0.38 | 23.23±1.30 |
| Toplam | Ortalama±SD | 1.06±0.19 | 5.20±0.51 | 20.09±2.48 | 2.12±0.41 | 25.10±2.36 |
| Erkek – Dişi p değeri | | p=0.80 | p=0.81 | p=0.85 | p=0.1 | p=0.002 |

Aynı kadavraya ait kemikler "*" ile işaretlenmiştir. İ.G.= İnterkondiler Genişlik, T.P.= Tibial Plato Genişliği, SD= Standart sapma.

## TARTIŞMA

Bu çalışmada Aksaray Malaklı ırkı köpeklere ait tibia kemiklerinin İnterkondiler Genişlik İndeksi, tibia uzunluğu, tibia kemiği gövde genişliği, tibial plato genişliği ve eminentia intercondylaris'in interkondiler genişliği ölçülmüştür. Bununla ilgili olarak non-travmatik CrCL kopmalarının multi-faktöriyel olduğuna inanılmaktadır ve köpeklerde karşılaşılan vakaların günden güne arttığı belirtilmiştir (Witsberger vd., 2008). Köpeklerde ön çapraz bağ kopuklarına özellikle 22 kg'ın üzerindeki köpek ırklarında daha fazla rastlandığı bildirilmiştir (Duval vd., 1999; Ünlü, 2011; Necas vd., 2000).

Ayrıca, CrCL kopmalarında yatkınlık anlamında ilk olarak iskelet faktörlerinin incelenmesi önerilmektedir (Tillman vd., 2002). Cinsiyet veya bireyler arasındaki anatomik farklılıklar CrCL rupturları açısından hazırlayıcı

etken olarak kabul edilmektedir (Hashemi vd., 2011). Bu nedenle anatomik faktörler üzerinde araştırmalar aktif olarak devam etmektedir (Griffin vd., 2006). Köpeklerde ırklar arası ölçüm farkının oldukça büyük olması nedeniyle mutlak bir ölçüm değeri kullanmanın zorluklara sebebiyet verdiği belirtilmektedir (Sabanci, 2022).

Köpek ırklarının çapraz bağ kopuklarının sağaltımında tibia'nın cranial'e subluksasyonunu engellemeyi amaçlayan tuberositas tibiae'yi öne taşıma tekniğinden (Çaptuğ, 2009) ve tibial plato düzeltme osteotomisi –TPLO (Aydın, 2010) operasyonlarından çok iyi sonuçlar alınmıştır.

Sabancı (2022), farklı köpek ırkları üzerinde ICWI ölçümü yaptığı bir çalışmada çapraz bağ yaralanmalarının tibial plato veya interkondiler genişlikte herhangi bir değişikliğe yol açmayacağı bildirmiştir. Aynı çalışmada 26 köpek ırkı üzerinde yapılan ölçümlerde ICWI indeks ortalamasının 19.2±2.72 cm, tibia uzunluğunun 19.7±4.72 cm, gövde genişliğinin 1.45±0.29 cm, tibial plato genişliğinin 3.53±0.68 cm, interkondiler genişliğin ise 0.67±0.14 cm olduğu bildirilmiştir. Yapılan çalışmada tüm köpekler için ICWI indeksi ortalaması 20.09±2.48 cm, tibia uzunluğu 25.10±2.36 cm, gövde genişliği 2.12±0.41 cm, tibial plato genişliği 5.20±0.51 cm ve interkondiler genişlik ortalaması 1.06±0.19 cm olarak ölçülmüştür. Elde edilen bu verilere göre Aksaray Malaklı köpeğinin tibia'sı ile ilgili ölçümlerin Sabancı (2022)'nın 26 köpek ırkı üzerinde yaptığı çalışmada bildirdiği ölçümlerden daha yüksek olduğu tespit edilmiştir.

Yapılan çalışmada Aksaray Malaklı köpeğinin erkeğinin tibia'larının ICWI indeksi 20.19±2.65 cm, dişisinin ise 19.98±2.50 cm olarak ölçülmüştür. Elde edilen bu verilerin Sabancı (2022)'nın Anadolu çoban köpeğinde bildirdiği veri ile (ICWI indeksi 19.9±1.76 cm) çok yakın olduğu belirlenmiştir. Literatürlerde (Barnes 1977; Denny ve Minter 1973; Whitehair vd., 1993) dişi köpeklerde CrCL ruptur insidansının erkeklere nazaran daha yüksek olduğu rapor edilmiştir. Aynı şekilde insanlarda da CrCL kopması ihtimali kadınlarda erkeklere nazaran daha yüksektir (Chandrashekar vd., 2006; Dienst vd., 2007).

Sunulan bu çalışmada da elde edilen verilere göre tibial plato genişliği (erkek 5.23±0.39 cm; dişi 5.16±0.63 cm) ve interkondiler genişliğin (erkek 1.08±0.17 cm; 1.04±0.23 cm) dişilerde erkeklere nazaran daha düşük olduğu tespit edilmiştir. Bu tespite göre tibial plato genişliği ve interkondiler genişliğin literatürlerde (Barnes 1977; Denny ve Minter 1973; Whitehair vd., 1993) bildirildiği gibi CrCL ruptur insidansını etkilediği düşünülebilir.

## SONUÇ

Sonuç olarak, büyük ve iri yapılı bir ırk olan Aksaray Malaklı köpeğinin CrCL ruptur vakalarında yapılacak olan tuberositas tibiae'yi öne taşıma tekniği ve tibial plato düzeltme osteotomisi –TPLO operasyonları için tibia hakkında anatomik bilgi verilmeye çalışılmıştır. Ayrıca, literatürde belirtildiği gibi köpek ırklarının çeşitliliğinin fazla olması ve anatomik ölçü farklarının büyük olması tür bazında mutlak ölçü değerleri kullanımını zorlaştırmaktadır. Bu nedenle ICWI ölçüm indeksi yapılan köpek ırklarının çeşitliliğinin artması, daha sonra bu çalışmalara eklenecek patolojik bozukluğu bulunan vakalarda aynı ölçümler ile yapılacak olan karşılaştırmalar, köpeklerin CrCL kopması konusunda anatomik yatkınlığının anlaşılması adına önem arz etmektedir.

## AÇIKLAMALAR

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# Serum pentraxin 3 levels in cats with feline parvovirus infection

Research Article


## ABSTRACT

Feline parvovirus (FPV) infection continues to be a serious problem in cats and therefore studies are ongoing to investigate all aspects of the disease. This study was designed to determine the levels of PTX-3 in cats with feline panleukopenia (FPL). Blood samples were taken from 12 cats of different breeds and gender with complaints of weakness, listlessness, anorexia, diarrhoea, vomiting and FPV positive on examination and from 7 cats found healthy on physical and laboratory examination. Whole blood, biochemical parameters, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSi), serum amyloid A (SAA), C-reactive protein (CRP) and pentraxin-3 (PTX-3) concentrations were determined in both sick and healthy cats. The results showed that there was marked panleukopenia and serum PTX-3 (58.69 pg/mL), SAA (59.91 µg/mL), TOS (14.35 µmol $H_2O_2$ Eq/L) and OSi (1.17 arbitrary unit) levels were significantly higher in cats diagnosed with FPL compared to healthy subjects. In conclusion, serum PTX- levels were measured for the first time in cats naturally infected with FPV and found to be elevated. Further clinical studies with large numbers of infected cats are needed to clarify these findings and to use PTX-3 as a reliable biomarker in FPV-infected cats.


**Keywords:** Acute phase proteins, cat, oxidative stress, panleukopenia, pentraxin-3


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## INTRODUCTION

Feline panleukopenia (FPL) is a highly contagious infectious disease of all Felidae caused by feline parvovirus (FPV). The disease is widespread throughout the world and mainly affects unvaccinated or partially vaccinated cats, especially kittens, with a high morbidity and mortality rate (Chowdhury et al., 2021; Dinçer and Timurkan, 2018; Truyen et al., 2009). The virus causes systemic infection after faecal-oral contamination. The initial replication site of infection is the oropharyngeal lymphoid tissue and then spreads to other tissues with high affinity to rapidly dividing cells (Awad, 2018; Greene, 2012). The most common clinical signs associated with FPL are anorexia, fever, depression, vomiting, diarrhoae, dehydration and the important consequence is sepsis-related death (Börkü, 2016; Truyen et al., 2009). The most characteristic laboratory finding is panleukopenia with a marked decrease in neutrophils and lymphocytes (Parrish, 1995).

Parvoviral enteritis and associated parameters have been well studied in details in dogs (Mazzafero, 2020), but there are limited number of studies evaluating parameters for diagnosis, prognosis and disease severity in cats with FPV (Petini et al., 2020).


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The status of some parameters such as C-reactive protein, serum amyloid A (SAA), haptoglobin, cholesterol, total thyroxine (tT4), complete blood count (leukocyte and their fractions, erythrocytes, thrombocytes etc.), blood chemistry (albumin, potassium etc.), redox balance (Vitamins D and E, glutathione peroxidase, superoxide dismutase, total antioxidant capacity, malondialdehyde) were related to progression and severity of the disease (Khoshvaghti and Nojaba, 2022; Kruse et al., 2010; Petini et al., 2020; Proporata et al., 2018). Recent studies revealed that the acute phase response develops after infections as a part of the innate host defense system that precedes the acquired immune response (Ceron et al., 2005; Paltrinieri, 2007; Schmidt and Eckersall, 2015) and has been used to assess FPL cases (Petini et al., 2020). However these studies are very limited both in number and content.

Pentraxin 3 (PTX-3), an acute phase proteins, has recectly gained attraction in human medicine in evaluating diagnosis, prognosis and severity of various diseases (Arslanoğlu, 2012; Bastrup-Birk et al., 2013; Liu et al, 2014). Pentraxin-3 (PTX3) plays a crucial role in innate immunity in inflammatory process (Zlibut et al., 2019), and its production in cells is particularly induced by inflammatory stimuli such as TNF-α and IL-1ß, and is stored in neutrophil granules (Günaştı et al., 2017). The main source of PTX-3 is also myeloid dendritic cells. It is produced by many cells such as fibroblasts, endothelial cells, monocytes, macrophages, smooth muscle cells, renal epithelial cells, synovial cells, chondrocytes, adipocytes and alveolar epithelial cells in response to proinflammatory cytokines (TNF-α, IFN-γ, IL-1ß, IL-8, IL-10), microorganisms and microbial products and has been reported to be associated with disease severity (Öztelcan, 2010). PTX-3 is a multifunctional protein and plays important roles in microbial recognition, immune regulation and tissue repair (Asgari et al., 2021). PTX-3 has been reported to inhibit and eliminate viral pathogens by binding to the sialic acid in the structure of PTX-3 and to haemagglutinin on the viral surface, thereby preventing its entry into the host cell, particularly dendritic cells, and binding to epithelial cells, and inducing antiviral immunity by regulating T-cell functions. It has also been reported that inhibition of the viral neuraminidase glycoprotein by PTX-3 prevents the release of newly formed viral particles from infected host cells. PTX-3 has also been shown to facilitate opsonisation and clearance of infected cells (Balhara et al, 2013; Deban et al, 2010; Perez 2019; Reading et al, 2008).

The fact that serum PTX-3 levels are less influenced by other inflammatory conditions and that plasma levels peak rapidly during infection has made it attractive for diagnostic and prognostic purposes in many pathological conditions. It has been shown that there is a positive correlation between the increase in plasma concentration and the severity of the disease (Inoue et al, 2011; Norata et al, 2010; Ristagno et al, 2019). However, to date there appear to be no studies on the status of PTX-3 in FPV-positive cats. Therefore, this study was designed to evaluate the concentrations of PTX-3 in cats infected with feline parvovirus infections.

## MATERIALS AND METHODS

### *Animals*

The animals used in this study were admitted to the Teaching Hospital of Faculty of Veterinary Medicine, Aksaray University and the Aksaray Private Veterinary Clinic for treatment or routine health checks. Written informed consent was obtained from the owners of each animal. The study was approved by the Local Ethical Committee for Animal Experimentation, Aksaray University.

The study involved 12 FPL positive and 7 healthy cats. Cats admitted with complaints of

anorexia, diarrhea, vomiting, fever lethargy and dullness and tested positive for FPV antigen (Vcheck V200, South Korea) were allocated to the FPL group. Those with no abnormal laboratory and clinical signs of any disease and tested negative for FPV antigen were assigned to the healthy control group. All cats in the FPL group received standard treatment for 7 days, including crystalloid fluid (isotonic saline, 44 mL/kg/day intravenously-IV, Polifeks perf., Polifarma, Türkiye), antibiotics (metronidazole, 25 mg/kg/day, IV, Polygyl %0.05 perf., Polifarma, Türkiye and amoxycillin+clavulanic acid, 20 mg/kg/day subcutaneously-SC, Synulox enj., Zoetis, Türkiye), supplemental therapy (inactive parapoxvirus extract, 1mL/cat, SC, Zylexis, Zoetis, Türkiye) (Greene, 2012).

### *Sampling*

Blood samples were taken from all cats on admission, followed by rectal faecal swabs. Blood from each cat was collected from the cephalic vein into plain and EDTA-treated tubes (BD microtainer, Switzerland) for biochemical and haematological analyses. Serum was collected after centrifugation at 5000 rpm for 10 min and stored at -80°C until analysis.

### *Laboratory analyses*

Faecal samples were tested in accordance with the manufacturer's instructions (Vcheck V200, Kore) and results were recorded as positive or negative.

Blood samples were analysed for complete blood count on a cell counter (Mindray BC30, Chine) for total leukocytes (WBC), erythrocytes (RBC), platelets (PLT), haemotocrit (HCT), haemoglobin concentration (HGB), neutrophils (NEU), lymphocytes (LYM), monocytes (MON) and eosinophils (EOS).

Biochemical analyses included alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transrase (GGT), blood urea nitrogen (BUN) and creatine (CRE) were performed on an automated analyser (FUJI DRI-CHEM NX600V IC, Japon). Acute phase proteins; C-reactive protein (CRP) and Serum amyloyid A (SAA) were determined using an automated analyser (Vcheck V200, South Korea).

Serum total oxidant status (TOS) and total antioxidant status (TAS) were measured calorimetrically (PowerWave XS, BioTek, Instruments, USA) using commercially available test kits (Rel Assay Diagnostics, Gaziantep, Türkiye) based on the method developed by Erel (2005). TAS was expressed as mmol Trolox Eq/L and TOS as µmol $H_2O_2$ Eq/L. Oxidative stress index (OSI) was calculated as OSi (arbitrary unit) = [(TOS, µmol/L)/(TAS, µmol Tro equivalent/L) × 100 (Gökçe et al., 2022).

Serum pentraxin-3 (PTX-3) was measured spectrophotometrically (PowerWave XS, BioTek, Instruments, USA) using a commercially available ELISA kit (Cat Pentraxin-3 ELISA kit, ELK Biotechnology, China) according to the manufacturer's instructions.

### *Statistical analyses*

Statistical analyses were performed using SPSS® (version 26.0, Chicago, IL, USA). Normality of data was tested by Shapiro-Wilk test and visually by histogram and Q-Q graph method. Groups were compared by independent samples t-test when data were normally distributed and by Mann-Whitney U test when data were not normally distributed. The stated sensitivity threshold was 13.5 pg/mL for PTX-3 and 5 µg/mL for SAA. Serum PTX-3 and SAA were tested below the sensitivity stated in the test kits for control cats, therefore these values were not included in the statistical analysis. The level of significance was set at $P<0.05$. Variables are presented as mean ± standard error.

## RESULTS

### *Clinical findings*

Of the cats diagnosed with FPL, 8 were female (66.6%) and 4 were male (33.4%). Two of these

cats were vaccinated (16.6%), 1 had unknown vaccination status (8.3%) and 9 were unvaccinated (75%). The mean age of the cats in the FPL group was 7.1 months (range 3-14 months). The mean age of the control group was 7.6 months (range 3-36 months) and all were vaccinated, three were males and four were females.

On clinical examination, common complaints on admission were anorexia (75%, 9/12), latergia (75%, 9/12), vomiting (75%, 9/12), diarrhoea (66.7%, 8/12), purulent nasal discharge (8.3%, 1/12) and no clinical signs (8.3%, 1/12). Of the treated cats, 3 died during the treatment (25%, 3/12).

### *Laboratory findings*

Haematological results showed a significant decrease in leukocytes and their fractions in the FPV group compared to the control group (Table 1). In the FPL group, total leukocytes (1.67±0.44/$10^9$/L), NEU (0.64±0.24/$10^9$/L), LYM (1.22±0.23/$10^9$/L), MON (0.12±0.03/$10^9$/L) and EOS (0.28±0. 0.06/$10^9$/L) were significantly lower than those of healthy cats (WBC, 10.96±1.23 $10^9$/L; NEU, 5.41±0.88/$10^9$/L, LYM, 3.94±0.52 $10^9$/L; MON, 0.84±0.12 $10^9$/L; EOS, 0.76±0.11 $10^9$/L) (P<0.05).

**Table 1.** Changes in haemotological parameters in the study groups.

| Parameters | Groups | Mean±SE | P value |
|---|---|---|---|
| **WBC ($10^9$/L)** | FPL | 1.67±0.44 | <0.001 |
| | Control | 10.96±1.23 | |
| **NEU ($10^9$/L)** | FPL | 0.64±0.24 | <0.001 |
| | Control | 5.41±0.88 | |
| **LYM ($10^9$/L)** | FPL | 1.22±0.23 | <0.001 |
| | Control | 3.94±0.52 | |
| **MON ($10^9$/L)** | FPL | 0.12±0.03 | <0.001 |
| | Control | 0.84±0.12 | |
| **EOS ($10^9$/L)** | FPL | 0.28±0.06 | <0.001 |
| | Control | 0.76±0.11 | |
| **RBC ($10^{12}$/L)** | FPL | 12.02±2.29 | 0.32 |
| | Control | 8.81±0.95 | |
| **HCT (%)** | FPL | 37.64±2.70 | 0.85 |
| | Control | 36.75±3.63 | |
| **PLT $10^9$/L** | FPL | 228.27±41.12 | 0.36 |
| | Control | 297.17±64.50 | |

WBC: Total leukocytes, RBC: Erythrocytes, PLT: Platelets, HCT: Haemotocrit, HGB: Haemoglobin, NEU: Neutrophils, LTM: Lymphocytes, MON: Monocytes, EOS: Eosinophils, SE: Standard error, FPL: Feline panleukopenia

Biochemical anaylses resulted in no significant changes in ALT, AST, BUN and CRE in both groups (Table 2). Only GGT was significantly lower in the FPL group (2.03±0.59 U/L) when compared to the control group (4.37±0.67 U/L) (P=0.02).

Results of acute phase proteins and redox status are given in Table 3. Cats with FPL had serum PTX-3 concentration of 58.69±11.59

pg/mL but the values were not detectable in the control group as the test assay had a sensitivity below 13.5 pg/mL. Smilarly, the serum SAA concentration of FPL was 59.91±10.12 µg/mL and was undetectable in the control cats due to the sensitivity of the assay at 5 µg/mL. Although serum CRP concentrations were higher in cases (5.85±1.27 mg/mL) than in controls (3.46±0.81 mg/mL), this was not significant (P=0.2). Cats with FPL experienced oxidative stress as TOS and OSi values were significantly higher in cases (TOS, 14.35±1.28 µmol $H_2O_2$ Eq/L; OSi, 1.17±0.15 AU) than in controls (TOS, 9.36±0.22 µmol $H_2O_2$ Eq/L; OSi, 0.77±0.02 AU) (P<0.05).

**Table 2.** Changes in biochemical parameters between the groups.

| Parameters | Groups | Mean±SE | P value |
|---|---|---|---|
| **Alanine aminotransferase (U/L)** | FPL | 83.08±13.40 | 0.58 |
| | Control | 72.14±10.53 | |
| **Aspartate aminotransferase (U/L)** | FPL | 22.08±1.93 | 0.49 |
| | Control | 24.57±3.19 | |
| **Gamma glutamyl transferase (U/L)** | FPL | 2.03±0.59 | 0.02 |
| | Control | 4.37±0.67 | |
| **Blood urea nitrogen (mmol/L)** | FPL | 4.99±0.39 | 0.39 |
| | Control | 5.94±0.97 | |
| **Creatine (µmol/L)** | FPL | 33.08±7.37 | 0.81 |
| | Control | 30.44±5.14 | |

SE: Standard error, FPL: Feline panleukopenia

**Table 3.** Changes in acute phase protein and oxidative parameters measured in groups.

| Parameters | Groups | Mean±SE | P value |
|---|---|---|---|
| **PTX-3 (pg/mL)** | FPL | 58.69±11.59 | - |
| | Control | UD | |
| **SAA (µg/mL)** | FPL | 59.91±10.12 | - |
| | Control | UD | |
| **CRP (mg/mL)** | FPL | 5.85±1.27 | 0.20 |
| | Control | 3.46±0.81 | |
| **TOS (µmol $H_2O_2$ Eq/L)** | FPL | 14.35±1.28 | 0.01 |
| | Control | 9.36±0.22 | |
| **TAS (mmol Trolox Eq/L)** | FPL | 1.28±0.04 | 0.33 |
| | Control | 1.22±0.02 | |
| **OSI (Arbitary Unit)** | FPL | 1.17±0.15 | 0.02 |
| | Control | 0.77±0.02 | |

UD: Undetectable, -: No statistic was applicable, PTX-3: Pentraxin-3, CRP: C-reactive protein, SAA: Serum amyloyid A, TOS: Serum total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index, SE: Standard error, FPL: Feline panleukopenia

## DISCUSSION

Feline panleukopenia is of great concern to cat owners and shelters because of its high mortality and difficulty of treatment. Studies have been undertaken to elucidate the pathogenesis of the disease and thereby predict its outcome (Börkü,

2016; Chowdhury et al., 2021; Dinçer and Timurkan, 2018; Truyen et al., 1996). In the present study, we aimed to determine serum levels of PTX-3, a novel biomarker for predicting disease severity and prognosis, in cats infected with FPV.

In the present study, the majority of cases (75%) were in unvaccinated cats and also about 1 in 5 cases were vaccinated as reported previously (Porporato et al., 2018), which is an alarming finding in both circumstances, as it not only indicates the importance of vaccination in prevention, but also requires more attention to the vaccine and vaccination process. The clinical signs (anorexia, vomiting, diarrhoea, dehydration etc.) and haematological findings (panleukopenia) observed were consistent with previously reported studies (Greene 2012; Parrish, 1995; Truyen et al., 2009). These findings are known consequences of pathogenesis of FPL (Greene, 2012). Biochemical parameters also did not change significantly as previously reported (Porporato et al., 2018), which is unexpected given the organs affected by FPV. It can be speculated that the stage of the disease when the cases were admitted to hospital which might have allowed earlier intervention and the age of the cases presented (mainly young adults and adults) who might be expected to be more resistant, may have played a role. This speculation may also be supported by the higher survival rate (75%), as it can be assumed that the organ damage might not have been severe enough to cause biochemical changes.

Like many other infectious agents FPV is also known to cause inflammation in various host tissues, where it causes cellular damage followed by organ dysfunction (Aydoğdu et al., 2018; McMichael, 2007). Oxidative stress has been reported to be involved in cellular damage in parvo viral infections in dogs (Aydoğdu et al., 2018). This was also the case in our study, as a reflection of oxidative stress TOS and OSi levels were significantly higher in FPL cats when compared to healthy cats. Similarly Khoshvaghti and Nojaba (2022) reported decreased antioxidants (gluthatione peroxidase, vitamin D) and increased malondialdehyde an indicator of cell wall damage in FPV infected cats. These findings may further support the idea that oxidative stress may play a key role in the pathogenesis of FPL.

Previous studies have shown that infectious agents induce an acute phase response in the host, leading to an increase in serum acute phase proteins such as PTX-3, SAA, CRP and haptoglobulin, in an attempt to limit inflammation and tissue damage and to eleminate infectious agents (Bastrup-Birk at al., 2013; Ceron et al., 2005; Gökçe et al., 2009; Hamed at al., 2017; Liu et al., 2014; Paltrinieri 2007, Reading et al., 2008; Perez, 2019; Petini et al., 2020; Schmidt and Eckersall 2015). Therefore, acute phase proteins are commonly used to determine the diagnosis and prognosis of many infectious diseases (Ceron et al., 2005; Gökçe et al., 2009; Liu et al., 2014; Hamed at al., 2017; Perez, 2019). A previous study by Petini and colleagues (2020) reported higher concentrations of haptoglobulin and SAA in surviving cats with FPL, but CRP was not markedly changed, but a study by Gülersoy and colleagues (2023) reported higher serum CRP levels in FPL cases than control. Similar results were found in our study, where cats diagnosed with FPL had significantly higher levels of SAA, but this increase was not significant for CRP.

In the present study, PTX-3 was measured for the first time in cats with FPL. Serum PTX-3 was higher in the diseased cats than in the control cats in which PTX-3 was undetectable. The increase in serum PTX-3 was consistent with the previous studies conducted in other species (Aygün and Yıldız, 2018; Hamed et al., 2017; Koç, 2021; Ramery et al., 2010; Townsend and Singh, 2021;

Wang et al., 2020). PTX-3, a multifactorial acute phase protein, is synthesised and released during inflammation induced by various factors, including infectious agents. PTX-3 synthesis and release is initiated by proinflammatory cytokines (IL-1β, TNF-α), lipopolysaccharide (LPS), various microbial moites and microorganisms (Kunes et al., 2012). During the inflammatory process PTX-3 activates the classical, alternative and lectin pathway of the complement system (Günaştı et al., 2017). PTX-3 recognises most microorganisms including viruses and activates a number of antimicrobial effector mechanisms. Its interaction with P-selectin in the inflammatory response plays an immunoregulatory role and modulates complement system activation (Foo et al., 2015). In sepsis, a condition that can lead to multiple organ failure, as in FPL, PTX-3 is thought to play a major role in early diagnosis and follow-up of the disease process, as PTX-3 is not affected by IL-6, serum PTX-3 levels reflect the severity of the infection and are less affected by other inflammatory effects that may develop simultaneously (Aygün and Yıldız, 2018; Hamed et al., 2017).

The main limitations of this study were the limited number of sick and healthy cats, the inappropriate sensitivity threshold of the ELISA kit to determine PTX-3 levels in healthy cats, which made it impossible to compare PTX-3 levels between cases and controls, and the undefined PTX-3 levels in cases during and after treatment, which made it impossible to evaluate treatment outcome The lack of comparable data is another drawback as this is the first study in this area.

## CONCLUSION

In conclusion, the serum concentrations of PTX-3, an acute phase protein, were increased in cats infected with FPV and that PTX-3 levels may be associated with oxidative damage in these cases. Further clinical studies with large numbers of infected cats are needed to clarify these findings and to use PTX-3 as a reliable biomarker in FPV-infected cats.

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# The impact of gender on growth performance, live weight gain, and survival rate in Pekin ducks

**Research Article**


## ABSTRACT

This study aimed to investigate the effect of gender on growth performance, live weight gain, and survival rate in Pekin ducks raised in a family-type farm in Kars province. A total of 150 ducklings (62 males, 88 females) were observed from day one. Weight measurements were recorded regularly, and mortalities were noted for survival rate analysis. No significant gender differences were observed in live weight up to 10 weeks of age, indicating comparable growth rates initially. However, at 10, 13, and 16 weeks, male ducks had higher live weights compared to females. Daily live weight gains were significantly higher during the first two weeks, with a decreasing trend as the ducks grew older. Gender significantly affected daily weight gains during the initial two weeks. The influence of gender on weekly live weight gains was not statistically significant. Live weight gains decreased over time, while overall live weight increased. Regression models indicated higher growth rates in males. Survival rates remained high throughout the 16-week period, with no significant difference between genders. The hazard ratio analysis suggested no substantial difference in mortality risk between male and female ducks. In conclusion, gender influenced live weight and daily weight gains in Pekin ducks, with males generally exhibiting higher weights. However, gender did not significantly affect average live weight gain and survival rates.


**Keywords:** Gender, growth performance, live weight gain, Pekin duck, survival


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## INTRODUCTION

Ducks, especially Pekin ducks, hold significant importance as a commercial poultry species in the global market. The meat duck industry has experienced rapid growth, witnessing a substantial surge in recent years. Asia, France, Myanmar, the United States, and the United Kingdom are among the leading regions in duck meat production. Pekin ducks, known for their rapid growth, are widely bred due to their ability to quickly reach the desired market weight. On the other hand, Muscovy ducks have a slower growth rate, lower fat content, and exhibit variations in body weight based on gender (Chen et al., 2021; Huang et al., 2012). The growth and development of Pekin ducks, the most widely used domestic duck breed in commercial settings, are influenced by various factors (Cherry & Morris, 2008; Debnath, 2022; Su, 2022). Genetics, nutrition, environment, and management practices all play a crucial role in their growth. Genetic factors significantly impact growth, body composition, bone development, muscle mass, and feather structure. Nutrition, including a balanced diet with adequate protein, energy, vitamins, and minerals, is vital for optimal growth. Environmental factors such as temperature, humidity, air quality, and lighting also affect growth. Providing favorable conditions reduces stress and promotes healthy growth (Cherry & Morris, 2008; Debnath, 2022; Jalaludeen & Churchil, 2022).

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When provided with the appropriate genotype and optimal conditions, Pekin ducks can achieve an average weight of 3.3 to 3.7 kg at 6-8 weeks of age. This represents a substantial weight gain within a relatively short time frame. Additionally, the feed conversion ratio has been improved to a range of 1.88 to 1.97, indicating a more efficient utilization of feed for weight gain. These improvements in growth performance are the result of continuous selection and breeding programs that prioritize traits associated with rapid growth and enhanced feed efficiency. The objective is to meet the market demand for larger, well-developed ducks within a shorter production cycle. The ongoing enhancements in the growth performance of Pekin ducks highlight the importance of genetic selection and advancements in the poultry industry. Not only have these developments increased productivity, but they have also contributed to the economic viability of Pekin duck farming (Debnath, 2022; Huang et al., 2012; Jalaludeen & Churchil, 2022; Su, 2022).

The aim of this study is to investigate the effect of gender on growth performance, live weight gain, and survival rate in Pekin ducks raised in a family-type farm located in Kars province, Türkiye. The study aims to determine potential differences in growth and live weight gain based on gender and provide better insights to breeders. Additionally, the impact of gender on the survival rate of ducks will also be evaluated. The obtained results will guide practical applications for farm owners and researchers engaged in Pekin duck farming.

## MATERIALS AND METHODS

### *Location*

The study was conducted in the province of Kars, which is located at coordinates 40°36'18"N and 43°5'48"E, at an altitude of 1760 meters above sea level. Kars province is situated in the easternmost region of Türkiye and shares a border with Armenia.

### *Animal and feeds*

In the conducted study, a cohort of 150 Pekin ducks was examined, comprising 62 males and 88 females. The ducklings, which were only one day old, were placed in heated brooder batteries with continuous lighting, ensuring consistent environmental conditions for all individuals. The male and female ducklings were subsequently relocated to floor pens equipped with deep bedding, where they were provided with wooden shavings measuring approximately 8-10 cm in thickness as their bedding material. The pens maintained a stocking density of 4 ducklings per square meter.

Starting from the second week onwards, the ducklings were exposed to a light-dark cycle consisting of 16 hours of light followed by 8 hours of darkness each day. Initially, during the first week, the ambient temperature was carefully set at a range of 32-34°C. Subsequently, over subsequent weeks, the temperature gradually decreased by approximately 3-5°C per week. By the conclusion of the 28-day period, the ambient temperature was successfully reduced to a minimum level of 19-20°C. Subsequently, the ducklings were transferred to an environment with free-range access, providing them the opportunity to explore and grow in a more unrestricted setting.

All ducks were fed *ad libitum* diets during the initial four weeks, containing 22% crude protein and 3000 kcal/kg of metabolizable energy. Subsequently, from weeks 5 to 16, ducks were subjected to restricted feeding (Cherry, 1993) with diets containing 18% crude protein and 3100 kcal/kg of metabolizable energy. This approach adhered to the conventional method employed by the farmers (Debnath, 2022), without any modifications or supplementation. Throughout the entire experimental period, ducks had *ad libitum* access to water.

The live weights of ducklings were measured every two weeks during the initial four-week

period following hatch, and then every three weeks until they reached 16 weeks of age. These weight measurements were conducted to determine their growth progress. Additionally, any mortalities that occurred during the respective periods were recorded to calculate the survival rate, thereby assessing the overall health and viability of the ducklings in the study.

***Statistical analyses***

The normal distribution of the data was assessed visually through histogram and Q-Q plot methods, as well as statistically tested using the Kolmogorov-Smirnov test based on the sampling weeks. This comprehensive analysis confirmed that the data exhibited a normal distribution, ensuring the validity of subsequent parametric statistical tests. To determine the effects of gender on the daily or weekly live weight gains and their impact on growth performance, a two-way analysis of variance (ANOVA) was employed. The significance of differences identified through the ANOVA in multiple comparisons was assessed using the Tukey post hoc test. Data obtained only from surviving animals after hatch were included in the analyses. Linear regression models and Gompertz growth curve models were developed to examine the relationship between gender and live weights. Additionally, Kaplan-Meier survival curve analysis was conducted by recording the outcomes of deaths and survivals according to sampling weeks. The statistical software GraphPad Prism® version 9.5.1 (GraphPad Software Inc., San Diego, CA, USA) was utilized for performing the analyses. The data were presented as mean ± standard error of the mean (SEM), and statistical significance was determined at a threshold of $P < 0.05$.

## RESULTS

Detailed information regarding the mean live weights of Pekin ducks at different weighing weeks can be found in Table 1.

The average hatching weights of male and female Pekin ducklings were found to be 47.5 ± 0.8 g and 45.7 ± 0.5 g, respectively ($P > 0.05$, Table 1). Interestingly, there was no statistically significant difference between male and female ducks up to 10 weeks of age, indicating comparable growth rates during this early stage (Figure 1A, $P > 0.05$). However, a two-way analysis of variance revealed that gender had a significant impact on the overall live weight of Pekin ducks ($P < 0.001$, Figure 1A). Moreover, at 10, 13, and 16 weeks, there were notable differences in live weights between male and female ducks ($P < 0.05$, Figure 1A). By the 16th week, male Pekin ducks exhibited an average live weight of 2142.3 ± 30.6 g, while their female counterparts weighed an average of 2049.0 ± 23.1 g (Table 1).

The average daily live weight gains varying with the weighing weeks in Pekin ducks are provided in Table 1. During the first two weeks of their growth, Pekin ducklings exhibited significantly higher daily live weight gains compared to the subsequent sampling weeks (Table 1). However, as they advanced in age, there was a noticeable trend of decreasing daily weight gain. Notably, a two-way analysis of variance revealed that the gender of Pekin ducks had a significant impact on their daily live weight gain ($P < 0.001$, Figure 1B). Specifically, male and female ducklings displayed statistically significant differences in their daily weight gains, particularly during the initial two-week period ($P < 0.05$, Figure 1B).

Investigating the influence of gender on the average daily live weight gain across weighing week intervals (Figure 1C) and the weekly live weight gains (Figure 1D) in Pekin ducks, it was determined that gender did not exhibit a statistically significant effect ($P > 0.05$). However, the data revealed a noteworthy surge in live weight gains during the initial two weeks of growth (Table 1).

**Table 1.** Growth performance and changes in live weight gain in Pekin ducks according to weighing weeks.

| Parameters | Week | Male | | Female | |
|---|---|---|---|---|---|
| | | Mean ± SEM | n | Mean ± SEM | n |
| Live weight | 0 | 47.5 ± 0.8 | 62 | 45.7 ± 0.5 | 88 |
| | 2 | 747.7 ± 18.3 | 57 | 699.7 ± 13.8 | 83 |
| | 4 | 971.4 ± 24.9 | 57 | 924.1 ± 19.4 | 81 |
| | 7 | 1303.8 ± 28.4 | 56 | 1233.2 ± 20.4 | 79 |
| | 10* | 1633.9 ± 34.1 | 55 | 1531.9 ± 26.5 | 76 |
| | 13* | 1881.2 ± 33.5 | 54 | 1788.2 ± 25.3 | 74 |
| | 16* | 2142.3 ± 30.6 | 53 | 2049.0 ± 23.1 | 74 |
| The daily live weight gain until the weighing week | 0-2* | 50.0 ± 1.3 | 57 | 46.7 ± 1.0 | 83 |
| | 0-4 | 33.0 ± 0.9 | 57 | 31.4 ± 0.7 | 81 |
| | 0-7 | 25.6 ± 0.6 | 56 | 24.2 ± 0.4 | 79 |
| | 0-10 | 22.7 ± 0.5 | 55 | 21.2 ± 0.4 | 76 |
| | 0-13 | 20.1 ± 0.4 | 54 | 19.2 ± 0.3 | 74 |
| | 0-16 | 18.7 ± 0.3 | 53 | 17.9 ± 0.2 | 74 |
| Daily live weight gain | 0-2 | 50.0 ± 1.3 | 57 | 46.7 ± 1.0 | 83 |
| | 2-4 | 16.3 ± 0.9 | 56 | 16.4 ± 0.8 | 80 |
| | 4-7 | 15.3 ± 1.0 | 56 | 14.1 ± 0.6 | 79 |
| | 7-10 | 15.6 ± 0.9 | 55 | 14.5 ± 0.6 | 76 |
| | 10-13 | 11.9 ± 1.0 | 54 | 11.8 ± 0.8 | 74 |
| | 13-16 | 12.3 ± 1.1 | 53 | 12.5 ± 1.0 | 74 |
| Weekly live weight gain | 0-2 | 699.8 ± 17.9 | 57 | 653.9 ± 13.8 | 83 |
| | 2-4 | 227.8 ± 13.0 | 56 | 229.6 ± 11.9 | 80 |
| | 4-7 | 320.9 ± 20.5 | 56 | 296.6 ± 12.5 | 79 |
| | 7-10 | 328.1 ± 18.4 | 55 | 305.0 ± 12.5 | 76 |
| | 10-13 | 249.7 ± 21.4 | 54 | 247.9 ± 15.9 | 74 |
| | 13-16 | 264.2 ± 23.2 | 52 | 270.7 ± 19.9 | 72 |

SEM: Standard error of mean. *: Indicates a statistically significant difference between genders within the same line ($P < 0.05$).

Despite the implementation of restricted feeding practices in traditionally reared Pekin ducks under family farm conditions, the study observed continuous growth and live weight gain over the 16-week period (Table 1). Notably, as time progressed, the rate of live weight gain exhibited a declining trend, while the overall live weight of the ducks continued to increase.

Based on the weighing weeks, the regression coefficient for males was higher than that for females in both the linear regression model (Figure 2A) and the Gompertz growth model (Figure 2B). These models revealed the influence of age on live weight gain. Notably, as age increased, the patterns of live weight gain became more apparent. Furthermore, the gender-specific formulas obtained for age-related changes are presented in Figure 2.

The Kaplan-Meier survival curve illustrates the survival rates of male and female Pekin ducks from the day of hatching to 16 weeks of age (Figure 3).

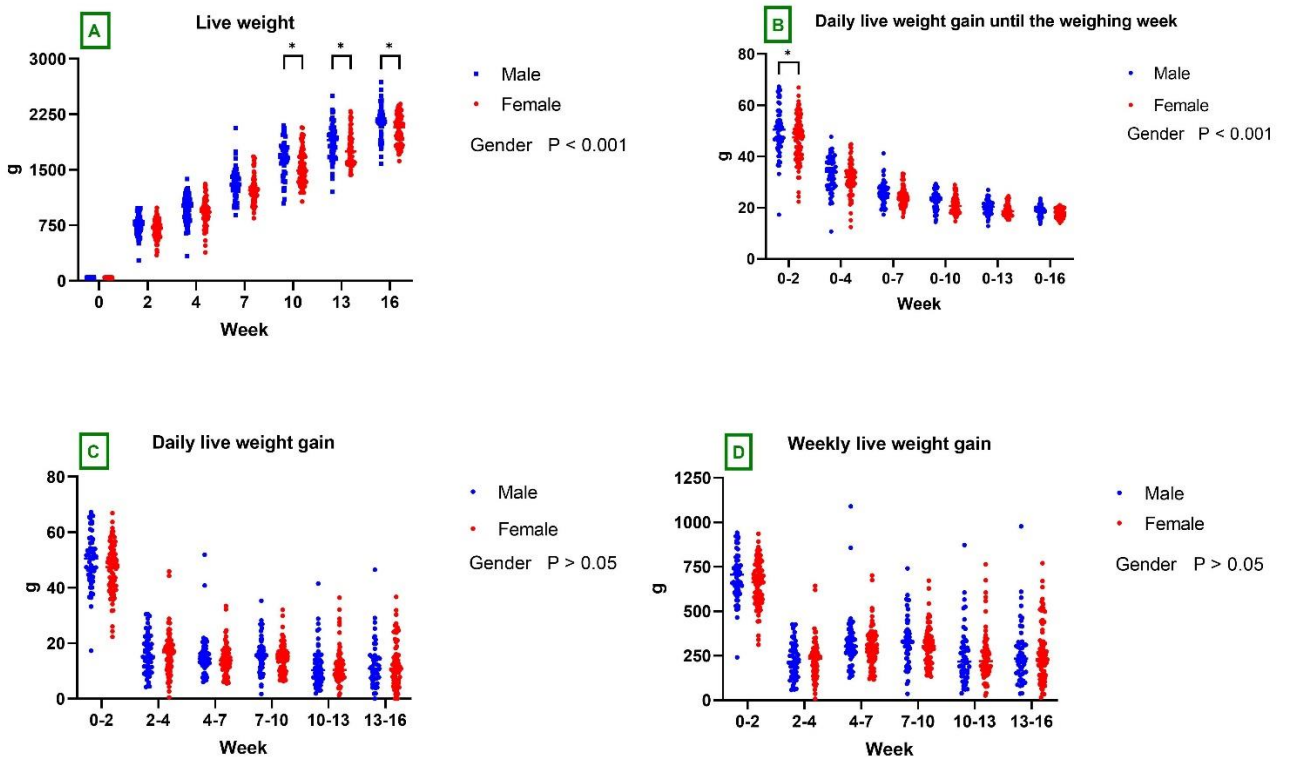


**Figure 1.** The growth performance (A), the daily live weight gain until the weighing week (B), daily live weight gain (C), and weekly live weight gain (D) of Pekin ducks across different weighing weeks. Our findings revealed a significant impact of gender on the growth performance of Pekin ducks. Specifically, at weeks 10, 13, and 16, male Pekin ducks exhibited higher live weights compared to females, and this difference was statistically significant (A). Additionally, the initial two-week period emerged as a crucial phase for live weight gain, characterized by substantial increases in weight. Moreover, our analysis suggested that gender could play a role in influencing live weight gain (B). Notably, there was a statistically significant difference in live weight gain between genders during the initial two weeks (B). *: $P < 0.05$

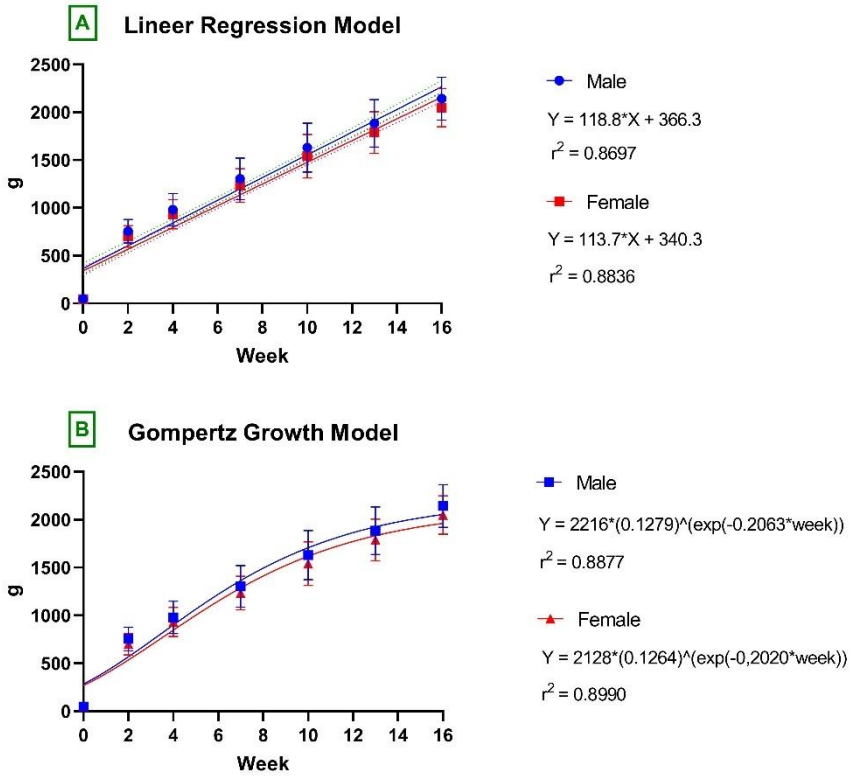


**Figure 2.** Growth curves of live body weight for male and female Pekin ducks according to linear regression model and Gompertz growth model.

The probability of survival gradually declines as the ducks approach the 16-week ages. However, the statistical analysis reveals that there is no significant decrease in survival rates throughout the 16-week period following hatching. At the conclusion of the 16-week timeframe, the survival rates were observed to be 86.9% for males and 84.2% for females, with no statistically significant difference between the genders ($P > 0.05$, Figure 3). Furthermore, the hazard ratio was calculated as 0.8195, with a 95% confidence interval ranging from 0.3458 to 1.942 and P-value was determined as 0.66. These findings suggest that there is no substantial difference in the risk of mortality between male and female Pekin ducks during the observed period.

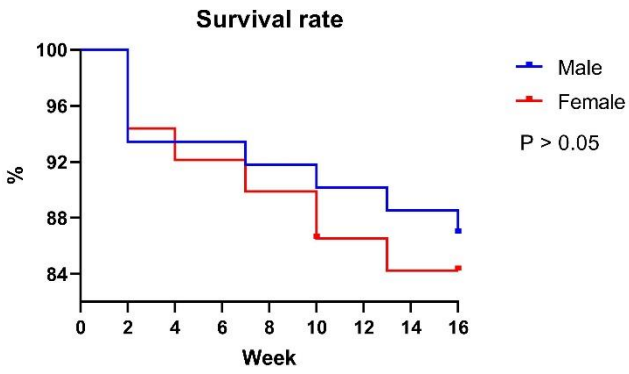


**Figure 3.** The Kaplan-Meier survival curve illustrates the survival rates of Pekin ducks from hatch to 16 weeks of age. The analysis reveals that there is no substantial decrease in survival rates over the 16-week period following hatch, indicating a relatively stable survival trend during this duration. The hazard ratio is calculated as 0.8195, with a 95% confidence interval ranging from 0.3458 to 1.942 and P = 0.66.

## DISCUSSION

Ducks play a vital role in the agricultural systems of many developing countries, where they contribute significantly to meeting daily protein requirements and generating additional income for farming households. Typically, duck farming is primarily undertaken by family members, especially in rural areas. However, there has been a noticeable decline in duck farming in recent years. This decline can be attributed to various factors, including natural feed resources, the depletion of natural water sources, and the excessive use of pesticides in agricultural fields. The conservation and sustainable management of ducks and other waterfowl are crucial for ensuring global food security (Jalaludeen & Churchil, 2022; Pingel, 2011). In many regions, duck farming continues to be carried out in a traditional system; it is reported that location-specific technological interventions are needed to improve the existing practices (Debnath, 2022; Rahman et al., 2017). We conducted this study in a small-scale family farm located in Kars province, aiming to assess the growth performance, daily live weight gain, and survival rates of Pekin ducks raised under a free-range system using traditional methods. For the first four weeks, the ducks were provided with unlimited access to feed, followed by a transition to restricted feeding based on traditional farm practices until they reached 16 weeks of age. Importantly, we maintained a hands-off approach to the feeding regime throughout the study. It was observed that in family farms in the Kars region, feeding of ducks can continue until 20 weeks of age, after which they are slaughtered (Sarı et al., 2012).

In various studies on Pekin ducks, the average live weight at different ages has been documented. For instance, at 6 weeks of age, the reported average live weight ranges from 1214-1218 g (Zhang et al., 2018) to 2812-2904 g (Yan et al., 2020). Similarly, at 7 weeks of age, the average live weight is documented as 1692-1785 g (İşgüzar, 2006) to 3281-3461 g (Abo Ghanima et al., 2020), while at 8 weeks of age, it ranges from 2364-2568 g (Sari et al., 2013). At 10 weeks of age, the average live weight is reported as 1347-2143 g (Osman, 1993), and at 20 weeks of age, it ranges from 2457-2346 g (Sarı et al., 2012). In modern farms with ad libitum feeding, it is noted that Pekin ducks can reach an average live weight of 3.27-3.55 kg by 42 days of age (Cherry & Morris, 2008; Debnath, 2022; Jalaludeen & Churchil, 2022; Su, 2022). However, our study observed that the average live weight at 16 weeks of age ranged from 2049

to 2142 g, which is noticeably lower than the average values reported for modern farming at 6 or 7 weeks of age. It is worth mentioning that studies with limited feeding, similar to ours, have reported live weight ranges of 1792-2007 g at 12 weeks of age (Işguzar et al., 2002) and approximately 2.4 kg at 16 weeks of age (Cherry, 1993). The differences in live weight observed in our study can be attributed to both the variations in feeding methods and the fact that the ducks were raised under a free-range system. Additionally, it is important to consider that the growth performance of Pekin ducks can be influenced by the specific genotypes used in the studies.

In our study, we investigated the impact of gender on live weight changes in Pekin ducks over different sampling weeks. The results revealed a significant effect of gender on live weight, particularly during the 10-16-week period, where male ducks exhibited higher live weights compared to females. Additionally, male ducklings showed higher daily live weight gain in the first two weeks of the study. These results are consistent with previous studies (Erdem et al., 2015; Işguzar et al., 2002; Sari et al., 2013; Tığlı et al., 1991) that have reported gender-related differences in live weight, with male Pekin ducks generally having higher weights at slaughter time. Moreover, similar to our findings, it has been observed that gender can influence live weight gain during the early weeks after hatching (Erdem et al., 2015; Erdem & Akçapınar, 2012; Işguzar et al., 2002). Factors such as hatch weight and genotype are believed to play a significant role in these gender-related differences. Several studies conducted on Pekin ducks have demonstrated that both genotype and gender can impact growth performance and live weight gain (Kokoszyński et al., 2015, 2019). Furthermore, our study revealed a noticeable trend of increasing body weight with age in Pekin ducks, with significant interactions between age and gender. Male ducks consistently exhibited higher weights compared to females throughout the different weeks, and the period of highest body weight gain was observed to be the first three weeks of life (Onbaşılar et al., 2011). However, it is important to note that after the initial four weeks, the significant daily weight gains observed in the early weeks gradually decreased. This discrepancy in live weight gains compared to intensive modern farming practices may be attributed to differences in both feeding and rearing conditions. Therefore, it is crucial to modernize and optimize both management practices and feeding preferences to enhance daily live weight gains and maximize economic benefits in Pekin duck farming.

Modeling growth curves in scientific studies allows us to gain valuable insights into the patterns of growth over time and provides us with predictive equations to estimate the expected weight of animals at different ages. Numerous growth models have been developed to examine the complex relationship between age and body weight in animals (Kokoszyński et al., 2019; Maruyama et al., 2010; Onbaşılar et al., 2011). In our study, we employed two widely used models, namely the linear regression and Gompertz growth models, to analyze and forecast body weight changes over time. Intriguingly, we observed that male individuals exhibited a higher regression coefficient compared to female, suggesting potential gender-based variations in growth. Furthermore, both models consistently demonstrated that body weight tends to increase as animals age, indicating the importance of considering age as a critical factor in understanding growth dynamics.

Under normal rearing conditions, the mortality rates of ducks between 1-5 months of age are approximately 10%. This mortality can be attributed to various factors such as predator

attacks, mycotoxin contamination in feed, parasitic infections, pesticide levels in feed, feed availability, and disease outbreaks like duck plague, duck cholera, and avian influenza (Cherry & Morris, 2008; Sankaralingam & Mahanta, 2022). As ducks reach 8 weeks of age, the mortality rate typically decreases to below 10%, although it may still vary within the range of 10-20% (Sankaralingam & Mahanta, 2022). It is noteworthy that the 0-8 week period holds particular significance in duck farming, with mortality rates ranging from 2-3% (Debnath, 2022). In the context of Pekin ducks, a species-specific study reported a mortality rate of approximately 4-9% until the day of slaughter (Erisir et al., 2009). In the present study, employing Kaplan-Meier survival curve analysis, we observed mortality rates of 14.5% for males and 15.9% for females. Furthermore, our investigation underscores the crucial necessity of meticulously monitoring the mortality rate throughout the 16-week interval following the hatching of Pekin ducks. Nevertheless, gender did not exhibit a statistically significant impact on the observed mortality rate among Pekin ducks.

## CONCLUSION

In conclusion, the gender of Pekin ducks raised in family-type farms in Kars province has been found to impact their growth performance. However, no significant difference was observed in terms of survival rate between male and female ducks. It is worth noting that during the 16-week monitoring period, the live weights of these ducks were notably lower compared to the data obtained from Pekin ducks raised in modern farms. This difference in growth performance can be attributed to the negative effects of traditional methods and restricted feeding practices commonly employed in family-type farms. To address these challenges and improve the growth performance of ducks in family-type farms, it is highly recommended to provide education and seminars on modern farming conditions and care standards. By equipping farming families with knowledge about optimal farming practices, they can enhance the well-being and growth potential of their ducks. Additionally, conducting comparative studies in the future that encompass both traditional and modern farming conditions would be valuable. Such studies would highlight the potential economic benefits that farming families can achieve by adopting higher growth performance practices observed in modern farming conditions.

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Conflict of interest: The authors declared that there is no conflict of interest.

Ethical statement: This study was carried out after the approval obtained from the Kafkas University Animal Experiments Ethics Committee, (KAÜ-HADYEK / 2021-107) and the permission obtained from The Turkish Ministry of Agriculture and Forestry.

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