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RESEARCH ARTICLE

Toxic Effects of Cobalt II Chloride on Tissue Histopathology and Serum Proteins in *Capoeta capoeta capoeta* (Guldenstaedt 1772) [1]

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Summary

In this study, the effects of Cobalt (II) chloride ($CoCl_2$) on *Capoeta capoeta capoeta* (Guldenstaedt 1772) were investigated by electrophoretic and histopathological methods. The fish from Kars Creek were placed in 500 liters tanks and they made to adapt into the medium for 15 days. Later, they were divided into 3 groups. The fish in the 1st group were held in normal water, 2nd and 3rd groups were held in the water containing 1 mg/L and 2 mg/L $CoCl_2$, respectively for 10 days. At the end of this period, blood and tissue samples were taken from the fish for electrophoresis and histopathological examinations. Serum samples obtained were run in Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Tissue samples were fixed in %10 formaldehyde solution. Paraffin blocks were prepared by routine histological methods and slices 4-5 µ thickness were performed. The slices obtained were dyed with hematoxylen and eosin dying method and examined under light microscope. Thinnings in various protein types were observed in experimental groups in comparison to the control group in the electrophoregram. These thinnings were more in the group that 1 mg/L $CoCl_2$ applied. In addition, formation of a 32.4 kD new protein band was observed in the group that 1 mg/L was applied; and 33.3 kD, 30.6 kD, and 28.2 kD new protein bands were observed in the group that 2 mg/L $CoCl_2$ applied. In histopathological evaluations, an increase in the level of degeneration was observed in the livers and intestines tissues of the experimental fish groups in parallel to the increase of the dose. Due to the changes both in the serum protein expressions and in the tissues, we conclude that Cobalt can be dangerous at higher concentrations.

Keywords: *Cobalt chloride, Capoeta capoeta capoeta, Serum protein, SDS-PAGE, Histopathology*

Capoeta capoeta capoeta (Guldenstaedt 1772)'nın Serum Proteinleri ve Doku Histopatolojisi Üzerine Kobalt (II) Klorür'ün Toksik Etkileri

Özet

Bu çalışmada, *Capoeta capoeta capoeta* (Guldenstaedt 1772) üzerine Kobalt (II) klorür'ün etkileri elektroforetik ve histopatolojik yöntemlerle araştırıldı. Kars Çayı'ndan yakalanan balıklar 500 litrelik tanklara konularak 15 gün süreyle ortama adaptasyonları sağlandı. Daha sonra 3 gruba ayrılarak I. gruptaki balıklar normal su ortamında, II. ve III. gruptaki balıklar ise sırasıyla 1 ve 2 mg/L $CoCl_2$ içeren su ortamlarında 10 gün süreyle bekletildi. Bu süre sonunda elektroforetik ve histopatolojik çalışmalar için balıklardan kan ve doku örnekleri alındı. Elde edilen serum örnekleri Sodyum dodesil sülfat poliakrilamid jel elektroforezi (SDS-PAGE)'nde yürütüldü. Doku örnekleri ise %10'luk formaldehit solüsyonunda tespit edilerek rutin histolojik yöntemlerle parafin bloklar hazırlandı ve 4-5 µ kalınlığında kesitler alındı ve elde edilen kesitlerin tamamı hematoksilen ve eosin boyama metoduna göre boyanarak ışık mikroskobunda incelendi. SDS-PAGE'den elde edilen elektroforegramda kontrol grubuna göre deney gruplarındaki birçok protein bandında incelmeler olduğu belirlendi. Bu incelmeler 1 mg/L $CoCl_2$ uygulanan grupta daha fazla olduğu, bununla birlikte 1 mg/L'lik grupta 32.4 kD, 2 mg/L'lik grupta ise 33.3 kD, 30.6 kD ve 28.2 kD'luk yeni proteinlerin sentezlendiği saptandı. Histopatolojik incelemelerde ise; deney gruplarındaki balıkların karaciğer ve bağırsak dokularında doz artışıyla orantılı olarak artan derecelerde dejenerasyonlar gözlemlendi. Gerek serum protein ekspresyonlarındaki gerekse de karaciğer ve bağırsak dokusundaki değişikliklere göre, Kobalt'ın yüksek konsantrasyonlarının tehlikeli olabileceği sonucuna varıldı.

Anahtar sözcükler: *Kobalt klorür, Capoeta capoeta capoeta, Serum protein, SDS-PAGE, Histopatoloji*

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INTRODUCTION

Cobalt is an essential trace element and common all over the world. It is obligated for proper formation of vitamin B_{12}. Deficiency of cobalt in fish and other animals results in anaemia. However, the excessive intake of cobalt by organisms results in toxic effects. Cobalt concentrations in drinking water range from less than 0.1 µg to approximately 5.0 µg [1]. Cobalt level in the edible muscle tissue of *Capoeta capoeta capoeta* in Kars creek was found to be 0.0038-0.373 μg^{-1} [2]. Sediments collected in Kars Creek were tested for mutagenicity by means of peripheral erythrocyte in *Orthrias angorae*. Micronuclei frequencies (MN) of all the groups exposed to the sediments were higher than those of the control group. Researchers claimed that the presence of mutagenic agents in Kars Creek sediments [3].

Cobalt and its alloys are widely used in many industrial fields such as automobile, fuel, battery, paint, metallurgy, and cosmetic. Due to the common use of cobalt and its derivatives on countless fields, contamination of environment, especially contamination of the natural waters, is a big environmental concern [4]. Due to high levels of cobalt contamination, majority of organisms are in the risk. Despite the fact that cobalt possesses a serious environmental risk, the studies that evaluated the effects of cobalt compounds on serum proteins and other tissues are rare [4-6].

The aim of the present study was to investigate the effects of $CoCl_2$ on *Capoeta capoeta capoeta*. We particularly studied the histopathological changes in liver, intestine and the serum protein levels after the fish was exposed to Cobalt (II) chloride.

MATERIAL and METHODS

Experimental Design

Eighteen *Capoeta capoeta capoeta,* weighing 200-250 g, were caught in Kars Creek, Turkey. Water quality of the creek during the collection of fish was as follow; pH 7.8-8.0, dissolved oxygen 5.1-8.8 mg/L and temperature 17-18.5°C. Following the collection of the fish, they were divided into three equal groups and placed in 500-L aquariums. Tap water temperature was adjusted to 18±1°C with a thermostatic thermometer and dissolved oxygen was supplied at 5±0.3 mg/L concentration in the aquariums.

The fish in group I (control) were kept in a tank that contains tap water, and the fish in groups II and III were kept in tanks that contain 1 mg/L and 2 mg/L cobalt (II) chloride, respectively, for 10 days. The fish were fed daily with standard fish-feed.

Electrophoresis

Blood samples were taken from the dorsal aorta of fish by using an injection syringe, they were centrifuged for 10 min at +4°C and 805X g, the serums were separated and total protein contents were measured by the Biuret method [7]. SDS-PAGE was performed according to the methods of Laemmli [8] and O'Farrell [9]. The gels were photographed and molecular weights of proteins were calculated according to the method of Weber et al.[10]. Calf albumin (66 kD), egg albumin (45 kD), carbonic anhydrase (29 kD) and β-lactoglobulin (18 kD) were used as standard protein. The percentages of the changes in serum protein bands were calculated according to,

$$\frac{\text{The number of protein bands changed}}{\text{The number of total protein bands}} \text{ X } 100$$

formula.

Histopathology

The tissue samples collected for histopathological analyses were fixed in 10% phosphate buffered formaldehyde solution for 48 h. Gill samples were decalcified with Osteodec (Bio-Optica, Italy). Paraffin blocks were prepared from the collected tissues by routine methods, and slices of 4-5 µm thickness were taken. All the obtained slices were stained according to hematoxylen and eosin staining method, and examined under the light microscope.

RESULTS

It was determined that in the SDS-PAGE obtained through electrophoregram there were thinning in various protein types of experimental groups in comparison with control group and these thinning were more in the group that 1 mg/L $CoCl_2$ applied. Furthermore, it was found that the fish kept in 1 mg/L $CoCl_2$ synthesized a new 32.4 kD protein band, and those kept in 2 mg/L synthesized three new protein bands of 33.3 kD, 30.6 kD, and 28.2 kD *(Fig. 1)*. As a result of exposure to cobalt (II) chloride, the percentages of change occurring in the serum protein bands were shown in *Table 1*.

Table 1. *As a result of exposure to cobalt (II) chloride, the percentages of change occurring in the serum protein bands*

Tablo 1. *Kobalt klorür maruziyetine bağlı olarak serum protein bandlarında meydana gelen değişim yüzdeleri*

Concentrations	Thinnings	New Protein Bands
1 mg/L	%80	%10
2 mg/L	%30	%30

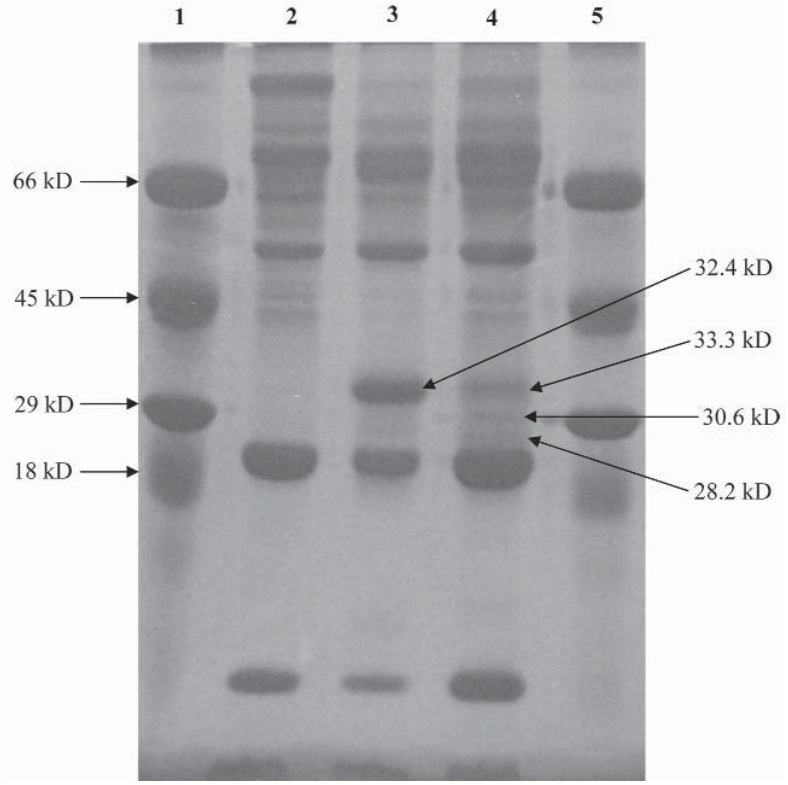

Fig 1. Electrophoregram obtained from the SDS-PAGE of the serum proteins of the fish exposed to cobalt (II) chloride. **1-5.** Standard proteins; **2.** Control group; **3.** 1 mg/L dose group; **4.** 2 mg/L dose group

Şekil 1. Kobalt (II) klorür'e maruz kalan balıkların serum proteinlerinin SDS-PAGE'den elde edilen elektro-foregramı. **1-5.** Standart proteinler; **2.** Kontrol grubu; **3.** 1 mg/L'lık grup; **4.** 2 mg/L'lik grup

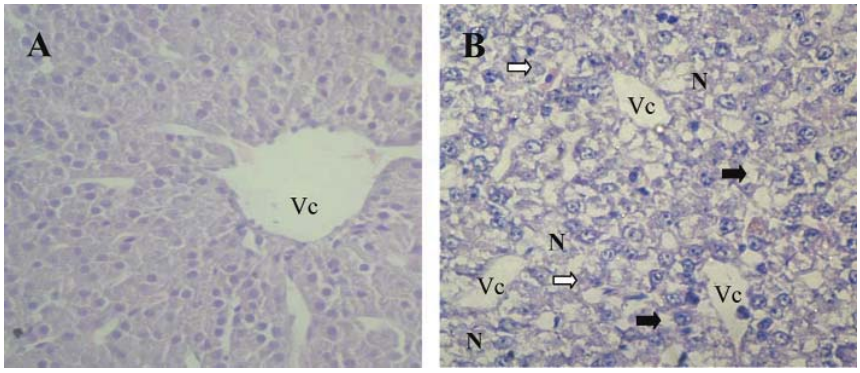

Fig 2. A- Liver tissue in the control group, **B-** Widespread areas of necrosis (N) of hepatocytes in the liver tissue of the fish exposed to $CoCl_2$, hidropic (white arrows) and areas of dissociation with vacuolar degenerations (black arrows) (H&E X 40)

Şekil 2. A- Karaciğer dokusu kontrol grubu. **B-** $CoCl_2$ uygulanan balıklardan elde edilen karaciğer dokusunda yaygın nekroz alanları (N), hidropik (beyaz oklar) ve vakuolar (siyah oklar) ile birlikte dissosiasyon alanları (H&E X 40)

When slices obtained from liver and intestine examined under the light microscope, the most remarkable histopathological changes were found in liver. Liver *(Fig. 2A)* and intestine *(Fig. 3A)* slices of the control group did not deviated from the normal architecture. In histopathological evaluations, common growing areas of necrosis, hidropic degenerations and dissociation with vacuolar degenerations were observed *(Fig. 2B)* in parallel to increase in the dose. Hemorrhage, desquamation and necrosis in villous epithelium were detected in the intestinal tissue *(Fig. 3B, C)*.

DISCUSSION

For humans, food and beverages represent the main source of cobalt intake. Traces of cobalt are also present in cement and various household products. In industry, the potential for exposure to cobalt is particularly important during the production of cobalt powder, the production, processing and use of hard metals, the polishing of diamonds with cobalt containing disks, and the processing of cobalt alloys [11].

Effects of cobalt toxicities on various organisms have been documented [4,12,13]. Effects of cobalt on hepatotoxicity in adult rats and their suckling pups have been investigated and histological studies revealed an infiltration of mononuclear cells and vascular congestion in liver pups and their mothers [12]. In another study, genotoxic and histopathological effects of heavy metal accumulation (Cd, Cu, Co, Pb and Zn) on *Chondrostoma*

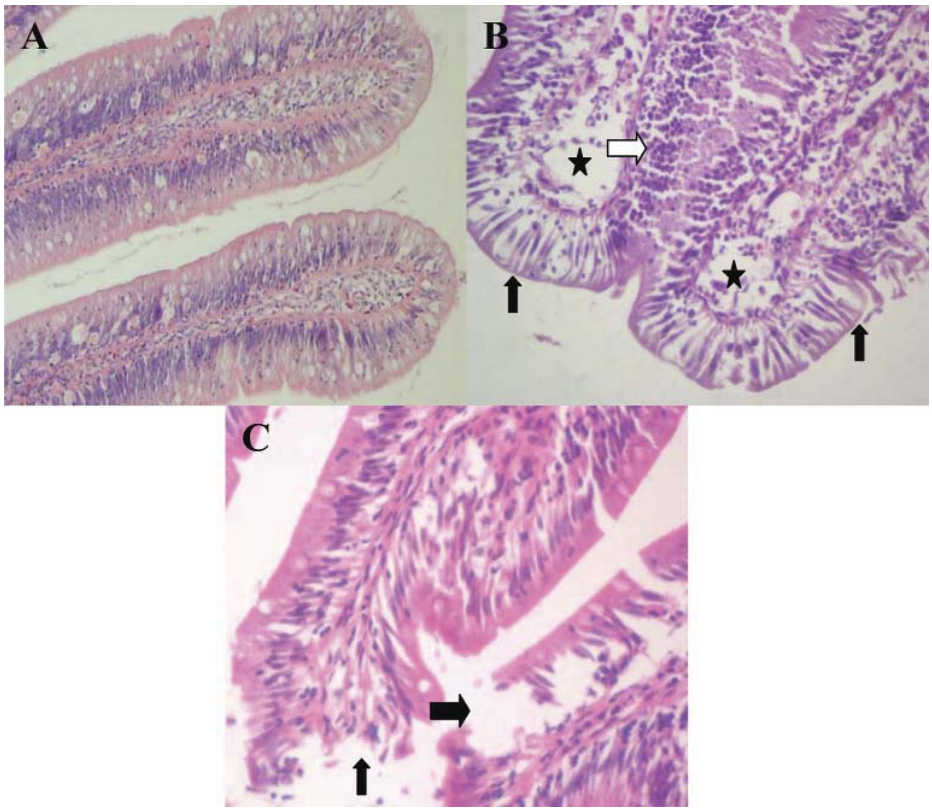

Fig 3. A- Intestine tissue in the control group, **B, C-** Hemorrhage, desquamation in intestine tissue of the fish exposed to $CoCl_2$ (white arrows), necrosis in villous epitelium (black arrows) and degeneration in lamina propria (stars) (H&E X 40)

Şekil 3. A- Bağırsak dokusu kontrol grubu. **B, C-** $CoCl_2$ uygulanan balıklardan elde edilen bağırsak dokusunda deskuomasyon, hemoraji (beyaz ok), villus epitelinde nekroz (siyah oklar) ve lamina propriada dejenerasyon (yıldızlar) H&E X 40)

nasus and *Barbus capito pectolaris* in Buyuk Menderes River and Cine stream (Turkey) were investigated. The histopathological results indicated significant decreases of mean lengths of primary and secondary lamellae. The changes in gill epitelia included in cellular proliferation with secondary fusion, ballooning degenerations, chub deformation of secondary lamellae, and cystic structures in secondary lamellae. The changes in liver included in swollen and ruptured parenchymal cells, some loss of cord structure, vacuoles filled with cellular debris, focal necrosis, and a significant increase in Kupffer cells [14]. The concentration of the heavy metals (Fe, Zn, Cu, Pb, Cd and Co) in the water and various fish organs of *Oreochromis niloticus* and *Lates niloticus* obtained from Lake Nasser, Egypt were investigated by atomic absorbsion spectrophotometer. Likewise, histopathological alterations due to higher concentrations of these metals were observed. Results showed that several histopathological alterations were observed including vacuolar degeneration with focal areas of necrosis in liver, proliferation in the epitelium of gill filaments, fusion of secondary lamellae, severe degenerative and necrotic changes in the intestinal mucosa, seminiferous tubules, degeneration and atrophy in cardiac muscle fibers, and degeneration in muscle bundles [5].

Serum albumins are synthesized in liver, and changes in serum proteins are inevitable during the metal toxicity. In our previous study, toxic effects of cobalt parahydroxybenzoate in liver, gills and intestine and serum proteins of *Capoeta capoeta capoeta* were investigated. We observed a decrease in the expressions of proteins with molecular weights of 85.8, 82.6, 73.9, and 68.5 kD while an increases in the expression of the proteins with molecular weights of 23.0 and 15.2 kD. Therefore, we concluded that the change in serum proteins may be a response to cobalt exposure especially in the liver. In histopathologic examination, degeneration in hepatocytes and dissociation in hepatic cords were observed in the treatment groups. The severity of the degeneration and the dissociation increased, and necrosis was noted in the liver of fish exposed to higher concentration of cobalt parahydroxybenzoate. In gill, degeneration and occasional necrosis in the epithelial cells of secondary lamellae were detected in both groups. Degeneration and necrosis of villous epithelium in the intestine were observed only in fish exposed to higher concentration of cobalt parahydroxybenzoate [4]. Histological aspects of liver and intestinal tissue of the current study show similarities to the findings of abovementioned study. Nevertheless, the serum proteins showed differences in direction. In the previous study in which we evaluated the effects of cobalt parahydroxybenzoate application, synthesis of a new serum protein and inhibition in the expression of some other proteins were observed.

However, in the present study new proteins were synthesized when fish exposed to cobalt (II) chloride. Based on finding in this study, we propose that the newly synthesized proteins are a part of response to cobalt exposure.

In conclusion, different cobalt concentrations can have different toxic effect on fish, and the excessive cobalt exposure causes damage in the liver and the intestine of *Capoeta capoeta capoeta*. Results of this study can be used as a guide for biomonitoring studies of cobalt toxicity on fish.

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