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Acute Toxicity of Hexavalent Chromium on Behavioural Parameters and Histopathology Damages in Freshwater Common Carp, *Cyprinus carpio*

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Authors' contributions:

This work was carried out in collaboration among all authors. Author TD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GBS performed data generation and analysis with literature search. Author CSS managed the analyses of the study. All authors read and approved the final manuscript

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ABSTRACT

Water sources of almost all regions in India are carrying the unbearable burden of dangerous pollutants. The release of waste products and anthropogenic wastes run-off has contributed to aquatic pollution. The environment has become a storehouse for chemical pollutant which infiltrate into the aquatic environment, including estuarine, thus immobilizing the aquatic biota, among the heavy metals. Chromium is a heavy metal which has both beneficial and harmful effect on organisms. It is highly toxic and carcinogenic. Many industries are disposing of chemical pollutants to the water. These disposals contain many chemicals, including Chromium. The purpose of this review was to check the accumulation and concentration of heavy metals in different organs of freshwater fishes that come in contact with the water contaminated with heavy metals. The subjected fish were exposed to Chromium (Cr) at the sub-lethal level at a concentration of 40mg/L

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in 96 hours. During the observation period, the fishes shown some behavioral changes like erratic swimming, slow motility, suffocation, and the scales become thin and decolorized. In the gill region, the gill filaments become swollen, and gill rackers become thick, and curling of lamellae was noticed, and it is caused due to the Chromium build-upon gills. At the region of the caudal fin, permanent bending of the tail was observed. The Chromium also affects other vital organs like the spleen and gut region. LC50 was found in 96 hours. The result indicates that Chromium is highly toxic and has deleterious effects on aquatic life. Humans are also affected by the intake of fishes for primary people of those areas where the leading food is fish.

Keywords: Toxic effect; behavioral change; caudal fin; scales; vital organs.

1. INTRODUCTION

Heavy metals play an essential role in various biological activities of terrestrial and aquatic organisms and remain present in trace amounts in the body, not exceeding more than 0.05 μg/g (WHO and FAO). Heavy metals like Aluminum, Chromium, cobalt, copper, iron, nickel, and zinc are essential heavy metals for organisms. In some cases, the increasing concentration of heavy metals leads to toxicity in different organs of an organism. Heavy metals are essential contaminants of aquatic environments worldwide [1]. Metal pollution has increased with the technological progress of human society. Industry, mining, advanced agriculture, household waste, and motor traffic are all among the activities considered significant sources of metal pollution. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments [2,3]. In this metal pollution, Chromium is a significant pollutant that's affecting aquatic fauna. Industrialization leads to many tones of chromium ore are importing for the production of stainless steels, chrome-plated metals, pigments for inks and paints, and a wide variety of chemicals. In some continents like Asia, Europe and America reported lung disease and different respiratory disorders among workers involved in the manufacture of chromates. Chromium is considered one of the most ubiquitous pollutants in the aquatic environment, but the pure metallic form is naturally absent [4,5,6]. There are three oxidation states in the case of Chromium, namely Cr (II), Cr (III), Cr (VI). Cr (II) is the most unstable form, and (Cr+3) is an essential element in mammals as it plays an influential role in glucose, lipid, and protein metabolism.

Others document that land dumping of wastes from chromate production has been responsible for groundwater contamination. Chromium waste discharge into streams and lakes has caused damage to aquatic ecosystems [7]. The increase of heavy metals in water intimates that fish may help as an estimable indicator for contamination metals in aquatic systems because they respond with great sensitivity to changes in the aquatic environment [8,9,10]. Scientific evidence of toxicological impacts of Chromium on the fish reproductive system has to study. Recent studies in a few mammal species like monkeys, mice, and humans [11,12] determined that Chromium acts as a reproductive toxicant. The exposure of the freshwater catfish Saccobranchus fossils to the subtoxic level of Cr followed in reduced antibody production, decreased proliferation of splenic lymphocytes, and higher susceptibility to infections with the microorganism *Aeromonas hydrophila* [13,14]. Several investigations have assessed the effect of Chromium on immune cells and immune roles in vitro. Chromium (VI) irreversibly repressed DNA synthesis to 50% in a mouse cell line, defeated mitogen-induced human lymphocyte proliferation, and modulated then nitric oxide production of murine macrophages [8,15,16,17].

In the present study, common freshwater carp *Cyprinus carpio* was exposed to know the toxicity of Chromium. Common carp is a widely consumed fish that commonly inhabits native aquatic bodies. This fish is exposed to hexavalent Chromium through various anthropogenic activities, including but not limited to its release through industrial activities. In toxicological studies of acute exposure, changes in concentrations and enzyme activities often directly reflect Cell and organ damage in specific organs. Hexavalent Chromium affects almost every functional site of fish, often due to their bioaccumulation and inadequate excretion after exposing the fish to water containing Chromium, some behavioral changes like erratic swimming and slow motility due to oxidative stress. This study investigates Chromium-induced histopathology of the vital organs like the gill, kidney, and liver. In addition, the toxic impact on alteration in the behavioral pattern.

2. MATERIALS AND METHODS

Fish and acclimation conditions:- 100 living specimens of *Cyprinus carpio* of 10 to 15 cm and 30 to 50g weight were brought from the Fishery Department of Karnataka, Hebbal. They were maintained in the laboratory glass aquaria in clean tap water for 3 to 4 days before the experimentation. Water used in the aquaria had a pH of 6.9, a temperature of 24 ± 3°C, hardness of 160 ppm, alkalinity 87 ppm, and dissolved oxygen concentration of 7.5 ppm.

Test chemical and determination of LC50:- The potassium dichromate was used as the test compound for the determination of the lethal dose (LC50) of Chromium. The test concentrations were chosen on the basis of initial experiments to determine the LC50. The stock solution was prepared in distilled water, and the required concentrations were maintained in aquaria, renewing the water every day. The day before and during the test, the fish were not fed. The control group without Chromium was maintained simultaneously. The mortality of fish was recorded during 96 h of exposure in each concentration of the toxicant. The data was used to estimate the LC50 of potassium dichromate.

Experimental design:- The fish were divided into three groups, each containing 10 individuals. Group I was kept as control, with no chromium added to the water, and the other two groups were exposed to potassium dichromate. Groups II and III were acutely exposed for 96 h to 20 mg/L and 40 mg/L, respectively. The whole exposure medium was changed every day in both the treatment groups to maintain chromium salt concentration. The water in the control group was also changed at the same time. During the exposure, the mortality and behavior of the fish were monitored. The behavioral pattern was also monitored regularly.

Tissue preparations and histology:- At the end of exposure time. Gill, kidney, and liver of alive treated fish were dissected out, fixed in the Bouin's solution for 24 h, and then were processed for paraffin (melting point-62^{0}C) embedding. Paraffin blocks of gills, kidneys, and liver were cut at 6µm thickness using a microtome and stretched on sterilized glass slides. The sections were well stained with the Haematoxylin and Eosin for 30 minutes. Mount with a coverslip and observed under light microscopy. The histopathological changes in the tissues were examined by selecting sections from each fish. Histopathological changes induced by treatments in the tissues were photographed using a Nikon photomicroscope.

3. RESULTS

In the 40 mg/L out of 10, the five fishes died during the 96 h acute exposure. The fish exposed to 40 mg/L chromium exhibited abnormal behavior like erratic swimming, slow motility, and loss of equilibrium due to oxidative stress. The exposed fish swam to the surface more often than the control fish. Scales become thin. The caudal fin shows noticeable bending due to the Chromium. The gill of *Cyprinus carpio* is made up of primary lamellae arranged in the double rows present on the lateral sides of secondary lamellae. In the acute Chromium exposed fish lamellar fusion, epithelial lifting and curling of secondary lamellae were observed. The gills were swollen. The summary of these histopathological changes observed in gills is shown in Fig. 1. (a).

The histology of the kidney, the excretory organ of *Cyprinus carpio,* was characterized by the renal tubules and Bowman's capsules with glomeruli cells. Hypertrophy of epithelial cells of the renal tubules was observed, and some of the epithelial cells showed distinct vacuolization. Contraction of glomeruli cells was also observed in 40 mg/L groups. A summary of the histological changes noticed in the kidney during the experiment is shown in Fig. 1. (c).

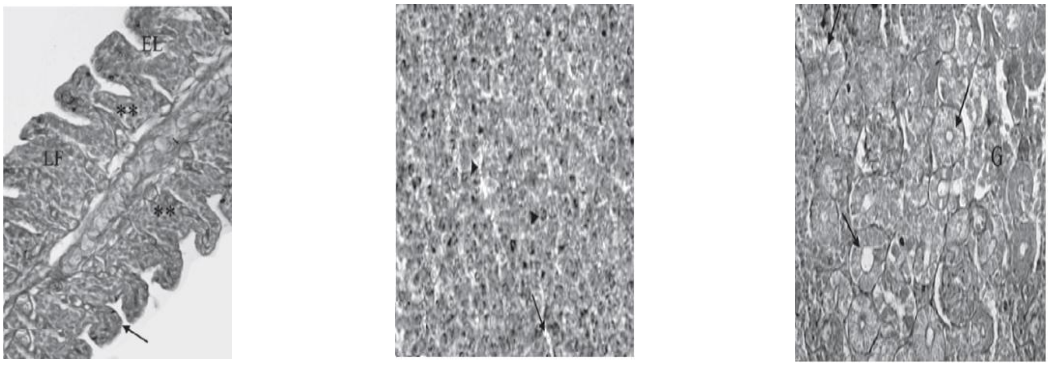
The histology of the liver shows the parenchymal architecture of the hepatocytes. The shrinkage of hepatocytes with increased sinusoidal blood spaces was noticed. The changes in the liver tissue of Chromium exposed fish are shown in Fig. 1. (b).

When fish was initially encountered to Chromium, it undergoes various behavioral modifications like suspending feeding behavior, uneven swimming, and accelerated operculum. It may trigger structural changes such as hypertrophy and paraplegia at the gill epithelium and weakens the body's immune system [8]. In this study, acute toxicity effects of hexavalent Chromium on behavior and histopathology of gill, kidney, and liver of common freshwater carp are noticed. An early report shows histopathology works and behavioral changes. Our report proposes that the accumulation of Chromium causes caudal fin bending, affects human health who consume those exposed fishes, and helps create sustainable metal waste management.

Table 1. Determination of LC50 value of hexavalent Chromium in a common carp, *Cyprinus carpio*, for 96 h

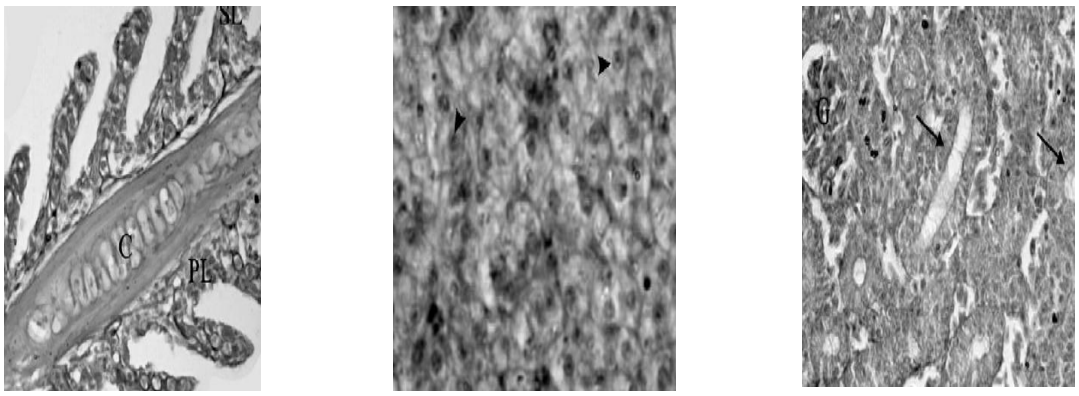
Concentration (mg/L)	Concentration difference	Number of alive fish	Number of dead fish	Mean death	Mean death X Concentration Difference
0	-	10	0	0	0
15	15	9	1	0.5	7.5
25	10	8	2	1.5	15
35	10	6	4	3.0	30
45	10	4	6	5.0	50
55	10	3	7	6.5	65
65	10	2	8	7.5	75
75	10	0	10	9.0	90
					∑= 332.5

LC 50 = LC 100- ∑ (Mean death X Concentration difference) / Number of fish per group = 75-332.5/10 = 75-33.25 = 42.75 mg/l (LC 50)



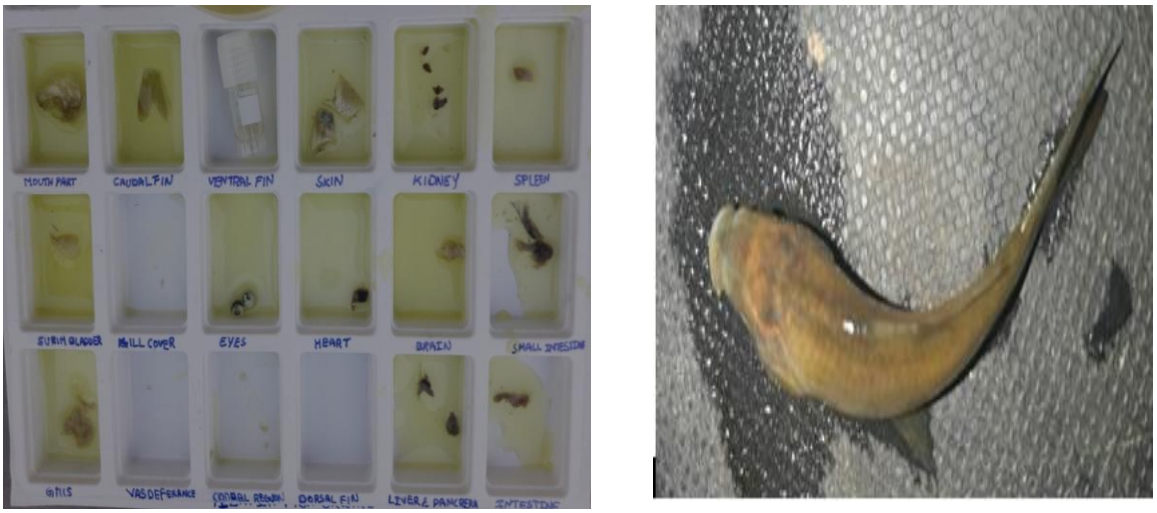
(a) (b) (c)

Fig. 1. Transverse sections showing histopathology of gill (a) lamellar fusion (LF), epithelial lifting (EL), curling of secondary lamellae (↑). Histopathology of liver (b) Shrinkage of hepatocytes, increase in sinusoidal spaces, dilation of blood sinusoids (↑), hepatocytes nucleus (▲). Histopathology of kidney (c) Renal tube atrophy (↑), necrosis of glomeruli (G) [400X]



(a) (b) (c)

Fig. 2. Transverse sections showing histopathology of gill (a) normal appearance of primary (PL) and secondary lamellae (SL) in the control group. Histopathology of the liver (b) Parenchymal architecture of hepatocytes in the control group with centrally placed nuclei. Histopathology of the kidney (c) Normal appearance of renal tubules and Bowman's capsule in the control group



(a) (b)

Fig. 3. (a) Collection of vital organs for histopathology studies, (b) Bending of the caudal region

4. DISCUSSION

Behavioral manifestations of acute toxicity like loss of scales, discoloration, surfacing, and darting movements were observed in *Cyprinus carpio* exposed to concentrations of potassium dichromate for 96 h. The fishes showed apathy and erratic swimming, intimating loss of equilibrium at greater concentrations. At the point of death transient hyperactivity was also observed. The stability and durability of aquatic animals depend on the physical state of the animals and the Physico-chemical characteristics of water and kind, toxicity, type, and expression to the toxicant [18,19,20]. In the present study, the fatality raised with a rise in concentration and exposure duration. Behavioral manifestations of acute toxicity in *Cyprinus carpio* were more or less similar to those reported in other fishes exposed to Chromium [21,22,8,23]. There is no clear-cut explanation of the exact mode of action of different metals causing mortality in aquatic animals. It is possible that the cumulative action of Chromium at various metabolic sites could be responsible for *Cyprinus carpio* [23]. In the present study, we elucidated acute toxicity effects of hexavalent Chromium on behavior and histopathology of gill, kidney, and liver of an Indian freshwater common carp *Cyprinus carpio*. The 96 h LC50 value of chromium salt, potassium dichromate, was determined to be 41.75 mg/L for *Cyprinus carpio*. The histopathological alterations observed in the gill tissue of *Channa* might have caused hypoxic conditions, which in turn affected the locomotor activity as reported earlier [24,25]. The hexavalent chromium exposure exhibited marked degenerative changes in the histology of gills, kidneys, and liver tissues. Chromium exposed fish, epithelial hyperplasia, lamellar fusion, epithelial lifting, necrosis and desquamation, aneurism, and curling of secondary lamellae were observed with excessive mucous secretion in the gill of Chromium exposed fish. Similar toxicity effects were noticed earlier in fish exposed to other toxicants, including heavy metals [26,27]. The kidney of the fish receives the largest proportion of post branchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution [28,29]. The exposure of fish to toxic agents such as pesticides and heavy metals induces histological alterations in several components of the trunk kidney [30,23,28,31]. In the present study, hypertrophied epithelial cells of renal tubules, reduction in tubular lumens, and contraction of glomeruli with the expansion of space inside the Bowman's capsules were observed in the kidney tissues of fish exposed to 20 mg/L of Chromium. The toxic effects in the 40 mg/L group were different. The atrophy and degeneration of the renal tubules and Bowman's capsules were observed. The liver is the vital organ of detoxification. The alterations in the liver due to toxicity impact are often associated with a degenerative necrotic condition [30,32,13]. The changes induced by Chromium in the liver hepatocytes, such as vacuolization, necrosis,

and nuclear condensation, were also reported for copper exposure [33]. The observed abnormal behavior and altered histopathology of vital organs demonstrate the severe adverse effects of acute exposure of hexavalent Chromium in *Cyprinus carpio* species. Thus acute chromium exposure may cause serious physiological problems, ultimately leading to the death of fish.

5. CONCLUSION

Aquatic ecosystems are the primary recipient of agricultural and industrial pollutants, including those from vehicle traffic emissions. Significant causes of industrial pollution are the discharges of heavy metals. Hexavalent Chromium was selected as a reference toxicant because it meets the following criteria: it has universal toxicity, it is persistent and non-degradable, it is highly water-soluble, and is readily quantified. Aquatic pollution is going to be a substantial threat to the ecosystems. Cr toxicity was greatly affected by a slight change in pH. There is a need for monitoring the industrial effluents for Cr concentration level. The heavy metals have toxic effects on various organs of fishes, but higher toxicity of Cr was noted in the liver and gills.

Cr induced toxicological pathology is significantly affected by certain factors as species type, age, environmental conditions, exposure time, and concentration. The present study elucidated Chromium induced histopathology of the vital organs like the gill, kidney, and liver. In addition, the toxic impact on alteration in the behavioral pattern. Present study alarming the need for monitoring the industrial effluents for Cr concentration level.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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