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RESEARCH ARTICLE

Toxic Effects of Cadmium Sulphate on Tissue Histopathology and Serum Protein Expression in European Chub, *Leuciscus cephalus* (Linnaeus, 1758)

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Summary

Effects of high water cadmium sulphate hydrate ($3CdSO_4 * 8H_2O$) ($CdSO_4$) levels on the expression of serum proteins and tissue histopathology of the European chub *(Leuciscus cephalus)* was investigated. Three groups of fish (control, 1st, and 2nd), each containing 10 animals, were placed in separate tanks containing no, 1 mg/L, and 2 mg/L $CdSO_4$, respectively, for 10 days. At the end of the study period, blood samples were taken and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Fish tissue samples were taken then routinely processed and stained with hematoxylin and eosin to observe histopathological changes. Depending on the increasing concentration of $CdSO_4$, a gradual decrease in protein bands was determined. Inhibition of 35.3 kilodalton (kD) and 100.5 kD proteins in the 1st group and of 44.5 kD and 47.3 kD proteins in the 2nd group was detected. Microscopic examination revealed degeneration in the secondary lamellar epithelia, hydropic degeneration and necrosis in chloride cells of the fish gill in $CdSO_4$ given groups. Hydropic degeneration and necrosis were also observed in light to severe levels in the liver depending on the groups. It was concluded that $CdSO_4$ has toxic effects on the expression of serum proteins and causes degenerative changes on gill and liver morphology in *Leuciscus cephalus*.

Keywords: *Cadmium sulphate, Serum protein, SDS-PAGE, Histopathology, Leuciscus cephalus*

Kadmiyum Sülfat'ın Avrupa Tatlısu Kefali, *Leuciscus cephalus* (Linnaeus, 1758)'un Doku Histopatolojisi ve Serum Protein Ekspresyonu Üzerine Toksik Etkileri

Özet

Yüksek düzeylerde kadmiyum sülfat hidrat ($3CdSO_4 * 8H_2O$) ($CdSO_4$) içeren suyun Avrupa tatlısu kefali *(Leuciscus cephalus)*'nin doku histopatolojisi ve serum proteinlerinin ekspresyonu üzerine etkileri araştırıldı. Balıklar her grupta 10 hayvan bulunan 3 gruba ayrıldı (kontrol grubu, 1. grup ve 2. grup) ve sırasıyla $CdSO_4$ içermeyen, 1 mg/L, and 2 mg/L $CdSO_4$ içeren tanklarda 10 gün süreyle bekletildi. Çalışma süresi sonunda, kan örnekleri alınarak sodyum dodesil sülfat-poliakrilamid jel elektroforezi (SDS-PAGE) yapıldı. Daha sonra balıklardan alınan doku örnekleri rutin yöntemlerle hematoksilen ve eozin ile boyanarak histopatolojik değişiklikler izlendi. $CdSO_4$ konsantrasyonundaki artışa bağlı olarak protein bantlarında kademeli bir incelme tespit edildi. 1. grupta 35.3 kD ve 100.5 kD'luk proteinlerde, 2. grupta da 44.5 kD ve 47.3 kD'luk proteinlerde inhibisyon tespit edildi. Mikroskobik incelemede ise $CdSO_4$ verilen balıkların solungaç dokusunda sekonder lamel epitelinde dejenerasyonla birlikte klorid epitel hücrelerinde de nekroz ve hidropik dejenerasyon tespit edildi. Karaciğer dokusunda da gruplarda önemli seviyelerde hidropik dejenerasyon ve nekroz gözlendi. $CdSO_4$'ın *Leuciscus cephalus*'da toksik etki yaparak serum proteinlerinin ekspresyonları, karaciğer ve solungaç morfolojisi üzerinde dejeneratif değişikliklere neden olduğu sonucuna varılmıştır.

Anahtar sözcükler: *Kadmiyum sülfat, Serum protein, SDS-PAGE, Histopatoloji, Leuciscus cephalus*

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INTRODUCTION

Cadmium (Cd) is an abundantly toxicant substance in the aquatic environment. As a non-degradable cumulative pollutant, it presents a significant health problem in living organisms. It is a widely used heavy metal in ship building, batteries, dying and electronic industries [1]. Water pollution of the metal mostly becomes leaches from these industries.

Cadmium in water is taken in through respiration and digestion and then it passes through other organs. Liver plays a major role in detoxification through inducing the expression of metal binding molecule metallothionein [2]. Later, Cd-metallothionein complex enters the blood. Although liver involves in Cd detoxification the metal builds up mostly in kidney causing renal tubular degeneration especially in chronic toxicities [3-5].

Studies on Cd toxicity are quite limited compared to the other metals. In this study, effects of high $CdSO_4$ exposure on the tissue histopathology and the expression of serum proteins of *Leuciscus cephalus* were investigated.

MATERIAL and METHODS

Experimental Design

Thirty *Leuciscus cephalus,* weighing 125-234 g, were caught by electro shocker in Kars Stream, Turkey. Water quality of the river during the collection of fish was as follow; pH 7.8-8.0, dissolved oxygen 5.1-8.8 mg/L, mean conductivity 210 ms/cm^2, mean ammonium (NH_3) 414 µg/L, total phosphate (PO_4) 56.6 µg/L, nitrate (NO_3) 0.245 mg/L and temperature 17-18.5ºC. Cadmium levels in the edible muscle tissues of *Capoeta capoeta capoeta* in Kars creek were found to be 0.0018-0.0029 µg^{-1} [6].

Fish were divided into three equal groups and placed in 500-L aquariums. Water temperature was adjusted to 18±1ºC with a thermostatic thermometer and dissolved oxygen was supplied at 5±0.3 mg/L concentration in the aquariums. While fish in control group were kept in tap water with no additional cadmium in it fish in 1st and 2nd groups were placed in aquariums containing 1 mg/L and 2 mg/L cadmium sulphate hydrate ($3CdSO_4 * 8H_2O$) (Merck, 102027), respectively, for 10 days. The fish were fed daily with standard fish-feed.

SDS-PAGE

At the end of the study period, blood samples were taken from the dorsal aorta of fish and then immediately centrifuged for 10 min at +4ºC and 805X g. Serums were removed and total protein amounts were measured by the biurate method [7]. Afterwards, serum proteins were modified by Laemmli [8] and O'Farrell [9] methods, and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS -PAGE) was performed. The gels were photographed, and molecular weights of proteins were calculated [10]. Calf albumin (66 kD), egg albumin (45 kD), glyseraldehite-3-phosphate dehydrogenase (36 kD) and β-lactoglobulin (18 kD) were used as standard proteins.

Histopathology

Fish were dissected without using anesthetic agent and then tissue samples were collected and fixed in 10% phosphate buffered formaldehyde solution. Gill samples were decalcified with Osteodec (Bio-Optica, Italy). Paraffin blocks were prepared and sections of 4-5 µm thickness were taken. Tissue sections were stained with hematoxylin and eosin, and examined under a light microscope to observe histopathological changes.

RESULTS

In microscopic examination, while liver samples did not change in the control group *(Fig. 1A)*, the histopathologic changes have occurred in the trial groups exposed to $CdSO_4$. Light to moderate degeneration in the 1st group and moderate to severe degeneration in the 2nd group was observed in liver. Although degenerate and necrotic cells were present in both groups, compared to the 1st group *(Fig. 1B)* seemingly a proportional increase in the amount of necrotic cells as well as increased Kuppfer cell activation was noted in the 2nd group *(Fig. 1C)*. Localization of necrotic cells did not show any apparent presentation. Gill histology also changed, compared to the control *(Fig. 2A)*, in $CdSO_4$ exposed fish. Hydropic degeneration was the main finding in gills in the 1st group *(Fig. 2B)*. In the 2nd group, hydropic degeneration and necrosis were observed in the secondary lamellar epithelia as well as swelling of chloride cells *(Fig. 2C)*. The severity of the changes in gills was also increased with the higher test dose.

Electrophoretic separation of serum proteins revealed inhibitions in the expression of proteins with molecular weights of 100.5 kD and 35.3 kD in the 1st group and 47.3 kD and 44.5 kD in the 2nd group compared to the control group *(Fig. 3)*.

DISCUSSION

Metal toxicities in fish have dual importance due to the effects caused in fish themselves and humans that consumed them. Many metals, alone or in combination, have been studied on many different kinds of fish. In a study, effects of $CoCl_2$ on *Capoeta capoeta capoeta* were investigated, and thinnings in various protein types were observed in experimental groups in comparison to the control group, formation of a 32.4 kD, 33.3 kD, 30.6 kD, and 28.2 kD new protein bands in the groups that $CoCl_2$ applied, however, an increase in the level of degeneration in the livers and intestines tissues of the experimental fish groups in parallel to the increase of the dose were

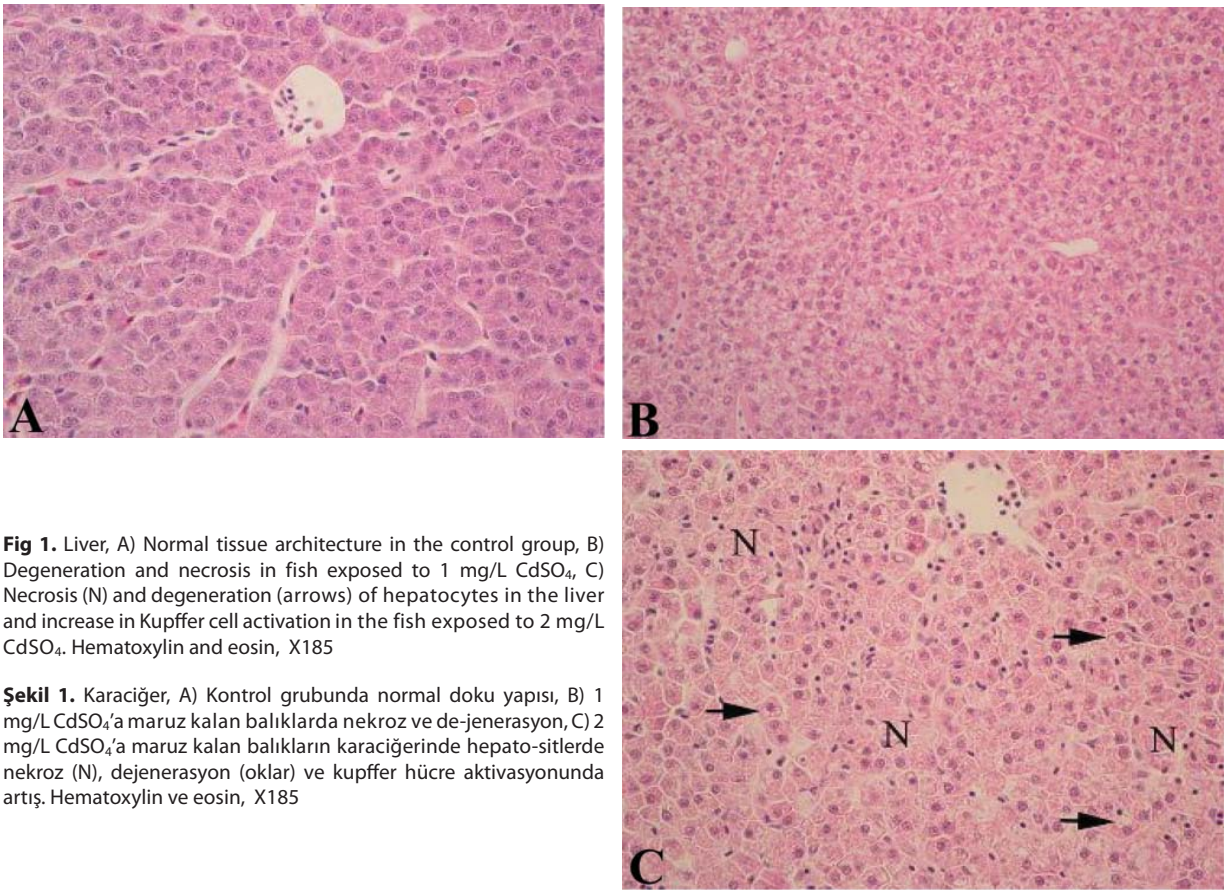

Fig 1. Liver, A) Normal tissue architecture in the control group, B) Degeneration and necrosis in fish exposed to 1 mg/L $CdSO_4$, C) Necrosis (N) and degeneration (arrows) of hepatocytes in the liver and increase in Kupffer cell activation in the fish exposed to 2 mg/L $CdSO_4$. Hematoxylin and eosin, X185

Şekil 1. Karaciğer, A) Kontrol grubunda normal doku yapısı, B) 1 mg/L $CdSO_4$'a maruz kalan balıklarda nekroz ve de-jenerasyon, C) 2 mg/L $CdSO_4$'a maruz kalan balıkların karaciğerinde hepato-sitlerde nekroz (N), dejenerasyon (oklar) ve kupffer hücre aktivasyonunda artış. Hematoxylin ve eosin, X185

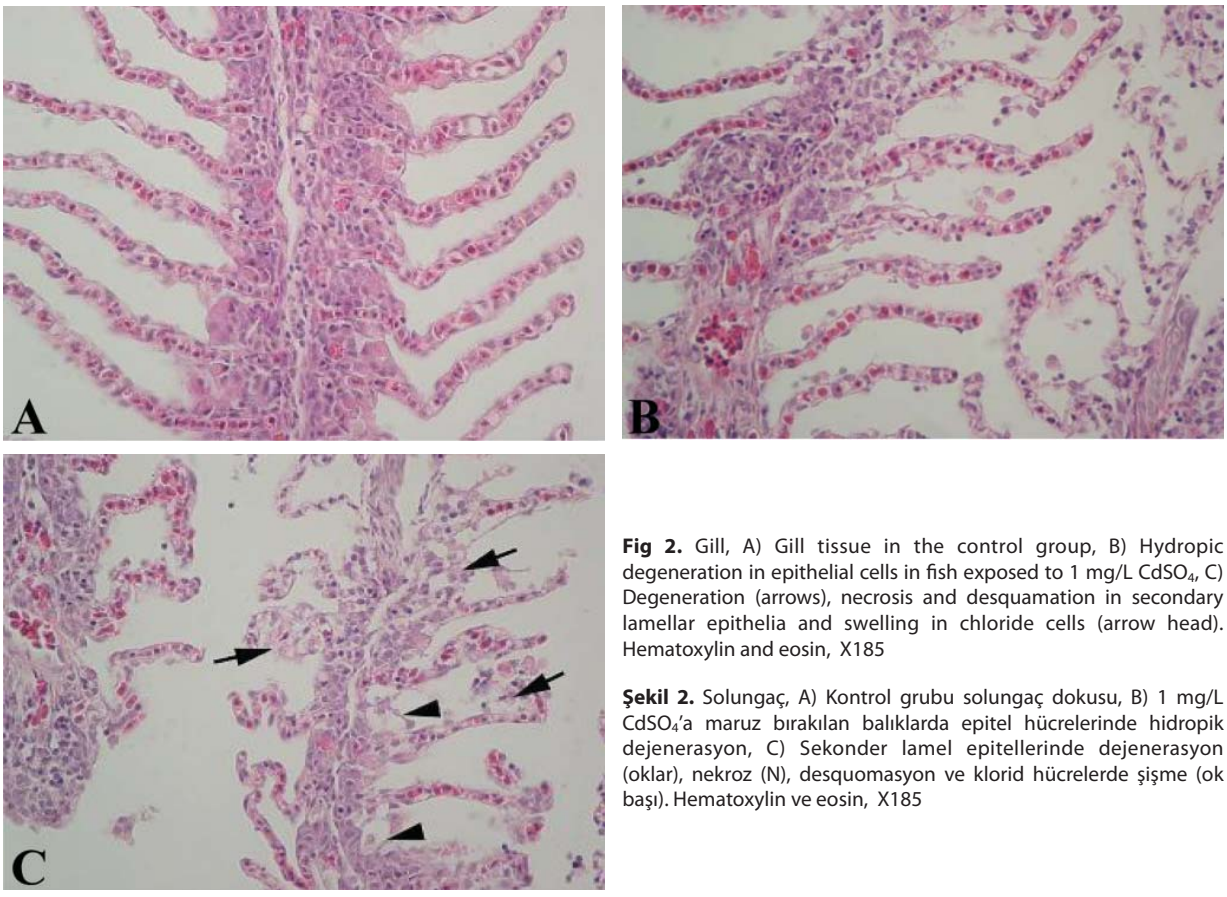

Fig 2. Gill, A) Gill tissue in the control group, B) Hydropic degeneration in epithelial cells in fish exposed to 1 mg/L $CdSO_4$, C) Degeneration (arrows), necrosis and desquamation in secondary lamellar epithelia and swelling in chloride cells (arrow head). Hematoxylin and eosin, X185

Şekil 2. Solungaç, A) Kontrol grubu solungaç dokusu, B) 1 mg/L $CdSO_4$'a maruz bırakılan balıklarda epitel hücrelerinde hidropik dejenerasyon, C) Sekonder lamel epitellerinde dejenerasyon (oklar), nekroz (N), desquomasyon ve klorid hücrelerde şişme (ok başı). Hematoxylin ve eosin, X185

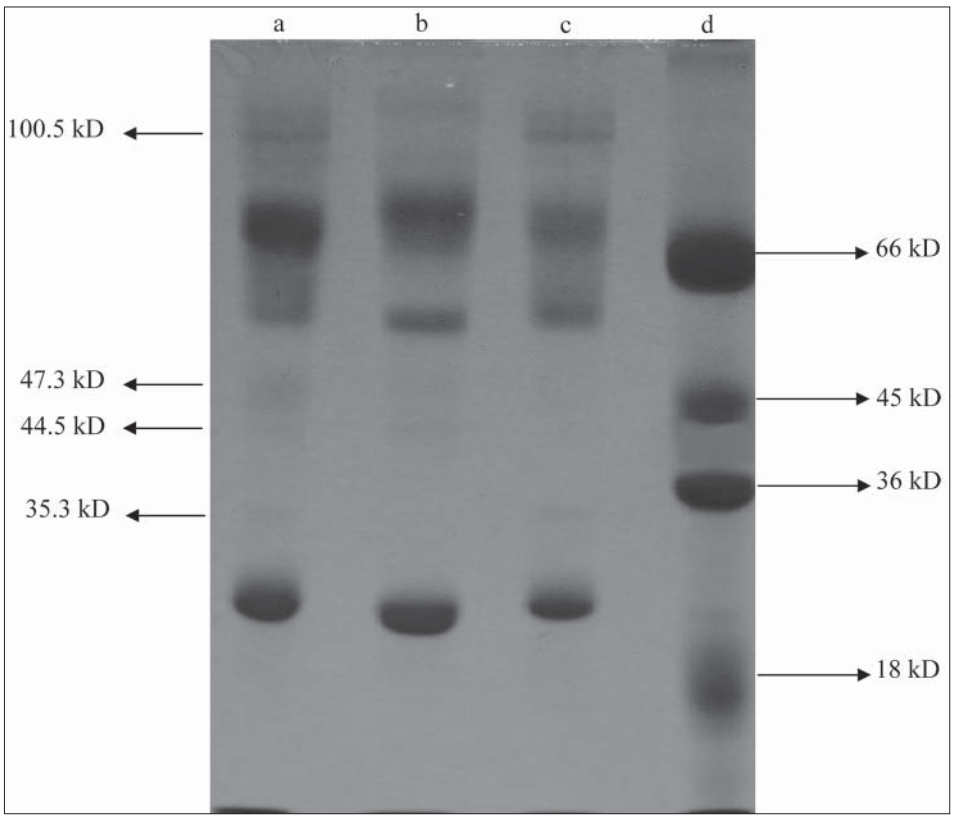

Fig 3. Electrophoregram obtained from the SDS-PAGE of the serums of fish exposed to $CdSO_4$. **a-** Control group; **b-** 1 mg/L dose group; **c-** 2 mg/L dose group; **d-** Standard proteins

Şekil 3. $CdSO_4$'a maruz bırakılan balıkların serum proteinlerinin SDS-PAGE'den elde edilen elektroforegramı. **a-** Kontrol grubu; **b-** 1 mg/L'lik grup; **c-** 2 mg/L'lik grup; **d-** Standart proteinler

reported [11]. In another study, Cobalt-*p*-hydroxybenzoate (CoPHB) exposure in *Capoeta capoeta capoeta*, and compared to control, over-expression and inhibition of proteins with 85.8, 82.6, 73.9, 68.5 kD and 23.0 and 15.2 kD molecular weights in the CoPHB groups. Also, depending on the application of cobalt in liver, intestine and gill tissue degeneration has been reported [12].

In this study, the effects of $CdSO_4$ exposure on the expression of serum proteins and tissue morphology in *Leuciscus cephalus* were investigated.

In the present study, microscopic examinations of the $CdSO_4$ exposed fish were revealed hydropic degeneration and necrosis of the secondary lamellar epithelia and swelling of chloride cells. Although similar results with Cd exposure were seen in other studies on different species of fish, many other histopathological changes were also reported. Cd exposure of *Gnathonemus petersii* at 1 mg/L concentration for 6 h was reported to cause big sub-epithelial spaces in the secondary lamella of gills and at 10 mg/L concentration lamellar aneurysm was shown [13]. In *Oreochromis niloticus*, 25 mg/L $CdCl_2$ for 4 days caused interstitial edema, swelling of lamella, lifting and cellular proliferation of the filamentar epithelium, lamellar fusion as a result of epithelial hyperplasia and hypertrophy, break-down of pillar cell system, and aneurisms with some ruptures and necrosis, especially in the filamentar epithelium [14]. In another study, 630 and 840 µg/L $CdCl_2$ exposure in *Puntius conchonius* resulted in decomposition in the secondary lamellar epithelium of gills, necrosis, cellular debris accumulation, capillary congestion and milting in pillar cell system, partial to total fusion in secondary lamella along with hyperplasia and hypertrophy in chloride cells [15]. In a study conducted by Thophon et al.[16], *Lates calcarifer* was exposed to $CdCl_2$ for either 10 mg/L for 96 h or 0.8 mg/L for 90 days. In gills, deterioration in pillar cell system as well as edema in epithelial cells, aneurysm in some ruptures of epithelial and chloride cells, and hyperplasia and hypertrophy was observed.

Gill is known to be one of the most affected organs in many types of toxicities since it is the port of entry of the toxicants dissolved in water. Degeneration of this organ, in general, results in unbalanced oxygen delivery, which then might trigger other functional problems in other metabolic organs such as liver and kidney. The severity of the lesions observed in gill cells in this investigation was comparably lower than the described in the literature. This might be explained by lower toxic potential of Cd in *Leuciscus cephalus*.

Degeneration and necrosis of liver cells were also detected in the current experiment. In the study of Thophon et al.[16], congestion in liver sinusoids, hydropic swelling and vacuolization in hepatocytes, and dark granule accumulation were reported. In the subchronic application, lipid droplets and glycogen content were also recorded in hepatocytes.

Liver is known to play a major role in detoxification process of Cd like many other metals. However,

investigations studying the detoxification function of liver were showed controversial results making difficult to analyze the findings. In a study, $CdCl_2$ exposure was reported to increase activities of catalase, superoxide dismutase and glutathione peroxidase in liver of *Salaria basilisca* [17]. Similarly, increased liver activities of glutathione and glucose-6-phosphate dehydrogenase were detected in *Oreochromis niloticus* [18]. Contrary to these studies, liver activities of catalase, superoxide dismutase and glutathione peroxidase were detected to decrease in *Oncorhynchus mykiss* [19] and *Carassius auratus gibelio* [20]. However, changes in liver metallothionine, glutathione-S-transferase, alanine aminotransferase and aspartate aminotransferase levels clearly indicates liver involvement in Cd metabolism [21-23].

Serum or organ proteins of fish are occasionally studied to estimate the toxic potential of many substances including metals [24-26]. Results of such investigations are valuable in observing the proteins involved in the metabolism of the toxic substance. $CdCl_2$ and $ZnCl_2$ exposure of chinook salmon embryo cells were shown to increase the expression of proteins with molecular weights of 84, 70, 68, 51, 46 and 28 kD. Increased expression of 10 kD metallothionein was also detected [27]. In the present study, expression of proteins with molecular weights of 100.5 and 35.3 kD in the 1st group and 47.3 and 44.5 kD in the 2nd group were determined to be inhibited compared to the control fish.

In conclusion, limited lesions observed in liver and gill and absence of other organ involvement such as kidney, which was reported in many other fish species, and absence of increased metallothionein expression indicates that $CdSO_4$ has limited toxic effects on *Leuciscus cephalus*. However, changing pattern of serum proteins expression depending on the dosage might success that different competing mechanisms involve in Cd toxicity under the studied test conditions.

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