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Research Article

The Protective Effects of L-Carnitine Against Lead (II) Acetate Toxicity in *Capoeta capoeta* (Guldensteadt 1773) [1]

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Abstract

In this study, the protective effects of L-carnitine (LC) against Lead (II) acetate ($Pb(C_2H_3O_2)_2$) toxicity in *Capoeta capoeta* were investigated by means of histopathologically, via electrophoretic and biochemical methods. Fish caught from Kars creek were divided into four groups, which include 10 fish each in 500 L tanks. Lead and liquid LC were added to water. Fish in the first group were adjusted as control. Fish in the II. group were applied 1 mg/L lead (as Lead (II) acetate) for 10 days. Fish in the III. group were administered 100 mg/L LC. Fish in the IV. group were administered 1 mg/L lead and 100 mg/L LC. Degenerations in liver, gill, intestine and kidney tissues were observed to reduce LC administration against the toxic effects of lead acetate. Electrophoretically, inhibitions of some protein bands in the group, which was applied lead acetate, were caused, and increases in protein expression in the group, which was applied L-carnitine, have occurred. While high a level of total protein in the group that was administered lead was found; in the group that was treated lead + LC, it was found to be lower ($P<0.05$). Levels of globulin in the group that was administered LC + lead were observed to be significantly lower ($P<0.05$). Total oxidant capacity (TOC) in lead treatment group were higher than the control group, TOC levels in lead + LC treatment group were determined to be between the control and lead group. LC was concluded to show a protective effect on *Capoeta capoeta* that were exposed to lead.

Keywords: *Lead (II) acetate, L-carnitine, Capoeta capoeta, Electrophoresis, TAC, TOC, Histopathology*

Capoeta capoeta (Guldensteadt 1773)'larda Kurşun (II) Asetat Toksisitesine Karşı L-Karnitinin Koruyucu Etkileri

Özet

Çalışmada, *Capoeta capoeta*'da Kurşun (II) asetat ($Pb(C_2H_3O_2)_2$) toksisitesine karşı L-karnitinin (LK) koruyucu etkileri histopatolojik, elektroforetik ve biyokimyasal yöntemlerle incelendi. Kars Çayı'ndan yakalanan balıklar 500 L'lik tanklarda her grupta 10'ar adet balık bulunan 4 gruba ayrıldı. Kurşun ve sıvı LK suya ilave edildi. I. gruptaki balıklar kontrol grubu olarak belirlendi. II. gruptaki balıklara 10 gün süreyle 1 mg/L kurşun asetat uygulandı. III. gruptaki balıklara 100 mg/L LK uygulandı. IV. gruptaki balıklar ise 1 mg/L kurşun ve 100 mg/L dozunda LK uygulandı. Karaciğer, solungaç, bağırsak ve böbrek dokularında kurşun asetatın toksik etki gösterdiği ve buna karşı L-karnitin uygulamasının koruyucu etki göstererek oluşan bu dejenerasyonların şiddetini azalttığı gözlemlendi. Elektroforetik incelemede, kurşun asetatın birçok protein bandında inhibisyona neden olduğu, LK uygulaması sonucunda da protein ekspresyonlarında artış meydana geldiği saptandı. Kurşun uygulanan grupta total protein düzeyinin yüksek, kurşun + LK verilen grubun ise düşük olduğu belirlendi ($P<0.05$). Globulin düzeylerinin, LK + kurşun verilen grupta istatistiksel olarak düşük olduğu saptandı ($P<0.05$). Kurşun uygulanan grubun Total Oksidan Kapasitesinin (TOK) kontrol grubuna göre yüksek, kurşun + LK verilen grubun TOK düzeyinin ise kontrol ile kurşun verilen grubun arasında olduğu belirlendi. Sonuç olarak; LK'in *Capoeta capoeta*'da kurşun toksisitesine karşı koruyucu özellik gösterdiği kanısına varıldı.

Anahtar sözcükler: *Kurşun (II) asetat, L-karnitin, Capoeta capoeta, Elektroforez, TAK, TOS, Histopatoloji*

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INTRODUCTION

Sources of lead in the environment are either natural or unnatural (anthropogenic) sources. While geological and volcanic events form natural resources of lead; anthropogenic sources include mining and resources in the industrial area (batteries, cables, pigments, oil, solder and steel) [1,2]. Lead in living organisms are not necessary for normal physiological events [3]. Lead exposure generally occurs through occupational or environmental contamination [4]. The main contamination form of lead in the environment is by air, and airborne. Lead can accumulate in soil and aquatic environments. Later, it can be transported to people through the food chain by living organisms in the aquatic environment and plants. Lead shows toxic effects on many organs and organ systems of living organism (blood, brain, kidney, heart and immune system) [1,2]. L-carnitine, being a vitamin-like compound, is endogenously synthesized from lysine and methionine that are essential amino acids mainly in the liver, kidney and brain [5]. For this synthesis, lysine, methionine, iron, vitamin C, vitamin B6 and niacin are needed [6]. The most important function in the organism of L-carnitine is to facilitate the entry into the mitochondria of fatty acids to be used in energy production [7,8]. In addition, L-carnitine plays an important role in reducing toxic effects of various drugs and chemical substances in the organism [6]. Nevertheless, carnitine is known to increase the blood antioxidant level [9]. Deficiency of L-carnitine emerges from immune function defects, such as systemic sclerosis and chronic fatigue syndrome [10].

In this study, investigation of productive effects of LC on *Capoeta capoeta* that was exposed to Lead (II) acetate was aimed.

MATERIAL and METHODS

Experimental Design

This study has been conducted under the approval (KAÜ-HADYEK/2015-043) of Kafkas University Animal Experiments Local Ethics Committee. *Capoeta capoeta* (200-270 g) caught by electro shock from Kars creek was divided into four groups, which included 10 fish each in 500 L tanks. Fish were fed with ad libitum until experimental studies. Feeding was stopped during experimental study. Fish in the first group were adjusted as control. Fish in the II. group were applied 1.00 mg/L lead for 10 days. Fish in the III. group were administered 100 mg/L L-carnitine. Fish in the IV. group were administered 1.00 mg/L lead and 100 mg/L L-carnitine.

The water temperature and oxygen concentration was adjusted to 18.0±0.20ºC by thermostat thermometer and 5.00±0.40 mg/L, respectively. At the end of the experimental period, blood samples from dorsal vein of fish for biochemical and/or electrophoretic analysis and, liver, kidney, intestine and gill tissue samples for histopathological analysis were taken.

In tanks, Kars creek water was used and water was changed daily. The quality of the water in the Kars creek was identified as pH: 7.80-8.40, O_2: 5.00-8.50, conductivity 213 mS/cm^2, NH_3: 408 mg/L, PO_4: 53.7 mg /L, NO_3: 0.25 mg/L and temperature: 16.3-19.0°C.

Histopathology

Tissues taken from the samples were passed through graded alcohol and xylene series after being fixed in 10.0% phosphate buffer formaldehyde solution. Gills were decalcified with Osteotec (Bio-Optica, Italy). Paraffin blocks from tissue samples were prepared by routine methods and slices from paraffin blocks were taken in 3.00 to 5.00 microns thickness. Slices were stained according to hematoxylin-eosin method (HE) and evaluated under a light microscope (Olympus BX51, JAPAN).

Sodium Dodecyl sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Blood samples were centrifuged at 4.00°C for 10 min at 3.000 rpm and serums were separated. Serums were stored at -20.0°C until analysis. The protein concentrations of the samples were measured by the biuret method [11]. SDS-PAGE was performed by the methods modified from Laemmli and O'Farrell [12,13]. Bovine albumin (66 kDa), egg albumin (45 kDa) and trypsinogen (24 kDa) were used as protein standards in electrophoresis. Molecular weight of the protein was performed according to the method of Weber et al.[14].

Biochemical Analysis

Antioxidant and oxidant levels of Serum were measured by Total Antioxidant Status and Total Oxidant Status Assay kit (Assay Rel Diagnostics, Clinical Chemistry Solutions, Gaziantep, Turkey) [15]. Blood glucose, total protein, albumin and globulin levels were measured using a commercial kit (ERBA DDS, Turkey).

Statistical Analysis

One-way analysis of variance for the statistical analysis of the differences between the groups (ANOVA), Tukey's test was used to determine differences between groups. as SPSS Statistics 18.0 software package was used. Values are expressed as mean ± standard deviation ($P<0.05$).

RESULTS

Histopathology

The livers of the fish in the control group did not exhibit any histopathological findings *(Fig. 1A)*. Structure of livers of fish, which were applied 100 mg/L L- carnitine, was

similar to the livers of fish in the control group *(Fig. 1B)*. The fish livers that was applied 1.00 mg/L lead acetate were observed to deteriorate in the remark cords with severe degenerative and necrotic changes. Large and small sharp-edged vacuolar degeneration in the cytoplasm of hepatocytes was detected. Hydropic degeneration were observed in a small number of cells. Due to changes in the cytoplasm of hepatocytes, cells demonstrated quite growth and loss of intercellular sinusoidal space. Focal necrotic areas were detected by the death of a few cells *(Fig. 1C)*. In the group, which was applied 1.00 mg/L of lead acetate +100 mg/L L-carnitine, the prevalence and severity of the degeneration of the liver were observed to decrease significantly compared to group, which was applied only lead acetate. Hepatocytes in this group have often been found small and sharp edged vacuoles in the few cells with hydropic degeneration. Necrosis was also detected in a few cells. Although the general structure of the remark cord was protected; particularly, the remark cord was found to be disrupted in necrotic areas. Light activation was formed in Kupffer's Star cells *(Fig. 1D)*.

There were no histopathological findings in the control group and LC of kidney tissue *(Fig. 2A-B)*. Kidney tissues of fish, which were applied 1.00 mg/L lead acetate, especially drew attention to leave the basal layer with hydropic degeneration in the proximal tubule epithelium and segmental necrosis. Integrity in the basal layer of the tubules was slightly lost. Tubule lumen were observed in a small number of pink hyaline cylinders. These degenerative changes were observed less in the distal tubule. Increased mesangial cells in glomeruli and thickening in bowman capsule were determined *(Fig. 2C)*. In kidney tissues of fish that were applied Lead acetate + L-carnitine, although reductions in the severity and prevalence of degeneration and necrosis occurring in tubules were observed compared to the group that was applied only lead; degenerations still continue. While glomerulus appear to be robust in some areas in the experimentals in this group; in some areas, especially in cytoplasms of the endothelial and epithelial cells close to the Bowman space, increase in eosinophilic staining and formation of pyknosis in nucleus attracted attention *(Fig. 2D)*.

In tissue samples taken from intestine, the control group and LC did not show any histopathological findings like in kidney tissues *(Fig. 3A-B)*. In the lead acetate applied group, the inner layer of the epithelium was observed to increase in goblet cells filled with secretory granules. Lamina propria was seen in mononuclear cell infiltration, edema and light hyperemia *(Fig. 3C)*. In the group, which was administered Lead acetate + L-carnitine, a decrease in degenerative and necrotic changes and a significant increase in goblet cells were seen, compared to the control and LC groups. However, compared to the group that was applied lead, it was quite a small number of increase in goblet cells. Mononuclear cell infiltration in the lamina propria was similar to control group and the one that was applied LC *(Fig. 3D)*.

The fish gill tissues in control and LC groups had normal histological appearance *(Fig. 4-A-B)*. In gill tissues of fish, which were applied 1.00 mg /L lead acetate, degeneration in the secondary lamellae epithelium, loss in necrosis, swelling of the chloride cells, and hydropic degeneration in the lamellar epithelium were observed *(Fig. 4C)*. In the

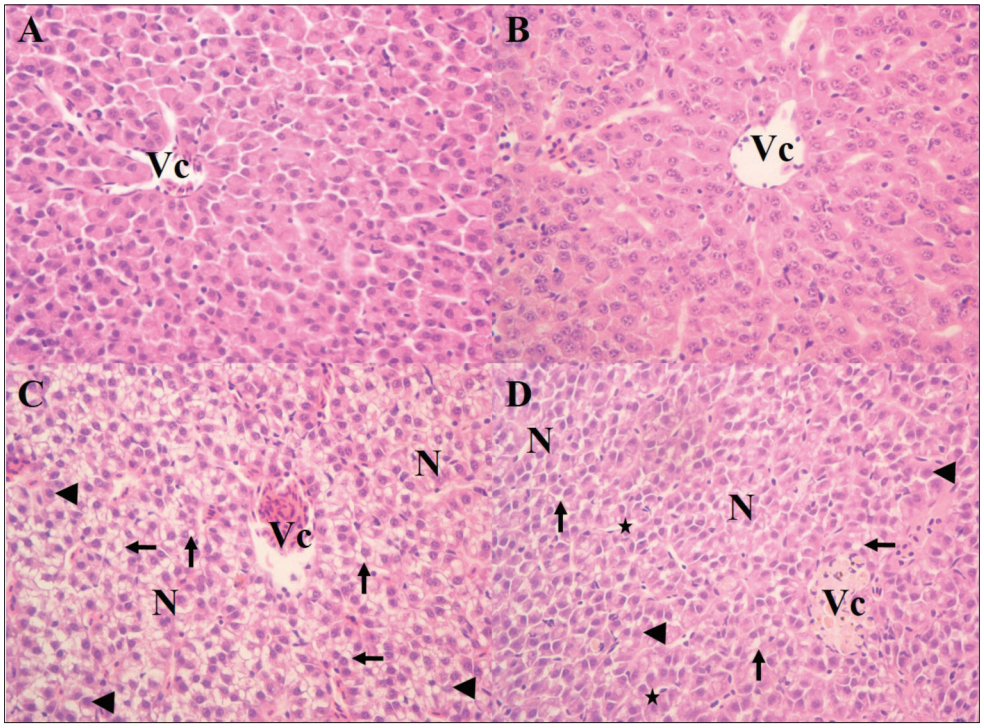

Fig 1. Liver tissue of fish in the control and experimental groups (Hematoxylin and eosin, x40). **A-** Control and **B-** liver tissues of fish in LC group, **C-** Liver tissue of fish applied 1.00 mg/L lead acetate, vacuolar *(arrows)* and hydropic *(arrowhead)* degeneration, focal necrosis (N), **D-** Liver tissue of fish that were administered 1.00 mg/L lead acetate +100 mg/L LC. Vacuolar *(arrows)* and hydropic *(arrowhead)* degeneration, focal necrosis (N)

Şekil 1. Kontrol ve deney gruplarındaki balıklara ait karaciğer dokusu (Hematoksilen ve eozin, x40). **A-** Kontrol ve **B-** LC grubundaki balıklara ait karaciğer dokusu, **C-** 1.00 mg/L kurşun asetat içeren tankta 10 gün süreyle tutulan balıklara ait karaciğer dokusu vakuoler *(oklar)* ve hidropik *(ok başı)* dejenerasyon, fokal nekroz alanları (N), **D-** 1.00 mg/L kurşun asetat ile birlikte 100 mg/L dozunda LK verilen balıklara ait karaciğer dokusu, vakuoler *(oklar)* ve hidropik *(ok başı)* dejenerasyon, fokal nekroz alanları (N)

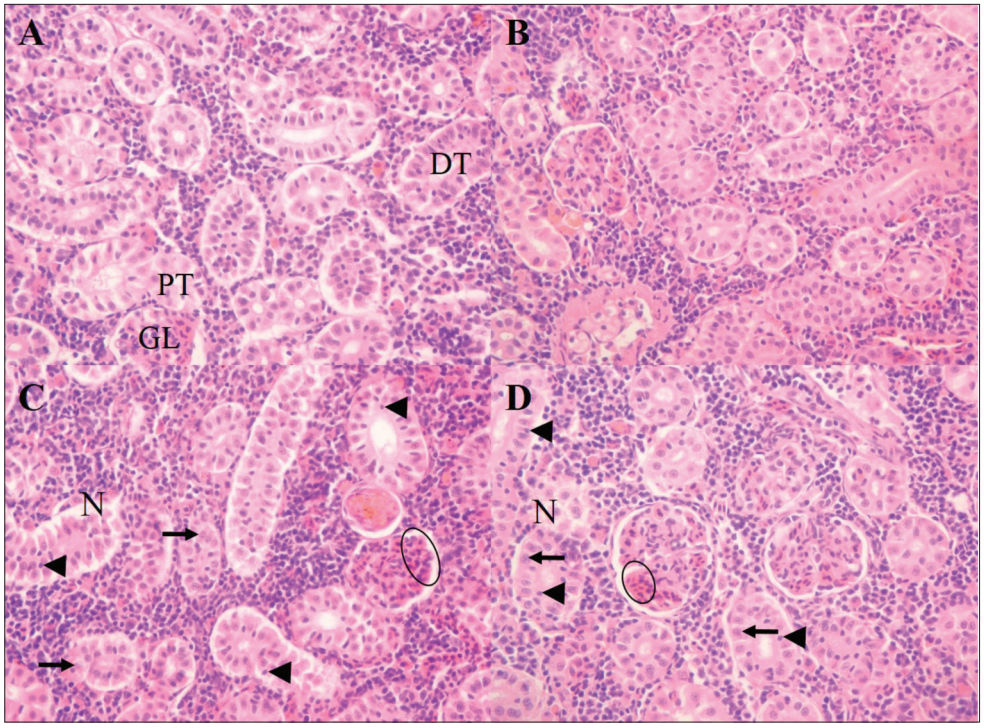

Fig 2. Kidney tissue of the fish in the control and experimental groups (Hematoxylin and eosin, x40). **A-** Control and **B-** kidney tissues of fish in group LC, **C-** Kidney tissue of fish,which were applied 1.00 mg/L lead acetate. Hydropic degeneration in the proximal tubule epithelium *(arrows),* pyknosis *(arrowhead),* segmental necrosis (N), **D-** Kidney tissue of fish that were applied 1.00 mg/L lead acetate + 100 mg/L LC. Hydropic degeneration in the tubule epithelium *(arrows),* pyknosis *(arrowhead),* segmental necrosis (N).

Şekil 2. Kontrol ve deney gruplarındaki balıklara ait böbrek dokusu (Hematoksilen ve eozin, X40). **A-** Kontrol ve **B-** LC grubundaki balıklara ait böbrek dokusu, **C-** 1.00 mg/L kurşun asetat içeren tankta 10 gün süreyle tutulan balıklara ait böbrek dokusu. Proksimal tubul epitellerinde hidropik dejenerasyon *(oklar),* piknoz *(ok başı),* segmental nekroz (N), **D-** 1.00 mg/L kurşun asetat ile birlikte 100 mg/L dozunda LK verilen balıklara ait böbrek dokusu. Tubul epitellerinde hidropik dejenerasyon *(oklar),* piknoz *(ok başı),* segmental nekroz (N)

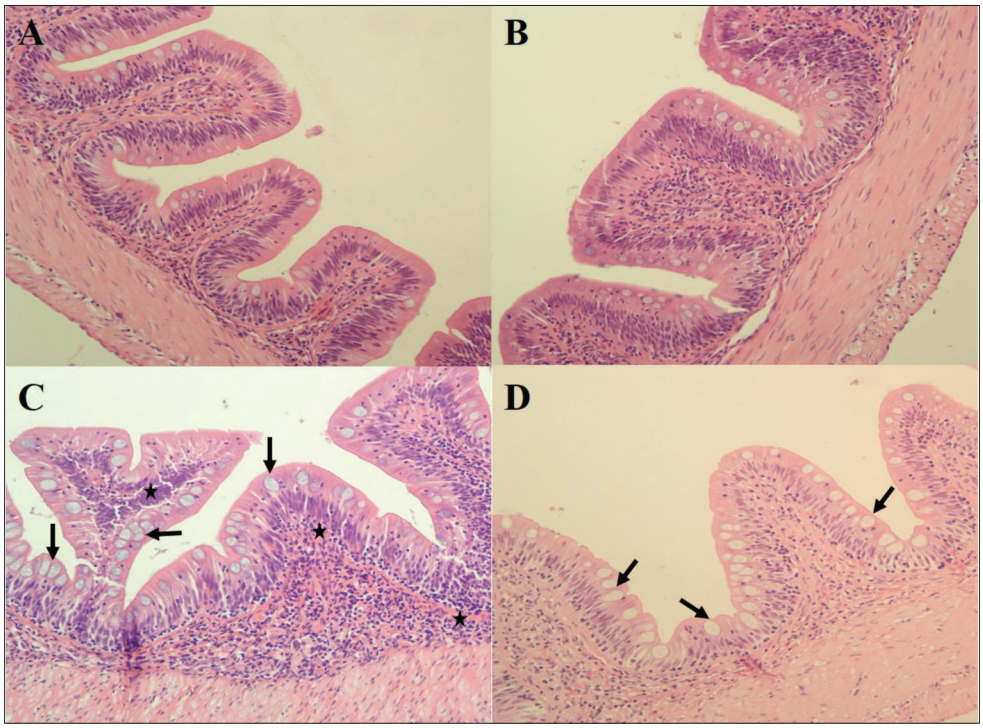

Fig 3. Intestinal tissues of fish in the control and experimental groups (Hematoxylin and eosin, x40). **A-** Control and **B-** Intestinal tissue of fish in the group, which was applied LC, **C-** Intestinal tissue of fish that were applied 1.00 mg/L of lead acetate. The increase in goblet cells *(arrows),* mononuclear cell infiltrate in lamina propria and edema, **D-** Intestinal tissue of fish, which were applied 1.00 mg/L of lead acetate +100 mg/L LC. Increase in goblet cells *(arrows)*

Şekil 3. Kontrol ve deney gruplarındaki balıklara ait bağırsak dokusu (Hematoksilen ve eozin, x40). **A-** Kontrol ve **B-** LK grubundaki balıklara ait bağırsak dokusu, **C-** 1.00 mg/L kurşun asetat içeren tankta 10 gün süreyle tutulan balıklara ait bağırsak dokusu. Goblet hücrelerindeki artış *(oklar),* Lamina propriyada mononükleer hücre infiltrasyonu, ve ödem, **D-** 1.00 mg/L kurşun asetat ile birlikte 100 mg/L dozunda LK verilen balıklara ait bağırsak dokusu. Goblet hücrelerinde artış *(oklar)*

gill tissues of lead acetate + LC applied fish, degenerations and necrosis in the secondary lamellae were significantly reduced and degeneration were only seen at the starting of secondary lamellae *(Fig. 4D)*.

SDS-PAGE

The electrophoretic examination, thinning in expressions of high molecular weight albumin bands and thickening in expressions of low molecular weight globulin bands were observed in lead applied fish. In the group, which was administered Lead + L-carnitine, compared to the control group, proteins with 98 kD and 43 kD molecular weights were over-expressed *(Fig. 5)*.

Biochemical Analysis

Glucose levels of groups treated with lead, LC and lead + LC were significantly lower than the control group ($P<0.001$). Total protein levels in the group of fish that

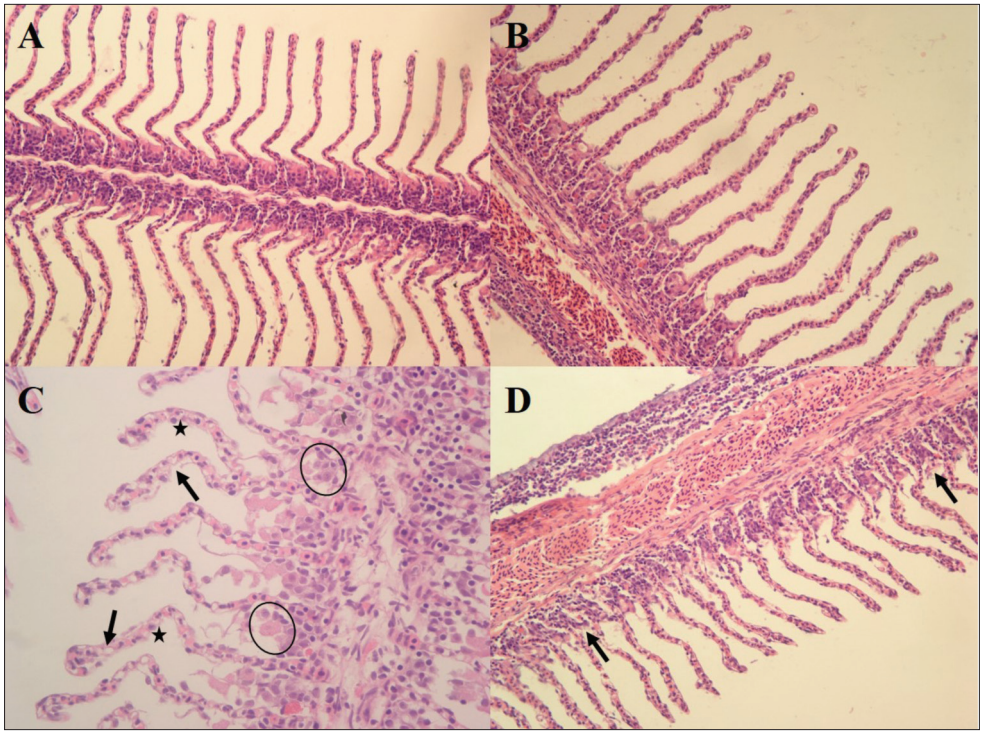

Fig 4. Gill tissue of fish in the control and experimental groups (Hematoxylin and eosin, x20). **A-** Control and **B-** Gill tissue of fish in the LC applied group, **C-** Gill tissue of fish that were applied 1.00 mg/L lead acetate. Degeneration in epithelial of secondary lamellae and necrosis, loss *(arrows),* swelling in the chloride cells (circle), **D-** Gill tissue of fish that were administered 1.00 mg/L of lead acetate + 100 mg/L LC. Degeneration in epithelial of secondary lamellae and necrosis *(arrows)*

Şekil 4. Kontrol ve deney gruplarındaki balıklara ait solungaç dokusu (Hematoksilen ve eozin, x20). **A-** Kontrol ve **B-** LC grubundaki balıklara ait solungaç dokusu, **C-** 1.00 mg/L kurşun asetat içeren tankta 10 gün süreyle tutulan balıklara ait solungaç dokusu. Sekonder lamel epitellerinde dejenerasyon ve nekroz, dökülme *(oklar),* Klorid hücrelerde şişme (daire), **D-** 1.00 mg/L kurşun asetat ile birlikte 100 mg/L dozunda LK verilen balıklara ait solungaç dokusu. Sekonder lamel epitellerindeki dejenerasyon ve nekroz *(oklar)*

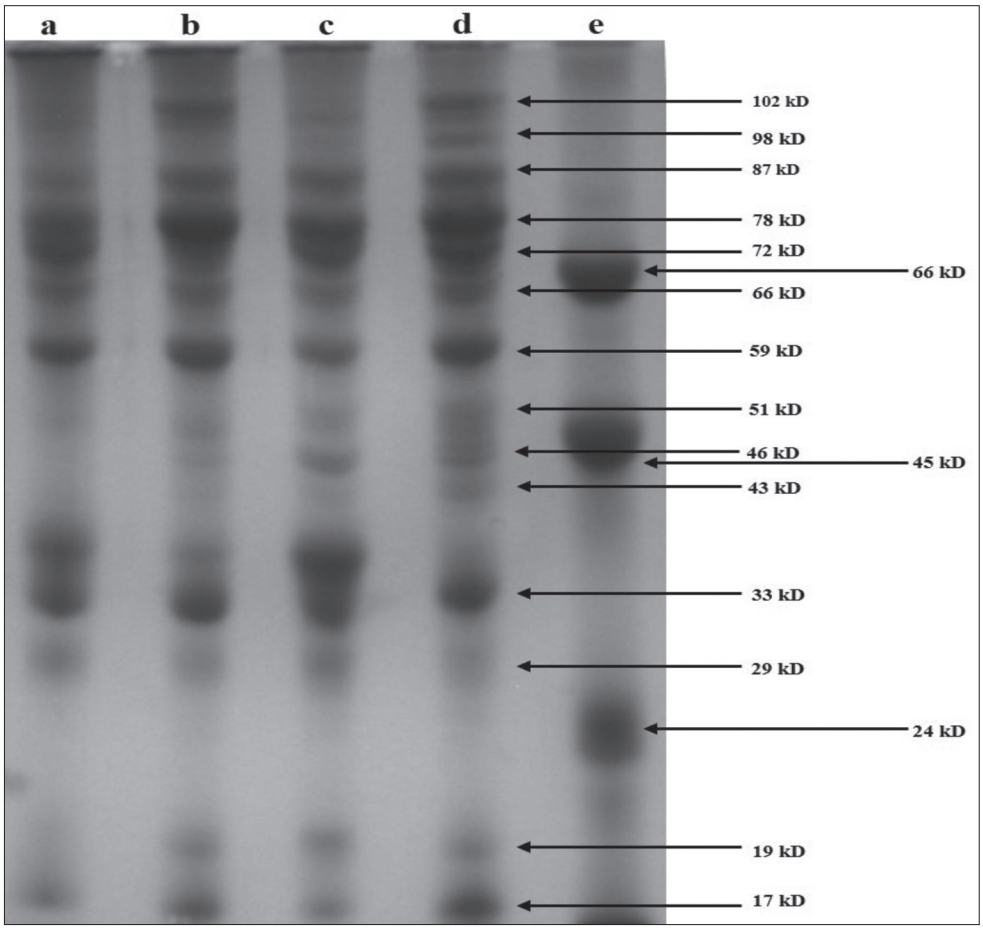

Fig 5. SDS-PAGE electropherogram obtained from fish, which were administered LC with Lead. **a-** Control group, **b-** Lead applied group, **c-** LC applied group, **d-** Lead+LC applied group, **e-** Standard proteins

Şekil 5. Kurşun ile birlikte LC verilen balıkların SDS-PAGE'den elde edilen elektroferogramı. **a-** Kontrol grubu, **b-** kurşun uygulanan grup, **c-** LC uygulanan grup, **d-** Kurşunla birlikte LC uygulanan grup, **e-** Standart proteinler

was applied LC were not observed to have a statistically significant difference; while total protein levels of the group that was applied lead were found to be high, and total protein level of group that was administered Lead + LC was determined to be low ($P<0.05$). Albumin level of the group that was applied LC was observed to have a

Table 1. *The biochemical analysis of fish that were applied LC and lead*
Tablo 1. *Kurşun ve LC uygulanan balıkların biyokimyasal analizleri*

Parameter	Control	Lead	LC	Lead + LC	P Value
Glucose (mg/dL)	73.19±3.03^{a}	57.06±3.16^{c}	49.74±3.67^{bc}	47.89±1.16^{b}	0.000
Total Protein (g/dL)	4.06±0.33^{ab}	4.26±0.07^{a}	4.02±0.15^{ab}	3.55±0.12^{b}	0.044
Albumin (g/dL)	1.95±0.11^{b}	2.18±0.04^{ab}	2.23±0.10^{a}	2.08±0.05^{ab}	0.117
Globulin (g/dL)	2.11±0.31^{a}	2.08±0.09^{a}	1.78±0.09^{ab}	1.46±0.08^{b}	0.018
Albumin/Globulin	1.11±0.19^{b}	1.07±0.07^{b}	1.27±0.07^{ab}	1.45±0.07^{a}	0.055
TAC (mmol Trolox Equiv./L)	0.47±0.06^{a}	0.46±0.03^{a}	0.45±0.01^{a}	0.51±0.02^{a}	0.658
TOC (μmolH_2O_2 Equiv./L)	9.68±1.62^{b}	17.02±1.37^{a}	10.91±2.21^{b}	13.20±1.79^{ab}	0.061

** Means with different superscript letters are statistically different in line (P<0.05)*

statistically insignificant increase (P>0.05), compared to the control group. Globulin level of the group that was applied lead +LC was significantly lower than the control group (P<0.05). Globulin levels of the LC applied group fish were found to be between total globulin levels of groups that were applied lead and lead+L-carnitine. Albumin/globulin ratio of group treated with Lead + LC statistically insignificant (P>0.05) compared to the control group, but was found to be high. TAC levels in the experimental groups and the control group were not observed to have a statistically significant difference. Total oxidant capacity (TOC) did not occur as a statistical difference between the groups (P>0.05). TOC levels in the group treated with lead were higher than the control group. TOC levels in group treated with lead + LC were determined to be between the control and lead group *(Table 1)*.

DISCUSSION

Although the literature about the effects of metals on aquatic organisms is quite wide, studies on fish are limited with lead. The use of LC in the aquatic environment is emphasized, which will be important in many ways. In particular, to accelerate the growth, to provide protection against xenobiotics and toxic levels of ammonia, to facilitate adaptation to environments of the fish in the changing temperature in water, the reduction of stress and increasing of reproductive performance of LC are some factors that make it important for fish [6]. In particular, the research relating to lead toxicity was found to have adverse effects on the antioxidant system of the lead. In studies conducted on the effects of different tissues and organs of fish exposed to lead, lead has been reported to cause oxidative damage in hepatocytes of liver [16], and kidney tissue [17]. In a histopathological study, formation of mononuclear cell infiltration and necrosis, expansion in sinusoidal space of in liver tissue depending on the lead toxicity have been indicated [18]. In another study, necrosis and cytoplasmic vacuolization in liver tissue of rat that were applied lead have been observed, whereas different degeneration and necrosis in kidney tissue have been reported [19]. Mutlu et al.[20] reported that degenerative and necrotic deterioration in liver tissue of lead acetate applied rats have occured. In the present study, while degenerations in the liver, kidney and intestines gill tissue of fish that were applied lead were formed, LC administration was observed to reduce the severity of degenerations.

There were no studies in the literature about the protective role of LC against lead toxicity on fish, electrophoretically. Changes in increase and decrease occurring in protein expression according to applied substance and the animal species have clearly been demonstrated in the toxicity studies [21,22]. In the present study, changes in protein expression due to lead the application have occurred, and the administration of LC showed an increase in expression of some proteins. The reason for increases in expression of these proteins was thought to be formed due to reduction of the toxic effects of lead exposure.

Proteins show the excessive sensitivity to free oxygen radicals [23]. In a study conducted in rats induced by acetaminophen, comparing to control group, globulin levels were significantly decreased, however, the application of LC did not change the level of globulin [24]. Another study, dietary protein and glucose levels have increased in the application of LC on *Oreochromis niloticus* [25]. In the present study, while lead application increased levels of the total proteins; Lead + LC administration was determined to decrease levels of the total proteins. However, globulin levels of the group treated with L-carnitine + lead were statically lowered more than the control group. Furthermore, glucose levels of the group treated with with L-carnitine + lead were statically lowered more than the control group.

Reactive oxygen species have quite increased in physiological and pathological conditions [26]. LC shows features similar to antioxidants and the harmful effects reactive oxygen species caused by various toxic substances reduces [27]. In a study, it was found that LC showed to be protective against oxidative damage induced by ethanol [28]. In another study, researchers reported that LC reduces the peroxidation of lipids and increases activities of antioxidant enzyme in rats fed with fish oil [9]. Protective effect of LC

against oxidative damage generated in Tilapia *(Oreochromis niloticus)* that were applied cylindrospermopsin has been determined [29]. In another study, oral administration of LC increased the level of antioxidant enzymes, whereas reduced the level of oxidative enzymes against lead stress in rats [30]. In the present study, in terms of TAC levels between treatment groups and the control group, a statistically significant difference was not observed. In terms of TOC levels, while there were increases in levels of TOC in the group that was administered lead, there were decreases in levels of TOC in the group treated with lead + LC. According to these data, LC reduces the oxidative damage and shows an antioxidant feature.

In conclusion, while there were histopathologically degenerations in livers, gills, intestines and kidney tissues of fish exposed to lead, LC caused a reduction in the severity of the toxicity of lead.

Increases and decreases in the expression of some proteins in lead toxicity occurred, electrophoretically, increase in expressions of proteins with 98 kD and 43 kD molecular weights in the group that was administered lead + LC were observed.

In the biochemical analysis, while levels of total protein in the group which applied lead were found to be high, levels of total protein in the group that was applied lead + LC were found to be low.

LC increases the albumin, insignificantly.

Compared to control group, globulin levels in the group that was applied LC + lead were found to be lower ($P<0.05$).

TAC levels against the lead toxicity were found to show a protective effect of L-carnitine.

While depending on the dose and duration lead acetate administered revealed various toxic effects on *Capoeta capoeta*, LC showed protective effect on fish that were exposed to lead.

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