Research Article

Hematological and Biochemical Studies in Commercially Important Fish *Labeo rohita* Exposed to Cadmium Chloride

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Abstract | The present study comes to assess how heavy metal (cadmium chloride) affects the hematological and biochemical parameters in the *Labeo rohita* (Rohu: Hamilton, 1822). Freshwater fishes *Labeo rohita*, (n = 60), with body weight (70– 120 g), were randomly divided into four experimental groups Treatment T1, Treatment T2 and Treatment T3 (0.44mg/l, 0.89mg/l, and 1.34 mg/l), while the fourth group (T0) served as a control (0.00mg/l). Sampling was done on 7, 14 and 21- day. Results showed significant increase in WBC (white blood cell), whereas red blood cells count, Hb (hemoglobin) and Hct (hematocrit) were significantly reduced in treated groups compared to the control. The mean corpuscular hemoglobin (MHC) and mean corpuscular hemoglobin concentration (MCHC) showed a non-significant decrease in treated groups compared to the control group. Serum biochemical parameters, including total proteins, albumin and globulin, decreased significantly ($p < 0.05$). At the same time, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose and cholesterol were significantly ($p < 0.05$) increased in the treated groups compared to the control group. In conclusion, the study indicates that exposure to cadmium chloride, even in a low concentration, can cause adverse hematological and biochemical changes in *Labeo rohita*.

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Introduction

Cadmium chloride is a naturally elemental occurring component found in various organisms, rocks and soil. It is primarily insoluble in its trivalent form. While oxidation makes chromium chloride soluble, reduction keeps Cr insoluble in water (Jaishankar *et al.*, 2014). Cadmium chloride, a prevalent metal,

infiltrates aquatic environments, industrial waste released by sectors employing concentrated chromate compounds. These sectors encompass electroplating, wood treatment, photography, pharmaceuticals, mining, textiles, stainless steel production, printing, tanneries, and dyeing (Muthukumaravel and Rajaraman, 2013). Cadmium chloride exposure can result in a broad spectrum of, with its compounds known for their potential to induce cancer, cellular mutations, and harm both aquatic organisms and human beings. Cadmium chloride compounds resulted in DNA damage among the native fish population (Kousar and Javed, 2015). The acute toxicity studies of cadmium on the edible carp, *Catla catla*, have uncovered noteworthy alterations in the fish's biochemical constituents, including glucose, glycogen, total proteins, lipids, and free amino acids. The presence of hemorrhagic conditions in the deceased fish serves as a clear indicator of the toxic effects induced by cadmium (Sobha *et al*., 2010). Acute tests assessing the toxicity of cadmium in the edible carp, *Catla catla*, revealed significant alterations in fish biochemical parameters, including glucose, glycogen, total protein, lipid, and free amino acids. Interestingly, fish previously exposed to lower concentrations of heavy metals demonstrated increased resistance to higher concentrations, suggesting an adaptive response characteristic of vertebrates. Additionally, the study observed that adults exhibited greater susceptibility to the toxicant compared to the fingerlings (Remyla *et al*., 2008).

In the aquatic environment, heavy metals get deposited onto sediment, functioning as an ecological reservoir (Mataba *et al*., 2016). Due to variables like pH, temperature, and salinity, among others, there is a constant risk of metals transferring from sediment to the water column. As these metals swiftly integrate into the aquatic environment, they become a part of the food chain, affecting both aquatic organisms and human populations reliant on them (Chatha *et al*., 2023). At present, the problem of aquatic pollution has increased many times due to the introduction of modern technologies using heavy metals as raw materials for different functions (Naz *et al*., 2023b).

In recent decades, the accumulation of chemicals from agriculture and industry in the aquatic environment has emerged as a critical global concern, with water bodies (Naz *et al*., 2023c). Heavy metal pollution has recently become a top worry for environmentalists, especially in the aquatic ecology (Merola *et al*., 2021). Aquatic ecosystems are made up of a wide variety of habitats, from very oligotrophic highland lakes and streams to chemoautotrophic black smokers on volcanic deep sea ridges. Distinct stressors characterize each of these habitats, leading to a diverse array of conservation complexities and prospects for restoration (Geist, 2011). Over the past few years, freshwater ecosystems have encountered significant challenges due to human actions, including the discharge of industrial pollutants, agricultural practices, urban waste management issues, and the expanding urban landscape (Meijide *et al*., 2018). Rapid industrialization produces a lot of waste, much of which is frequently dumped into adjacent bodies of water (Naz *et al*., 2023d).

Water pollution is mostly caused by increased urbanization, industry, and rapid population growth beside water bodies like lakes, reservoirs, and rivers, this, in turn, leads to the degradation of the ecosystem (Lemessa *et al*., 2023). Contamination with heavy metals is a significant issue in expanding cities of emerging countries, mostly as a result of unchecked pollution levels brought by root causes which including industrial expansion and significant increases in traffic powered by petroleum fuels (Das *et al*., 2023). Both naturally occurring and human-caused environmental releases of heavy metals occur. Heavy metals originate from multiple sources, including soil erosion, mining activities, industrial discharges, urban runoff, wastewater discharge, crop pesticides, and the natural weathering of the Earths crust (Sudharshan and Sunitha, 2023).

Hematology serves as a vital biomarker in various environmental monitoring, investigation, and assessment domains, including toxicology, chemical risk evaluation, safety analysis, and environmental surveillance (Naz *et al*., 2021). After toxicants such as heavy metals enter the fish body, blood-forming tissues remain consistently exposed to their detrimental impacts (Ullah *et al*., 2021). Blood is a fragile tissue that is impacted by the environment, alterations in its parameters are the first indications of path physiological disorders brought on by various toxicants. Thus, they are a great tool to observe fish health. Fish health can be evaluated with the help of hematological and biochemical signs that are monitored (Ullah and Li, 2019).

The serum biochemistry encompasses measurements of various components, including serum proteins, albumin, triglycerides, HDL-C, cholesterol, total cholesterol, as well as enzymes such as Alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) (Mattioli *et al*., 2016; Toghyani *et al*., 2010). The previous research revealed that when fish are subjected to various exposure treatments that the levels of total protein significantly decrease ($p > 0.05$). Fish subjected to increasing amounts of metals and metal oxides also showed a significant increase in total lipid levels compared to the control group. In response to extended exposure and rising concentrations of metals or metallic oxides, the activities of AST and ALT simultaneously showed a substantial rise (Al-Asgah *et al*., 2015). In this study, our objective was to comprehend the impact of cadmium chloride on *Labeo rohita* at various concentrations, examining both hematological (blood) and biochemical aspects (serum). *Labeo rohita* is commonly consumed by humans, and as chromium chloride is a heavy metal, we aimed to investigate the potential effects of these substances on fish blood.

Materials and Methods

The study involved conducting an experiment on the *Labeo rohita* with approximately the same age and weight (70-120 g), length (15-20cm) and sex ratio1:2 (male and female) was collected from a Fisheries complex Bahawalpur in October 2022. Lives specimens (n= 60) were calculated using specialized nets. Following the collection phase, the fish samples were delicately positioned within plastic bags, which were filled to approximately halfway with water sourced from the pond. Subsequently, these bags were transported to laboratory. To ensure optimal hygiene, all glassware and containers were meticulously cleansed and subsequently rinsed with deionized water. Prior to the initiation of the experiment, the glass containers, each with a capacity of 60 liters, were systematically filled with tap water that had been dechlorinated and adjusted to attain the desired pH and hardness levels. Prior to the experimental phase, 60-liter aquaria were filled with dechlorinated tap water, constant aeration by compressed air pumps (Silver Lake super pump SL-2800). The light was maintained at 12:12 h light; dark cycle throughout the day using fluorescent light tubes (Deebow Aquarium Light (D-53 4W 20-30cm, Pakistan). Prior to the commencement of the experiment, a total of 60 fish were allocated to four separate aquariums. Out of 60 fish, 15 fish were kept in control group for an acclimatization phase lasting 7 days during which they were subjected to a natural photoperiod consisting of 12 hours of light and 12 hours of darkness while the remaining divided into three treatment groups. During the experimental period, Group T_0 consisting of fish were maintained under standard conditions with control water. After acclimatization phase, fish from groups T1, T2 and T3 were exposed to doses of cadmium chloride. The treatment duration was 21 days and was divided into three sampling intervals on the 7^{th}, 14^{th} and 21^{th}. At the conclusion of the trial, a sampling process was conducted to assess hematology and biochemical aspects in the (*Labeo rohita*).

Various solutions at different desired doses were prepared for 60-Liter capacity aquariums. These concentrations, specifically 0.44 mg/l (Treatment T1), 0.89 mg/l (Treatment T2), and 1.34 mg/l (Treatment T3) were derived from the referenced LC_{50} value of 74.35 mgL^{-1}, as originally presented by (Azmat *et al*., 2012). The preparation process involved dissolving the requisite quantity of cadmium chloride in distilled water. Subsequently, these solutions were administrated to the 60-liter aquariums on alternate days within the environmental conditions. Clinical and behavioral symptoms in fish treated with different concentrations of chromium chloride included loss of equilibrium, mucus secretion from the mouth and gills, air gulping, rapid operculum movement, bulging eyes, coordination loss, erratic swimming, and swimming in isolation. Within the control group, the fish demonstrated an absence of clinical symptoms.

The water temperature, pH and electrical conductivity of each aquarium were determined with the help of a pH tester (HI98107 pHep) and EC tester (HI98304 DiST4). The temperature of each aquarium was measured by using an electric thermometer. Water salinity, total dissolved solids, and dissolved oxygen was tested by using a salinity tester (MarineLine/HI98319), TDS tester (HI98302 DiST 2), and a DO meter (DO meter Hanna 2400). All physicochemical variables were tested on daily basis during the trial period. Blood samples were collected from the caudal vein of the fish at 7^{th}, 14^{th} and 21^{th} day. In the initial step, the blood was carefully transferred into tubes, which contained the anticoagulant ethylenediaminetetraacetic acid (EDTA) to facilitate

hematological analysis. Simultaneously, another portion of the blood was transferred into vacutainers these blood samples were maintained in this configuration, (MinlCentrifuge, Model: mC12). The separation of serum from the blood cells. The serum was subsequently isolated using a suction device and stored in tubes for subsequent laboratory analysis (Naz *et al*., 2021).

Hematological parameters were assessed using a digital analyzer from a local laboratory. These parameters encompass a range of quantifiable components present in blood, serving as crucial indicators for evaluating an individual's overall well-being and diagnosing a diverse array of medical conditions. These measurements offer valuable insights into the functioning of the hematopoietic system, which encompasses the creation and maintenance of blood cells. An automated haematology analyzer was used to measure hematological parameters such as RBC (red blood cell) count, WBC (white blood cell) count, Hb concentration, hematocrit (HCT), and mean corpuscular volume (MCV). Using common biochemical assays, biochemical markers including ALT (alanine aminotransferase), (AST), alkaline phosphatase ALP (aspartate aminotransferase), total protein, albumin, and blood glucose levels were examined. Biochemical parameters were examined to assess the influence of cadmium chloride on the freshwater fish *Labeo rohita*. Serum enzymes were determined by spectrophotometrically using commercially available kits. The data thus collected from hemato-biochemical, parameters were presented as means ± standard error (SE).

Results and Discussion

Clinical and behavioral signs due to exposure of chromium chloride

The current study noted no mortality in the treatment groups (T1, T2, and T3) during the trial. Clinical and behavioral signs, such as loss of equilibrium, mucus secretion from the mouth and gills, air gulping, increased swimming, rapid operculum movement, bulging eyes, coordination loss, erratic swimming, and swimming in isolation, were observed in the treated fish. The severity of these clinical and behavioral signs increased proportionally with the elevated concentration and duration of exposure to cadmium chloride (Table 1).

Table 1: *Intensity of various clinical signs exhibited by fish treated with various concentrations of cadmium chloride.*

Clinical ailments	Groups/treatments		
	T1 0.44 mg/l	T20.89 mg/l	T31.34 mg/l
Loss of equilibrium	+ – +	– - +	+ + -
Mucus secretion from mouth and gills	+ – +	– - +	+ + -
Air gulping	+ – +	– - +	+ + + -
Rapid operculum movement	+ +	– - + +	+ + + -
Bulging eyes	+ +	+ - +	+ + -
Coordination loss	+ – +	– - +	+ + -
Erratic swimming	+ +	+ ++	+ + + -
Swimming in isolation	++	+ _ +	+ + -
Average severity	Mild	Moderate	Severe

The sings show –: Absent; +: mild; ++: moderate; +++: sever.

Physio chemical parameters of water

The provided Table 2 illustrates the variations in different parameters across distinct treatment groups labeled as control Treatment T0, T1, T2, and T3, each associated with varying concentrations (T1 (0.44 mg/l), T2 (0.89 mg/l), and T3 (1.34 mg/l). The parameters include temperature (°C), pH, electrical conductivity (µScm-1), dissolved oxygen (mg/l), and total dissolved solids (mg/l). The control group serves as the reference point against which the changes in the other treatment groups are noted. In this context, it appears that as the concentration levels increase from control to Treatment T3, there is a general trend of decreasing values across the parameters. For instance, the temperature reduces progressively from 27.12 ± 0.23 (control) to 25.92 ± 0.12 (T3), indicating a decline in temperature as the concentration level rises. Similarly, the pH demonstrates a slight decrease from 7.45 ± 0.03 in the control to 7.11 ± 0.01 in T3. The electrical conductivity, dissolved oxygen, and total dissolved solids also depict a declining pattern, suggesting a correlation between increased concentration levels and reduced values across these parameters. Overall, this table highlights a trend where an increase in concentration is associated with a decrease in the measured parameters, indicating a potential relationship between the varying concentrations and the observed changes in the specified characteristics within the treatment groups.

Table 2: *Physio chemical parameters of water during the trial of cadmium chloride.*

Parameters	Groups/ treatments			
	Control	T1(0.44mg/l)	T2(0.89 mg/l)	T3(1.34 mg/l)
Temperature (°C)	27.12 ± 0.23	26.71 ± 0.42	26.32 ± 0.31	25.92 ± 0.12
pH	7.45 ± 0.03	7.45 ± 0.03	7.34 ± 0.12	7.11 ± 0.01
Electrical conductivity(μScm^{-1})	3.01±0.30	2.76±0.25	3.11±0.33	2.85±0.34
Dissolved oxygen (mg/l)	5.79±0.43	5.22±0.13	5.40±0.18	5.51±0.24
Total dissolved solids (mg/l)	185.32±1.12	182.21 ± 1.10	179.16 ± 0.88	176.24 ± 1.11

Table 3: *Variation in hematological profile of fish Labeo rohita exposed to different doses of cadmium chloride during treatments and control.*

Parameters	Days	Control	T1	T2	T3
White blood cells (WBC)	7	172.97±3.19	226.45±3.15	262.73±2.11	281.19±2.05*
	14	192.07±8.90	203.79±9.18	216.24±3.38*	221.88±9.75*
	21	196.35±7.62	207.02±8.15	218.76±4.29*	223.91±9.12*
Red blood cells (RBC)	7	8.78±0.06	8.48±0.02	8.55±0.03	8.34±0.02
	14	8.79±0.02	8.46±0.025	8.33±0.02*	8.01±0.061*
	21	8.82±0.03	8.49±0.027	8.36±0.025*	8.04±0.057*
Hemoglobin (HGB)	7	8.27±0.51	7.07±0.21	6.12±0.49	6.68±0.29*
	14	8.73±0.35	7.58±0.37	7.13±0.23	5.89±0.42*
	21	8.89±0.42	7.74±0.39	7.28±0.27	5.98±0.45*
Hematocrit (HCT)	7	27.93±2.63	28.15±2.30	22.34±2.55*	30.12±1.93
	14	36.6±1.65	24.37±1.45*	25.72±0.95*	28.04±0.63
	21	37.9±1.73	25.02±1.55*	26.38±1.05*	28.72±0.78
Mean corpuscular volume (MCV)	7	156.47±11.54	180.67±9.85	142.84±20.91*	205.29±11.91
	14	204.4±9.29	163.12±12.85*	187.49±10.50	270.98±7.62
	21	208.9±9.87	167.88±12.31*	190.76±11.04	274.35±8.27
Mean corpuscular hemoglobin (MCH)	7	46.33±1.59	46.89±0.81	39.85±3.21*	47.92±2.06
	14	48.73±1.68	53.78±2.38	54.62±2.44	58.13±7.52*
	21	60.91±1.92	60.84±2.58	60.76±2.62	60.29±7.86
Mean corpuscular hemoglobin concentration (MCHC)	7	29.67±1.20	26.15±1.02*	28.02±5.67	24.47±0.74*
	14	23.9±1.87	31.95±3.60*	28.15±1.35	21.09±1.95
	21	24.8±1.95	32.07±3.75*	28.85±1.52	22.17±2.12
Platelets (PLT)	7	73.67±4.16	85.21±2.45	112.07±4.90*	127.46±2.98*
	14	81±4	85.19±6.32	165.08±7.28*	102.27±3.06
	21	84±4.25	88.63±6.68	168.92±7.91*	104.05±3.28

*Values (Mean +SE) with * asterisks in each row differ significantly (P<0.05) from untreated fish.*

Hematological parameters

To determine the effect of cadmium chloride on the blood profile of freshwater fish (*Labeo rohita)*, hematological parameters were evaluated. In present study, the effect of cadmium chloride was studied on hematological parameters of fish (*Labeo. rohita*) .The results obtained are given in (Table 3).

The control group exhibited a white blood cell (WBC) concentration of (172.97±3.19), whereas in the groups exposed to cadmium chloride (T1, T2, and T3), the WBC concentrations were (226.45±3.15), (262.73±2.11), and (281.19±2.05), respectively, on the 7th day. On the 14th day, the control group displayed WBC levels of (192.07±8.90), while the respective levels for the exposed groups were (203.79±9.18), (216.24±3.38), and (221.88±9.75). In the control group, the white blood cell (WBC) concentration was (196.35±7.62), while in the cadmium chloride-exposed groups (T1, T2, and T3), the respective concentrations were (207.02±8.15), (218.76±4.29), and (223.91±9.12). Significant changes (P<0.05) in white blood cell (WBC) levels were observed in both short-term and long-term experiments involving the chromium chloride-exposed groups, as indicated in (Table 3). These findings are further elucidated in the accompanying (Figure 1).

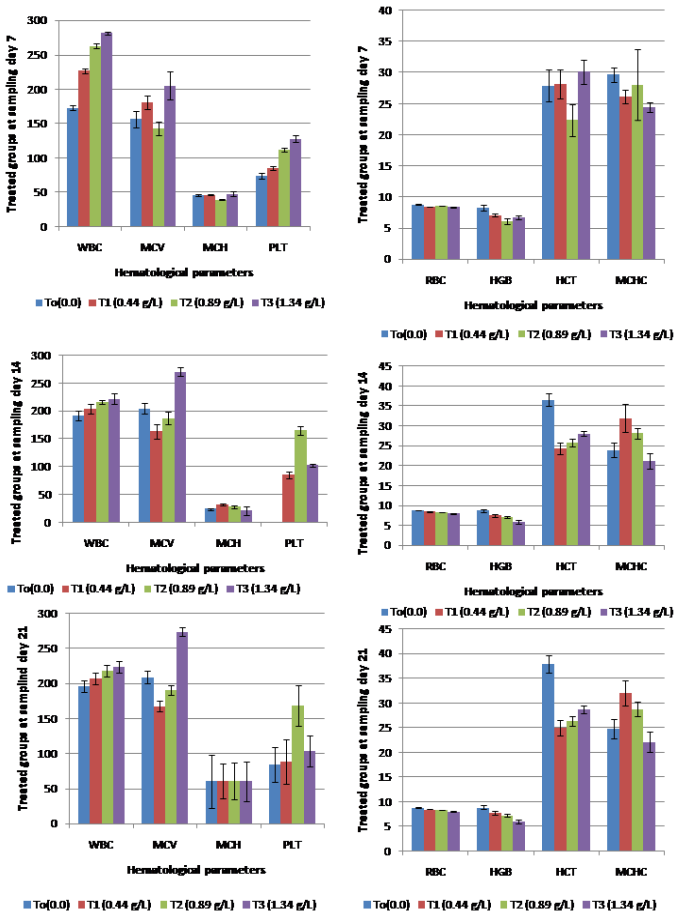

Figure 1: *Hematological parameters of Labeorohita exposed to cadmium chloride.*

The red blood cell (RBC) concentration in the control group was (8.78±0.06), while in the T1, T2, and T3 groups on the 7th day, it was (8.48±0.002), (8.55±0.03), and (8.34±0.02), respectively. On the 14th day, the RBC concentration in the control group remained (8.79±0.02), whereas in the cadmium chloride-exposed groups, it was (8.46±0.025), (8.33±0.02), and (8.103±0.061), respectively. On the 21st day, the RBC concentration was (8.82±0.03) in the control group and (8.49±0.027), (8.36±0.025), and (8.04±0.057) in the cadmium chloride-exposed groups (Treatment T1, T2, and T3). Notably, there were significant changes in white blood cell (WBC) counts in both short-term and long-term experiments involving cadmium chloride-exposed groups, as presented in (Table 3). These findings are also elaborated in the accompanying (Figure 1).

The hemoglobin (Hb) concentration in the control group was (8.27±0.51), while in the treatment groups T1, T2, and T3, it was (7.07±0.21), (6.12±0.49), and (6.68±0.31) respectively on the 7th day. On the 14th day, the Hb levels were (8.73±0.35) in the control group and (7.58±0.37), (87.13±0.23), and (5.89±0.42) in the cadmium chloride-exposed groups, respectively. By the 21st day, the Hb concentration was (8.89±0.42) in the control group and (7.74±0.39), (7.28±0.27), and (5.98±0.45) in the cadmium chloride-exposed groups (T1, T2, and T3). Notably, there were significant changes in Hb levels in both short-term and long-term experiments involving chromium chloride-exposed groups, as presented in (Table 3). These findings are also detailed in the accompanying (Figure 1).

The hematocrit (Ht) percentages in the control group were (27.93±2.63), and in the cadmium chloride-exposed groups treatment (T1, T2, and T3), they were (28.15±2.30), (22.34±2.55), and (30.12±1.93) respectively. After 15 days, the Ht levels were (36.6±1.65) in the control group and (24.37±1.55), (25.72±1.05), and (28.04±0.68) in the treated groups, respectively. The hematocrit percentages in the control group were (37.9±1.73), and in the exposed treatment groups, they were (25.02±1.55), (26.38±1.05), and (28.72±0.78). Significantly, there were changes in Ht levels observed in both short-term and long-term experiments involving chromium chloride-exposed groups, as indicated in (Table 3). These results are also detailed in the accompanying (Figure 1).

Mean corpuscular volume in control group was (156.47±11.54) and cadmium chloride exposed groups were (180.67±9.85), (142.84±20.91) and (205.29±11.91), respectively. The level of MCV after 15 days were, (204.4±9.29) in control and other groups T1, T2, T3 were (163.80±12.85), (187.49±10.95), (270.98±7.62), respectively after 15 days. The level of MCV at 21th day (208.9±9.87) and in exposed treatments groups were (1.67.88±12.31), (190.76±11.04) and (274.35±8.27). The statically MCV not changed significantly in short term and long term and long term of exposure, given in (Table 3). All these results were also explained in (Figure 1).

The mean corpuscular hemoglobin (MCH) concentration in the control group was (46.33±1.59), while in groups treatment T1, T2, and T3 exposed to chromium chloride, it was (46.89±0.86), (39.85±3.21), and (47.92±2.06), respectively. At 14th, the MCH levels in the control group were (48.73±1.68), and in the cadmium chloride-exposed groups, they were (53.78±2.38), (54.62±2.44), and (58.13±7.52). These levels showed a gradual increase but were not statistically significant. Furthermore, the MCH concentrations in the control group were

(60.91±1.92), (60.84±2.58), and (60.29±7.86) as indicated in (Table 3). All of these findings are also depicted in the accompanying (Figure 1).

The mean corpuscular hemoglobin concentration (MCHC) values in the control group were (29.67±1.20), whereas in the cadmium chloride-exposed groups treatment (T1, T2, and T3), they were (26.15±1.02), (28.70±5.67), and (24.47±0.74), respectively. After 15 days, the MCHC levels were (23.9±1.87) in the control group and (31.95±3.60), (28.15±1.35), and (21.09±1.95) in groups T1, T2, and T3, respectively. The MCHC concentrations in the control group were (24.81.95), and in the exposed treatment groups (T1, T2, and T3), they were (32.07±3.75), (28.85±1.52), and (22.17±2.12). Importantly, there were no significant changes in MCHC observed in both short-term and long-term experiments involving cadmium chloride-exposed groups, as indicated in (Table 3). All of these results are further elucidated in the accompanying (Figure 1).

The platelet concentration in the control group was (73.67±4.16), while in the cadmium chloride-exposed groups (T1, T2, and T3), it was (85.21±2.52), (112.07±5.03), and (127.46±2.98), respectively. At 14th day, the platelet levels in the control group were (81±4), and in the other cadmium chloride-exposed groups, they were (85.19±6.51), (165.08±7.28), and (102.27±3.06). There was a gradual increase observed in group T1, but a significant increase was noted in groups T1 and T2. The platelet concentration (103/µl) in the control group was (84±4.25), and in the treatment-exposed groups (T1, T2, and T3), they were (88.63±6.68), (168.92±7.91), and (104.05±3.28) as indicated in (Table 3). All of these results are also detailed in the accompanying (Figure 1).

Serum biochemical parameter

Biochemical parameters were examined to assess the influence of cadmium chloride on the freshwater fish (*Labeo rohita*). Alkaline transaminase (IU/L) exhibited no significant differences between the short-term and long-term experimental groups. The ALT levels on 7 days were (32.48±3.25) in the control group and (27.94±2.91), (22.08±3.30), (13.75±2.50) in the treated groups. Similarly, the concentrations of ALT on 14th day were (35.78±1.63) in the control group and (31.46±1.28), (24.10±1.48), (17.52±1.18) in the other cadmium chloride-treated groups. On the 21st day, ALT levels in the control group were (36.75±1.70), while in treatment groups treatment T1, T2, and T3, they were (33.60±1.50), (25.30±1.70), and (18.60±1.50), respectively. Notably, a gradual reduction was observed in the three different treatments, and this reduction was statistically significant, as presented in (Table 4) all of these results are further elucidated in the accompanying (Figure 2).

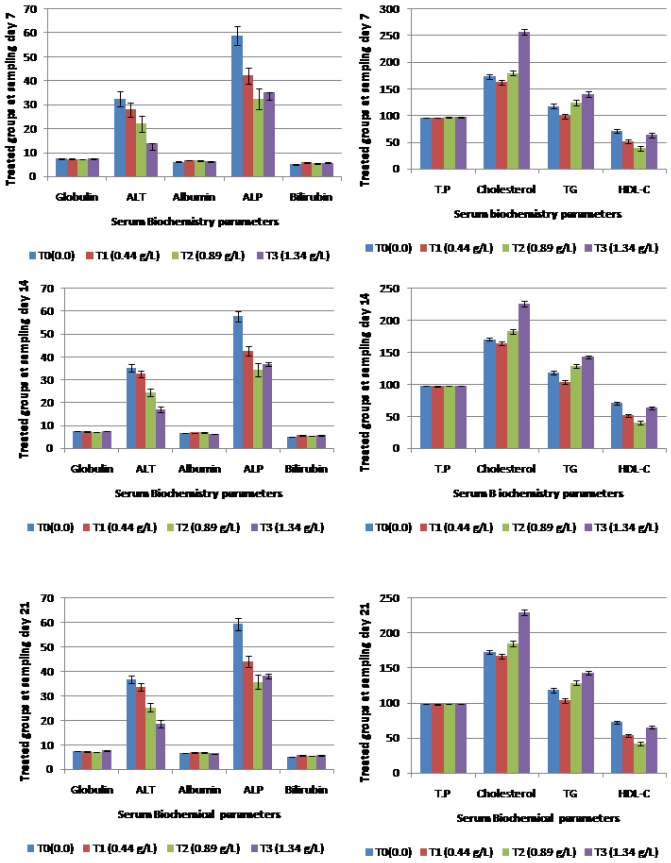

Figure 2: *The comparison graphs for serum biochemical parameters of fish Labeo rohita exposed to different concentration of cadmium chloride.*

Alkaline phosphatase exhibited no significant differences in the short-term experimental groups but showed significant changes after 14th days in comparison. The concentrations of ALP at 7th day was (58.90±4.12) in the control group and (42.28±3.38), (33.54±4.40), (35.18±3.03) in the other cadmium chloride-treated groups. Similarly, the levels of ALP at 14th day were (57.82±2.42) in the control group and (42.17±1.92), (33.23±3.90), (36.62±0.53) in the treated groups. On the 21st day, ALP concentrations in the control group were (59.40±2.40), while in treatment groups T1, T2, and T3, they were (44.10±2.20), (35.70±2.90), and (38.20±0.90), respectively, as presented in (Table 4). All of these results are further elucidated in the accompanying (Figure 2).

Table 4: *Variation in serum biochemistry parameters profile of Labeo rohita exposed to different doses of chromium chloride during treatments.*

Treatments	Time	Control	T1	T2	T3
Alanine Aminotransferase (ALT)	7	32.48±3.25	27.94±2.91	22.08±3.30*	13.75±2.50*
	14	35.25±1.60	32.70±1.40	24.50±1.60*	17.10±1.40*
	21	36.75±1.70	33.60±1.50	25.30±1.70	18.60±1.50*
Alkaline Phosphatase (ALP)	7	58.90±4.12	42.28±3.38	32.54±4.40*	35.18±3.03*
	14	57.80±2.30	42.80±2.00	34.40±3.00*	36.90±0.80
	21	59.40±2.40	44.10±2.20	35.70±2.90*	38.20±0.90
Total protein (T.P)	7	96.49±0.18	96.07±0.22	96.88±0.18	96.87±0.28
	14	97.55±0.12	97.08±0.15	97.92±0.10	97.95±0.10*
	21	98.60±0.13	98.10±0.16	98.94±0.11*	98.98±0.11*
Globulin	7	7.40±0.05	7.25±0.20	7.18±0.08	7.59±0.19*
	14	7.42±0.04	7.28±0.20	7.10±0.05	7.57±0.08*
	21	7.45±0.05	7.30±0.22	7.12±0.06	7.59±0.09*
Albumin	7	6.07±0.18	6.83±0.05	6.68±0.09	6.25±0.10*
	14	6.72±0.10	6.85±0.05	6.90±0.13	6.37±0.07*
	21	6.75±0.11	6.87±0.06	6.93±0.14	6.40±0.08*
Bilirubin	7	5.02±0.20	5.81±0.14	5.46±0.19*	5.65±0.20
	14	5.10±0.08	5.76±0.10*	5.48±0.12	5.68±0.12
	21	5.12±0.09	5.78±0.11	5.50±0.13*	5.70±0.13*
Cholesterol	7	173.50±4.50	162.80±4.50	180.00±4.80	257.40±5.80*
	14	170.50±2.50	164.60±2.90	182.80±3.40*	226.50±4.20*
	21	172.80±2.70	166.90±3.00	185.10±3.50*	229.80±4.40*
Triglycerides (TG)	7	117.80±4.20	98.56±4.20	124.40±5.00	140.12±4.70*
	14	118.30±2.80	103.80±2.90	128.70±3.20	142.80±2.40*
	21	121.00±2.90	105.50±3.00	130.40±3.30*	145.60±2.50*
High-Density Lipoprotein Cholesterol (HDL-C)	7	71.10±3.90	52.20±3.90	39.12±4.70*	63.80±4.20
	14	70.80±1.90	51.60±1.80	40.30±2.30*	63.10±1.70
	21	72.60±2.00	53.40±1.90*	42.10±2.40*	65.40±1.80

*Values (Mean +SE) with *asterisks in each row differ significantly (P<0.05) from untreated fish.*

Total protein exhibited significant differences between the short-term and long-term experimental groups in comparison. The levels of TP at 7th day were (2.49±0.18) in the control group and (2.07±0.22), (1.88±0.18), (0.87±0.28) in the treated groups. Similarly, the concentrations of TP at 14th day were (2.55±0.08) in the control group and (2.03±0.18), (1.89±0.13), (0.88±0.13) in the other cadmium chloride-treated groups. Total protein (g/dl) on the 21st day was (2.60±0.13) in the control group, while in treated groups T1, T2, and T3, it was (2.10±0.16), (1.94±0.11), and (0.98±0.11), respectively. Notably, a gradual decline was observed in the three different treatments, and this decline was statistically significant, as indicated in (Table 4). All of these results are further elucidated in the accompanying (Figure 2).

Globulin levels exhibited a significant gradual decrease in both short-term and long-term experimental groups, with this decline becoming more pronounced with higher levels of toxicity in comparison. The concentrations of globulin were (7.40±0.05) in the control group and (7.25±0.20), (7.18±0.08), (7.59±0.19) in the other chromium chloride-treated groups. Similarly, the levels of Globulin after 15 days were (7.40±0.06) in the control group and (7.25±0.19), (7.09±0.05), (7.55±0.09) in the other experimental groups. On the 21st day, Globulin (g/dl) levels were (7.45±0.05) in the control group, while in the treated groups T1, T2, and T3, they were (7.30±0.22), (7.12±0.06), and (7.59±0.09), respectively. These trends are presented in (Table 4). All of these results are further elucidated in the accompanying (Figure 2).

Albumin levels showed a significant decrease in both short-term and long-term experimental groups, with variations observed in different levels of toxicity (T1, T2, and T3). The concentrations of albumin after 96 hours were (6.07±0.18) in the control group and (6.83±0.05), (6.68±0.09), (6.25±0.10) in the other cadmium chloride-treated groups. Similarly, the levels of albumin after 15 days were (6.73±0.10) in

the control group and (6.81±0.08), (6.88±0.13), (6.31±0.05) in the other experimental groups. On the 21st day, albumin levels in the control group were (6.75±0.11), while in treated groups T1, T2, and T3, they were (6.87±0.06), (6.93±0.14), and (6.40 ±0.08), respectively. These findings are presented in (Table 4). All of these results are further elucidated in the accompanying (Figure 2).

Bilirubin levels exhibited significant differences between short-term and long-term experimental groups in comparison. The levels of bilirubin at 7th day was (5.02±0.20) in the control group and (5.81±0.14), (5.46±0.20), (5.65±0.20) in the treated groups. Similarly, the concentrations of bilirubin at 14th day were (5.08±0.09) in the control group and (5.77±0.08), (5.48±0.10), (5.66±0.12) in the other cadmium chloride-treated groups. On the 21st day, bilirubin levels in the control group were (5.12±0.09), while in treated groups T1, T2, and T3, they were (5.78±0.11), (5.50±0.13), and (5.70±0.13). Notably, it was observed that bilirubin reached its minimum level in the T3 treatment, as detailed in (Table 4). All of these results are further elucidated in the accompanying (Figure 2).

Cholesterol levels displayed significant differences between short-term and long-term experimental groups, particularly in treatments T1, T2, and T3. The levels of cholesterol at 7th day was (173.50±4.50) in the control group and (162.80±4.50), (180.00±4.80), (257.40±5.80) in the treated groups. Similarly, the concentrations of cholesterol at 14th day were (170.50±2.90) in the control group and (165.62±3.10), (182.15±43.90), (225.80±4.80) in the other cadmium chloride-treated groups. On the 21st day, cholesterol levels in the control group were (172.80±2.70), while in the exposed treated groups T1, T2, and T3, they were (166.90±3.00), (185.10±3.50), and (229.80±4.40), respectively. Notably, it was observed that cholesterol was at its highest level in T1 treatments and lowest in T2, which changed significantly. These findings are detailed in (Table 4). All of these results are further elucidated in the accompanying (Figure 2).

Triglycerides levels exhibited significance in both short-term and long-term experimental groups, with notable increases in T1 and T2 compared to other groups. At 7th day, the levels of TG was (117.80±4.20) in the control group and (98.56±4.20), (124.40.56±5.00), (140.12±4.70) in the treated groups. Similarly, at 14th day, TG concentrations were (118.95±3.30) in the control group and (103.12±3.02), (129.47±3.35), (143.22±2.40) in the other cadmium chloride-treated groups, respectively. On the 21st day, triglyceride levels in the control group were (121.00±2.90), while in the treated groups (T1, T2, and T3), they were (105.50±3.00), (130.40± 3.30), and (145.60±2.50), respectively. These findings are presented in (Table 4). All of these results are further elucidated in the accompanying (Figure 2).

High-density lipoprotein cholesterol (HDL-C) levels exhibited significant decreases in both short-term and long-term experimental groups, particularly in T1 and T2 when compared comparatively. At 7th day, HDL-C levels was (71.10±3.90) in the control group and (52.20±3.90), (39.12±4.70), (63.80±4.20) in the treated groups. Similarly, at 14th day, HDL-C concentrations were (69±2.0) in the control group and (68.20±1.90), (41.25±2.40), (63.40±1.80) in the other cadmium chloride-treated groups, respectively. In the control group, HDL-C was measured at 21th day (72.60±2.00), while in treated groups T1, T2, and T3, it was (53.40±1.90), (42.10±2.40), and (65.40±1.80), respectively. These findings are provided in (Table 4). All of these results are further elucidated in the accompanying (Figure 2).

One of the most significant environmental problems is heavy metal pollution (Anirudhan and Sreekumari 2011). Indeed, these metals often exhibit toxicity even at low levels, remain non-biodegradable, and commonly show resistance to conventional removal techniques. As a result, they have the potential to significantly lower drinking water quality. Heavy metal exposure can cause a variety of major human health problems, including cancer, neurological disorders, kidney pathology and respiratory problems. For example, chromium is a carcinogen and can cause skin lesions and respiratory problems (Mohammadi *et al.*, 2020; Naz *et al.*, 2023a).

Considering the intimate connection between fish and their environment, encompassing aspects like physical maturation, growth, and reproductive processes (Das *et al.*, 2012). Therefore, it is important to comprehend how environmental elements affect the health state of fish. This research aligns with similar work previously conducted by others in the field in *Labeo rohita* (Rajkumar *et al.*,, 2016; Naz *et al.*, 2020). In our research, we observed significant

alterations in hematological and biochemical parameters, consistent with previous reports. The potential release of cadmium chloride into aquatic ecosystems raises specific concerns, especially in light of research demonstrating its high toxicity to various freshwater aquatic species (Lee *et al*., 2007; Asharani *et al*., 2008; Griffitt *et al*., 2008; Naz *et al*., 2023b).

All previous reported findings that support the current study have concluded that exposure to cadmium chloride results in significant changes in hematological parameters. Specifically, there is a significant increase in white blood cells (WBCs) and a significant decrease in red blood cells (RBCs), hemoglobin (Hb), hematocrit, and platelet counts. Additionally, it has been determined that cadmium chloride exposure leads to increased levels of all serological parameters, except for total proteins. Furthermore, the serum enzyme levels rise in response to toxic substances such as chromium chloride (Naz *et al*., 2020, 2021). In the current investigation, the levels of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) did not show significant differences among the groups. This variation may be attributed to the selection of a different species of fish in the current experiment compared to previous ones. At 14th day in the present study, significant changes were observed in the levels of MCV, MCH, and MCHC. These significant parameter changes may be attributed to the increasing concentration of cadmium chloride. The results of current study coordinated with the previous findings which was reported by (Shaluei *et al*., 2013; Remya *et al*., 2015) at 14th day. These results align with the findings previously reported by (Perera and Pathiratne, 2012). In the current study, with the exception of ALP, total protein, and bilirubin, all other biochemical parameters showed no significant changes at 7th day and at 14th day of exposure to various concentrations of chromium chloride. Mohamed (2009) documented the occurrence of edema between the submucosa and mucosa attributed to the absorption of toxic metals. Histopathological examinations of the intestines of Rohu (*Labeo rohita*) fingerlings revealed severe degeneration and destruction of the intestinal mucosa. The observed necrosis led to cell death in the columnar epithelial cells, and the shortening of villi was directly associated with both increased dose and exposure time to cadmium chloride. This underscores the possibility that, despite the primary uptake of metals occurring through the gills, absorption may also take place via the intestinal epithelium, as previously suggested by Mohamed (2008).

Conclusions and Recommendations

The study was conducted over a 21-day period, during which the researchers used different concentrations of chromium chloride for exposure. These treatment groups (T1, T2, and T3) were exposed to different concentrations of cadmium chloride (0.44g/l, 0.89g/l, and 1.34 g/l), while a fourth group served as the control and was not exposed to cadmium chloride (0.00g/l). The results of the study indicated that exposure to cadmium chloride had significant effects on most of the hematological parameters. This suggests that the fish blood parameters were significantly altered due to the exposure to cadmium chloride. Additionally, various biochemical markers in the fish's serum were examined. These included HDL-C, bilirubin, ALP, ALP, total protein, albumin, cholesterol, and triglycerides. The levels of all these parameters exhibited significant differences between short-term and long-term experimental groups in comparison with control. The outcome of this study underscores the importance of evaluating the effects of environmental contaminants such as cadmium chloride on aquatic organisms. The observed alterations in hematological and biochemical studies provide valuable insights into the potential sub lethal impacts on fish health and ecosystem integrity. Continued research in this area will contribute to a better understanding of this risks associated with contaminant exposure and support strategies for sustainable aquatic resource management.

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Novelty Statement

The current study investigates the hematological and biochemical changes in freshwater fish *Labeo rohita* under exposure of Cadmium Chloride, this study will be highlighting the environmental issue of heavy metal contamination resulting from human activities,

which poses a significant threat to aquatic organisms.

Author's Contribution

Moazama Batool: Execution of study, formatting and analysis and reviewed the final manuscript..
Saima Naz: Planning research, supervision, arrangement of supplies, write-up.
Ghulam Abbas: Reviewed the manuscript and analysis.
Ahmad Manan Mustafa Chatha: Helped in conducting research and data analysis.
Mamoona Mahmood and Asma Aziz: Helped in data compilation and manuscript writing.
Fatima Yasmin: Performed the experiment in laboratory.

Conflict of interest
The authors have declared no conflict of interest.

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