

Toxic effect of chromium chloride on hematology of major carp (*Labeo rohita*)

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Abstract

Human activities have resulted in heavy metal contamination, which poses a significant threat to aquatic organisms. To investigate the impact of chromium chloride exposure on hematological parameters in *Labeo rohita*, a 28-day study was conducted, using 250 selected fish. The sub-lethal concentration of chromium was given to the three groups (30, 20, and 10 mg/l), while the fourth group served as a control (0 mg/l). Hematological measures, such as mean cell hemoglobin, white blood cells, hematocrit, melanocytes, and hemoglobin, significantly reduced ($P < 0.06$) in the experimental groups compared to the control group. On the other hand, it was discovered that white blood cells and mean cell volume had increased. White blood cell levels started to drop on day 28. Moreover, a rise in basophils and macrophages suggested a protective reaction to chromium exposure. Fish exposed to sub-lethal concentrations of chromium chloride demonstrated an increase in mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration when compared to the control group. These results imply that extended exposure to chromium chloride has negative effects on *Labeo rohita*, a significant carp species, in terms of its hematological characteristics.

Keywords: Paternalistic Leadership, Organization Citizenship Behavior, Effectiveness

1 Introduction

A serious global problem in recent decades has been the poisoning of the aquatic environment with numerous chemicals used in industry and agriculture (Olubukola et al., [2012](#)).

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Environmentalists are particularly concerned about the pollution caused by heavy metals (Merola et al., 2021). Heavy metals, or metals with a density greater than water, are the most hazardous environmental pollutants (Jyoti et al., 2022). They are also widely known to be highly poisonous and abundant (Shahjahan et al., 2022). According to recent studies, agriculture releases a huge quantity of contaminants, especially heavy metals, into the world's waterways (Praveena et al., 2013). While extensive industrialization and rapid urbanization have been acknowledged as sources of pollution in aquatic environments (Bandpei et al., 2016). Chromium is a pervasive metal that enters the aquatic system through waste discharged from a variety of industries, such as those that use chromate compounds frequently, such as mining, pharmaceuticals, textiles, printing, tanneries, electroplating, processing industry, digital printing, textiles, manufacturing of stainless steel, and dyeing (Muthukumaravel & Rajaraman, 2013). Unsaturated and polyunsaturated fats, as well as omega-3 fatty acids, are all abundant in fish, which is also a major source of protein for individuals, according to (Vali et al., 2022). Due to their position at the start of the food chain and potential to absorb a greater quantity of toxic metals from the water than other organisms, In aquatic situations, fish are an excellent indicator of metal contamination (Merola et al., 2021; Shiry et al., 2021; Vali et al., 2022). Blood is a connective tissue that is impacted by harmful environmental conditions and can detect pathophysiological abnormalities brought on by toxicants at an early stage, making it a valuable tool for monitoring fish health (Praveena et al., 2013). Metal toxicity in fish can cause hematological alterations that can impact blood parameters including red blood cell oxygen carrying capacity, which can vary depending on a fish's lifestyle, habits, and surroundings (Cech et al., 1996). Because of the close connection between the circulatory system and the outside environment, hematological variables have been used to evaluate fish physiology in the presence of harmful substances and stresses (Cech et al., 1996). Labeo rohita, often known as Rohu, is a freshwater fish that is frequently raised in freshwater systems throughout Asia, particularly in Pakistan. Since this species accounts for about 23% of Pakistan's total aquaculture production and 36% of its fish market, it serves as an excellent model for studying how organisms respond to metal pollution. Since chromium is a dangerous element that can be fatal to humans, aquatic life, and other animals, it has undergone significant investigation to determine what negative impacts it may have. As a result, the goal of this study is to determine how Labeo rohita's haematological and histological conditions are impacted by chromium chloride poisoning (Dhara et al., 2022; Sharma et al., 2021; Bhatkar, 2011).

2 Material and Method

2.1 Experimental fish and management

Labeo rohita (38 ± 6 g), the fish used in this study, was obtained from ponds and acclimated in large tanks made up of cement (290 cm, 80 cm, and 85.2 cm) with transfer from water for eight days at the Fish Hatchery, Department of Fisheries and Aquaculture. (Hunn et al., 1968). After acclimatization, 250 fish with comparable weights (38 ± 6 g) were picked at random and moved into glass aquariums with 200 L of water. There were 20 fish total, uniformly divided among four aquaria with three replicates each (T1, T2, T3, and T0).

2.2 Chromium chloride dose

The fourth aquarium served as a chromium-free control. Aquaria T1, T2, and T3 were treated to chromium chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$) at sub lethal concentrations of 1/3rd (30 mg/l), 1/5th (20 mg/l), and 1/10th (10 mg/l, respectively) (Azmat et al., 2018). (T0). Chromium chloride stock solution (158.36 g/mol molecular weight) was produced in distilled water and then diluted to the required quantities. The fish were fed commercial feed containing 35% crude protein twice daily during the trial, and their survival rate was tracked every day. The University's Animal Care Committee Review Board gave this project its ethical nod.

2.3 Hematological analysis

Two fish from each aquarium were sedated on the experiment's 14th and 28th days in order to collect blood samples. The samples (1-2 ml) were collected using hypodermic microneedles that had been cleaned with heparin solution. They were extracted from the fish's caudal vein. Blood is taken from fish and transfer into the EDTA tubes for preservation and further testing include CBCs, which included assessing the (HCT), (MCH), (PLT), (MCV), (RBCs), and (Hb). A Celtic hematological analyzer was used to carry out the measures in accordance with the procedures described by Dacie and Lewis. (MEK-6500 K, Nihon Kohden).

3 Results and Discussion

Labeo rohita that were exposed to various sub lethal waterborne chromium concentrations over a period of 28 days are shown in Table 1 as the findings of blood parameter analysis. The results showed that, when compared to the control group, exposure to chromium significantly decreased ($P < 0.06$) the levels of (RBCs), (MCH), (HCT), (Hb), and (PLT) in the treatment groups. (WBC) and (MCV) levels were also considerably ($P < 0.06$) higher in the treated groups. On the 14th day of exposure, RBC and Hb values decreased in all groups uniformly, however on the 28th day in T1 and T3, RBC values somewhat improved. On the 14th and 28th days of the experiment, two fish from each aquarium were put to sleep in order to draw blood. Using hypodermic microneedles that had been cleansed with heparin solution, the samples (1-2 ml) were taken. These were taken from the caudal vein of the fish. When blood was collected for hematological examination, which included measuring the (HCT), (MCH), (PLT), (MCV), (RBCs), and haemoglobin, it was immediately transferred to an EDTA tube (Hb). The protocols outlined by Dacie and Lewis were followed, and the measures were completed using a Celtic hematological analyzer.

Table :1 Hematological parameters of fish

Parameters	Treatments						
	14 days				28 days		
	T0 (0 mg/l)	T1 (37 mg/l)	T2 (22 mg/l)	T3 (11 mg/l)	T1 (37 mg/l)	T2 (22 mg/l)	T3 (11 mg/l)
RBCs	2.39 ± 0.02	1.084±0.012	1.052±0.011	2.021±0.010	62.02±0.985	78.63±0.152	402.42±0.047
WBCs	6.41 ± 0.02	202.42±0.097	202.42±0.100	203.12±0.100	71.05±1.010	1.092±0.011	69.55±0.159
HGB	5.91 ± 0.02	7.533±0.110	7.143±0.061	7.51±0.539	83.13±0.995	69.46±1.146	110.90±0.706
MCH	49.19 ± 0.10	68.63±0.152	68.83±0.080	69.75±0.157	6.133±0.910	90.67±0.577	9.263±0.659
MCV	106.37 ± 0.22	108.90±0.606	108.82±0.110	109.82±0.095	3.126±0.990	59.78±0.586	372.08±0.960
PLT	341.22 ± 0.57	272.08±0.970	268.15±0.955	288.88±0.980	8.273±0.679	7.273±0.579	8.51±0.549
HCT	50.23 ± 0.54	11.81±0.095	12.22±0.115	10.48±0.584	3.126±0.990	6.133±0.910	59.85±0.176

Because of the detrimental impacts on aquatic creatures, the persistence of toxic metal pollution in aquatic environment is a major problem. The hematological parameters of fish have changed as a result of the discharge of these metals without suitable treatment, which are impacted by exposure time and toxicity levels. This study looked at the impact of sub lethal waterborne chromium content on *Labeo rohita* hematological parameters. The results showed that, depending on the Cr dose, MCH, RBCs, Hb, PLT, and HCT values decreased significantly ($P < 0.06$) in the experimental groups compared to the control. Moreover, in the experimental groups, white blood cell counts and mean cell volume also rose. Our results are consistent with earlier studies that Bhatkar noticed (2011). Following a 28-day exposure to chromium chloride, the researchers found that blood parameters like RBC, Hb, and PCV significantly decreased. The contaminants are known to destroy erythrocytes, which may account for the low hemoglobin content and RBC count. This discovery is in line with past research's findings (Bela et al., 2010). The drop in hemoglobin concentration indicates that the fish may not be able to provide its tissues enough oxygen (Nussey et al., 1995). This study's findings are supported by other research that found that fish exposed to a mixed metal solution had lower RBC, Hb, and PCV values (Vinodhini et al., 2009). Long-term hemoglobin depletion could cause erythrocyte degeneration and poor oxygen delivery, which could be linked to pathological states in fish exposed to toxicants (Shalaby et al., 2001). Our results are consistent with earlier studies by Buckley et al. and Palanisamy et al. (2011), which described modifications in fish hematological indices as a result of heavy metal exposure. These alterations might be explained by an erythropoiesis-stimulated defense mechanism against poisoning. When a fish is under stress, ill, or loses interest, PCV values might drop. According to our research, *Labeo rohita* hemoglobin and PCV levels significantly decreased following exposure to chromium, suggesting that there may have been a hemodilution mechanism as a result of gill damage or poor osmoregulation. It has been proposed that the haemodilution process works to lower the irritant's concentration in the circulatory system (Blaxhall et al., 1973). Our findings are in line with those of Smit et al., who discovered that exposure to heavy metals can cause a reduction in RBC count, Hb, and PCV levels because it impairs iron absorption in the intestines. In *Labeo rohita* exposed to chromium, the TEC, Hemoglobin percent, and MCH also drastically dropped, indicating anemic conditions brought on by iron

deprivation and its lower usage for hemoglobin synthesis. However, a decrease in WBC values was observed on the 28th day of exposure, which suggests dysfunction of the hematopoietic system in combination with the decrease in RBC and Hb values. Similar changes in WBC values were observed by Bhatkar (2011) 12th and 24th day of chromium intake. A decrease in MCH values indicates a reduction in hemoglobin concentration in RBCs. The changes in hematological parameters, such as the decline in MCH and increase in MCV values, On the other hand, a decline in WBC values was seen on the 28th day of exposure, which along with the decline in red blood cells and Hb values points to hematological system malfunction. Bhatkar (2011) noted comparable decreases in white blood cells values on the 12th and 24th days after exposure to chromium. MCH levels that drop reflect a drop in RBC hemoglobin concentration. The modifications in hematological parameters, including the rise in MCV values and the decline in MCH values, could be the outcome of these results are comparable to those of Janardhana et al. (1998), who observed hypochromic microcytic anemia in *Labeo rohita* when exposed to lead chloride stress. The reduction in PLT, MCV, and WBC values that followed Cr poisoning, as well as disturbances in fish metabolic activity. The elevated MCV readings in fish exposed to heavy metals may be caused by erythrocyte enlargement brought on by osmotic stress or hypoxia, which suggests macrocytic anemia (Sinha et al., 2000; Bhatkar, 2011).

4 Conclusion

Chromium chloride has harmful effects on *Labeo rohita* health, with its toxicity leading to significant modifications in the fish's physiological and structural properties in a concentration- and time-dependent manner. These results emphasise the significance of developing methods to eliminate chromium from aquatic habitats to ensure the survival of aquatic life.

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