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RESEARCH ARTICLE

Toxic effect of some heavy metals on hematology and histopathology of major carp (*Catla catla*)

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Abstract

The current study was conducted to assess the hematological and histopathological changes in major carp (*Catla catla*) exposed to different concentrations of copper (Cu) and cadmium (Cd). For this purpose, *Catla catla* fish, weighing approximately 230–235 g, were randomly divided into four groups and then exposed to acute doses of Cu (1.25 ppm), Cd (4.5 ppm), and their mixture (2.25 ppm) for 96 h and then 20, 30, and 40% respectively for a period of 30 days. Results showed that red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), lymphocyte, and monocyte decreased significantly, while the total white blood cell count and neutrophil population significantly increased in experimental groups as compared with the control one. Histopathological examination of liver tissues showed karyorrhexis, hepatic cells degeneration, congestion, and hemorrhages. Microscopic analysis of gills' sections revealed lamellar atrophy, telangiectasia, and necrosis of lamellar epithelial cells. In the kidneys, different histopathological ailments like atrophy of glomeruli, necrosis of renal tubular cells, increased urinary spaces, degeneration of renal tubules, and melanomacrophage aggregates were observed, while in the intestine, atrophy of villi, sloughing of epithelial villi, and congestion were seen after 30 days of exposure. In conclusion, the study indicates that exposure to Cu and Cd for longer period of time causes adverse hematological and histopathological changes in *Catla catla* fish.

Keywords Alterations · Degeneration · Fish · Hematology · Heavy metals · Histopathology

Introduction

Heavy metals are natural environmental components and considered potential marine pollutants. Large quantities of these heavy metals are accumulated as a result of land-based activities in the aquatic ecosystem (Javed et al. 2017; Shah et al. 2020). Nowadays, heavy metal residues have become a matter of serious concern because of their continuous increase in air and aquatic environment (Abah et al. 2016; Javed and Usmani 2019). Moreover, due to the increased human population with their anthropogenic activities, both underground and surface water supplies are now affected with the heavy metals which ultimately results in depletion of the aquatic organisms (Waqar et al. 2013). 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 (Wong et al. 2001). The contamination of aquatic ecosystem with heavy metals is regarded to be dangerous not only for aquatic fauna but also for the human as the consumer of fish for food (Sabullah et al. 2015).

Among metals, cadmium (Cd) occurs naturally as a non-essential trace element and has the tendency to accumulate in the living organisms due to which it is considered one of the most dangerous environmental pollutants (Abbas et al. 2019). Copper (Cu) is also one of the most poisonous metals that

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affects aquatic species and ecosystem (Singh et al. 2008). One of the well-known mechanisms of Cu toxicity in fish is that it results in oxidative stress (Lushchak 2011). Fish blood is being studied as an indicator of pathological changes and environmental monitoring system (Walter et al. 2006). Therefore, it is important to diagnose the functional and systemic state of fish exposed to toxicants (Hassan et al. 2018). Metals can cause alteration in hematological indices in fish. Increase or decrease in blood parameters is considered to be the symbol of unhealthy state, environmental stress, or tissue injury (Li et al. 2010; Hassan et al. 2018). The histopathological changes due to exposure of heavy metals are helpful in evaluating their toxic effects in different species of fishes (Clemente et al. 2013). Previous studies have shown that the liver of fish exposed to heavy metals showed congestion of the central vein, edema, and nuclear pyknosis and degeneration in hepatic cells of different species (Kaoud and El-Dahshan 2010; Su et al. 2013). The histological alterations due to heavy metals in the intestines of fish include atrophy in the muscularis, necrotic changes in the intestinal mucosa, and sub-mucosa with degenerative cells in the intestinal lumen (Al-Balawi et al. 2013; Kaoud et al. 2013; Padrilah et al. 2018). Thus, the current study was planned to investigate the effect of induced toxicity of Cd and Cu on the hematology and the histopathology of *Catla catla* fish.

Materials and methods

A total of 160 freshwater fish (*Catla catla*) about 3 months old (weight 230–235 g; length 12–15 cm) were collected from a public fish seed hatchery located in district Bahawalpur, Punjab, in plastic bags supplemented with sufficient amount of oxygen. The fishes were then transferred to the laboratory of Toxicology, Department of Zoology, Government Sadiq College Women University, Bahawalpur. Experimental fishes were acclimatized to the laboratory conditions for 15 days. During acclimatization period, water of the entire aquarium was changed on a daily basis. Air pump attached with a capillary system was used to supply air to the test media. Temperature (30 °C), hardness (255 mg/l), and pH (6.5) were maintained throughout the experimental period. Feed was offered at the rate of 2% of their body weight. Cu, Cd, and their mixture was used to investigate their effects on hematology and histopathology of the experimental fish. All the chemicals used in this experimental research were obtained from Sigma-Aldrich (St. Louis Missouri, USA) and Merck (Germany). The different physicochemical parameters of water including temperature, pH, electrolytes, electrical conductivity (EC), and dissolved oxygen (DO) were maintained during the whole experiment (Table 1).

Table 1 Physicochemical analysis of water for experimental fish

Parameters	Values
Water temperature (°C)	23.8 ± 0.31
Calcium (mg/L)	38.9 ± 0.81
Potassium (mg/L)	1.8 ± 0.30
pH	9.11 ± 0.04
Alkalinity (mg/L)	179.7 ± 0.47
Total dissolved solid (mg/L)	181.8 ± 4.02
Electrical conductivity at 25 °C (μmhos/cm)	410.0 ± 2.99
Chlorides (mg/L)	11.10 ± 0.04
Dissolved oxygen (mg/L)	8.11 ± 0.12

Experiment 1

After acclimatization, 40 fish were divided into four groups with 25 fishes in each group. These experimental groups were exposed to different concentrations of heavy metals such as Cu (1.25 ppm), Cd (4.5 ppm), and their mixture (2.25 ppm) for an acute period of 96 h, while the control group was free of any heavy metal treatment.

Experiment 2

In the second experiment, fish were shifted to another aquarium and exposed to sublethal doses prepared at the rate of 20, 30, and 40% of Cu, Cd, and mixture (Cu + Cd), respectively, from mother solution of experiment 1.

Hematological study

After 96 h and 30 days of experimentation, blood samples (3–4 ml) were collected from the caudal vein of each fish in EDTA vacutainers using a 26-guage hypodermic needle. Hematological parameters such as RBC's count, hemoglobin (Hb), hematocrit (Hct), and total and differential leukocyte counts were determined as described by Ghaffar et al. (2017).

Histopathological processing

For histopathological study, all the fish were dissected and tissue samples like the gills, liver, intestines, and kidneys were collected from the control and experimental fish after 30 days of exposure. The samples were fixed in 10% neutral buffered formaldehyde solution, embedded, and processed as described elsewhere (Hussain et al. 2018).

Statistical analysis

The data obtained were statistically analyzed by using one-way analysis of variance (ANOVA), while the means±SEM

of different groups were compared through Tukey's test at $p < 0.05$ level of significance.

Results

During this study, no mortality was noticed in the control group and all fish remained active and healthy. In the current experiment, different behavioral and clinical ailments such as tremors of fins, increased swimming area, erratic swimming, air gulping, loss of equilibrium, hypersecretion of mucus, operculum movement, lying on one side, and increased surface breathing were observed.

The obtained results in this study indicated significant changes in the blood values of the control and experimental groups exposed to Cu, Cd, and their mixture for acute (96 h) and chronic (30 days) period. Hematological parameters such as red blood cells (RBC's), hemoglobin (Hb), hematocrit (Hct), monocyte, and lymphocyte decreased significantly, while the total white blood cells and neutrophil population increased significantly after an acute exposure of heavy metals and their mixture (Table 2). Our results showed that the exposure of Cu metal showed lower values of RBCs, Hb, and Hct than their mixture and Cd metal alone (Table 2).

Table 2 showed that the RBCs, Hb, and Hct were reduced in fish exposed to sublethal doses. The values decreased significantly after the exposure of 40% dose as compared with 20 and 30%. Total and neutrophil counts showed an increase in their values after the exposure of different concentrations (20, 30, and 40%) of heavy metals. The results indicated that 40% concentration of heavy metals reduced the blood parameters more significantly as compared with 20 and 30%. The results also indicated that the increased duration of exposure severely affected the hematological parameters. The overall trend of toxicity of metals during acute and chronic exposure was Cu > Cu + Cd > Cd (Table 2).

The microscopic study of different organs such the gills, liver, intestine, and kidney of the control fish showed normal histo-architecture. On the other hand, the structure of the gills, liver, kidney, and intestines exposed to heavy metals showed alterations in vital organs. Histological examination of the gills of treated fish showed epithelial hyperplasia, epithelial necrosis, lamellar atrophy, and curling of secondary lamellae after 96 h of acute toxicity (mixture) and after 30 days of heavy metal exposure (Fig. 1). Microscopically, different changes such as melanomacrophage aggregates,

Table 2 Hematological changes in *Catla catla* fish exposed to different concentration of heavy metals and their combination during an acute and chronic exposure

Groups/treatment	Hematological changes in fish exposed to different heavy metals				
	Erythrocyte count (10^6/μL)	Hemoglobin (g/dL)	Hematocrit (%)	White blood cell count (10^3/mm^3)	Neutrophil count (%)
Acute exposure (96 h)					
Control	2.39 ± 0.07	5.43 ± 0.11	25.81 ± 0.21	19.24 ± 2.74	14.11 ± 0.13
Cd (4.5 mg/L)	1.78 ± 0.05	4.11 ± 0.12*	19.11 ± 0.37*	20.04 ± 1.10	16.13 ± 0.22
Cu (1.25 mg/L)	1.81 ± 0.08	4.05 ± 0.11*	16.17 ± 0.25*	27.05 ± 1.49*	15.30 ± 0.29
Cu + Cd (2.25 mg/L)	1.64 ± 0.03	3.77 ± 0.08*	17.22 ± 0.35*	29.14 ± 4.16*	24.23 ± 0.15*
Chronic exposure (30-day)					
Cadmium metal					
Control	3.12 ± 0.07	6.11 ± 0.13	33.01 ± 0.45	20.01 ± 1.98	15.71 ± 0.36
T1 (20% of acute dose)	1.18 ± 0.08*	4.01 ± 0.14	22.71 ± 0.72*	22.56 ± 1.78	17.08 ± 0.34
T2 (30% of acute dose)	1.06 ± 0.08*	4.16 ± 0.14*	20.88 ± 0.66*	34.54 ± 1.13*	23.08 ± 0.37*
T3 (40% of acute dose)	0.93 ± 0.08*	4.33 ± 0.14*	18.90 ± 0.67*	35.88 ± 1.34*	26.17 ± 0.44*
Copper metal					
Control	3.12 ± 0.07a	6.11 ± 0.13	33.01 ± 0.45	20.01 ± 0.98	14.71 ± 0.36
T1 (20%of acute dose)	1.02 ± 0.09 cd	4.30 ± 0.15*	19.37 ± 0.91*	26.66 ± 0.24*	16.88 ± 0.45
T2 (30% of acute dose)	0.85 ± 0.08*	4.01 ± 0.15*	17.34 ± 0.87*	31.86 ± 2.46*	25.64 ± 0.44*
T3 (40% of acute dose)	0.75 ± 0.08*	3.78 ± 0.15*	15.97 ± 0.89*	33.51 ± 4.34*	29.73 ± 0.49*
Mixture (Cu + Cd)					
Control	3.12 ± 0.07	6.11 ± 0.13	33.01 ± 0.45	21.01 ± 1.98	14.81 ± 0.36
T1 (20% of acute dose)	1.10 ± 0.08*	4.49 ± 0.16*	20.44 ± 0.78*	27.33 ± 2.07*	22.90 ± 0.70*
T2 (30% of acute dose)	1.03 ± 0.09*	4.42 ± 0.16*	20.12 ± 0.76*	36.69 ± 3.89*	27.09 ± 0.65*
T3 (40% of acute dose)	0.93 ± 0.09*	4.39 ± 0.16*	19.48 ± 0.67*	37.12 ± 1.23*	29.91 ± 0.70*

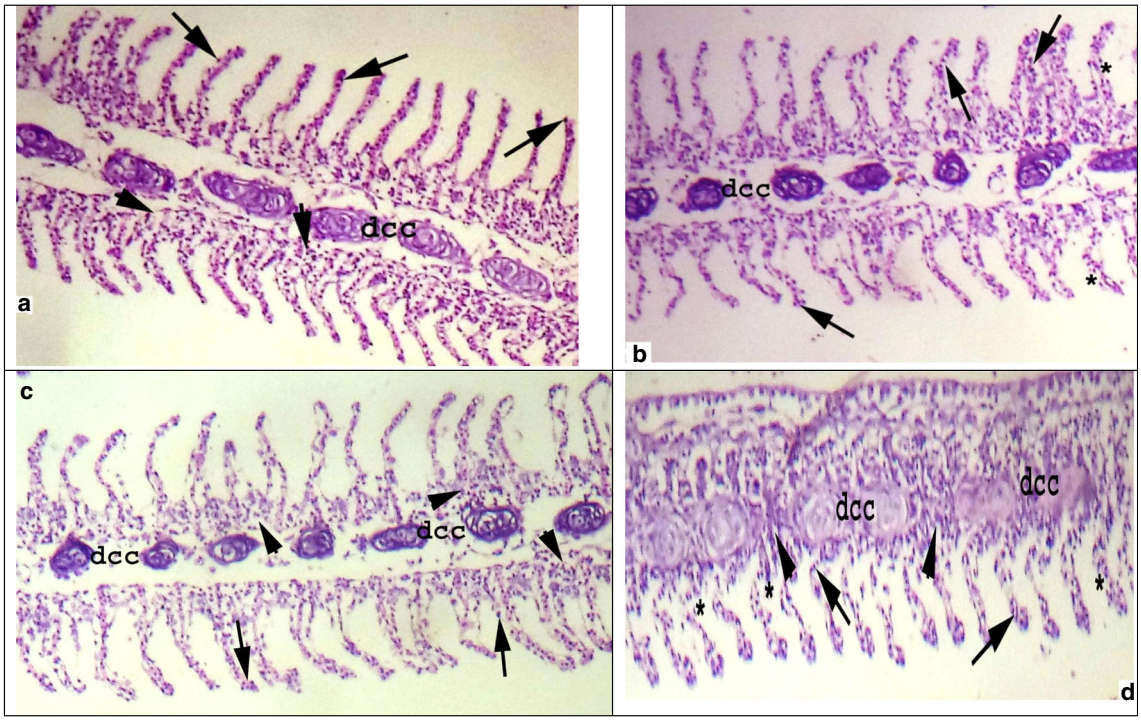

Fig. 1 Photomicrograph of the gills of treated fish showing (A, B, C, D) lamellar epithelial cell necrosis (arrows), sloughing of lamellar epithelium (*), disorganization of primary lamellae (arrow heads), atrophy and curling of secondary lamellae, and disorganization of cartilaginous core

thyroidization, nuclear hypertrophy, deterioration of glomerulus, dilation of tubular lumen, and cellular necrosis were observed in the kidneys of fish exposed to heavy metals (Fig. 2). Histopathological examination of different sections of the liver of treated fish showed degenerated hepatocytes, dilated sinusoids, vacuolar degeneration, eosinophilic infiltration, central vein contraction, and necrosis (Fig. 3). Intestinal tissues exhibited sloughing of the epithelial villi, atrophy of villi, congestion, and necrosis of epithelial cells after the 30th day of experiment (Fig. 4). Histopathological examination revealed that the tissue changes were much prominent in fish exposed to Cu than Cd and their mixture on the 30th day (Table 3).

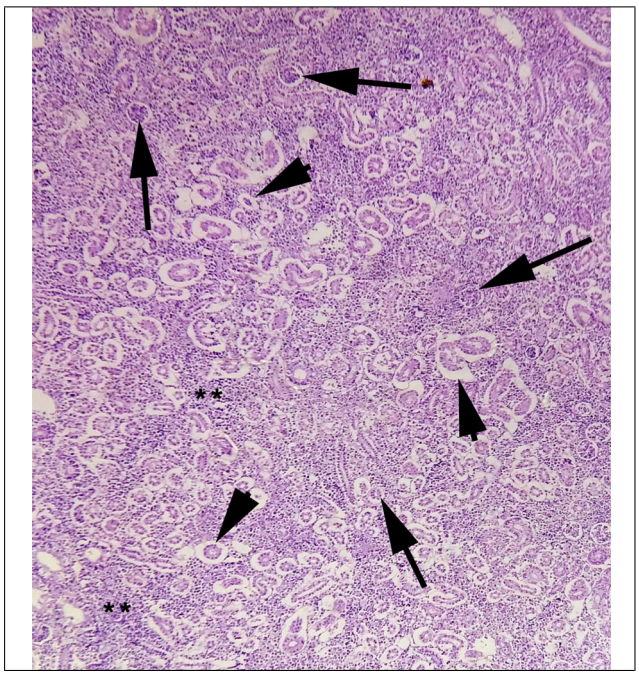

Fig. 2 Photomicrograph of the kidneys of treated fish showing melanomacrophage aggregates (**), deterioration of glomerulus (arrow heads), dilation of tubular lumen, cellular necrosis, and renal tubular degeneration (arrows)

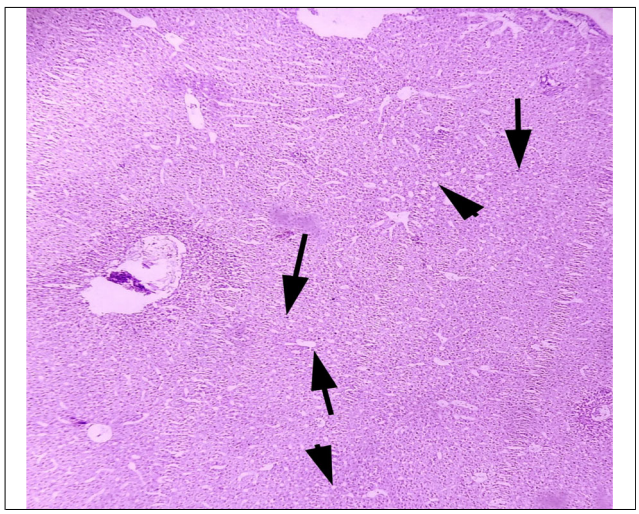

Fig. 3 Photomicrograph of the liver of treated fish showing degenerated hepatocytes, dilated sinusoids, eosinophilic infiltration, central vein contraction, necrosis of hepatocyte (arrows), and vacuolar degeneration (arrow heads)

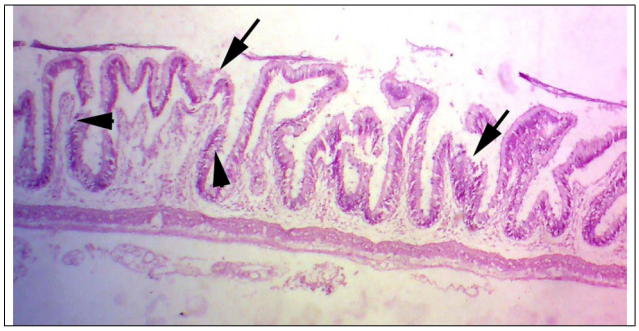

Fig. 4 Photomicrograph of sloughing of epithelial villi (arrows), atrophy of villi, cellular infiltration, and necrosis of epithelial cells (arrow heads)

Discussion

Due to environmental pollution, many researchers have documented changes in the blood indices of fish (Azarin et al. 2012). The variations in their characteristics depend on the intensity and length of toxicity of metals to fish and their physiological and environmental importance (Burgos-Aceves et al. 2019). In this study, the exposure of various metals to fish resulted in significant decrease in fish contents of RBCsC, Hb, and Hct compared with the control in both acute and chronic phases. The concentration of metals and time shows decline in the blood parameters. The 96-h exposure resulted in maximal negative effects on these parameters than the chronic one. Previous studies also reported a decrease in the content of RBCsC, Hb, and Hct in freshwater fish exposed to Cd and Ni (Hedayati and Ghaffari 2013). Alkahemal-Balawi et al. (2011) reported similar findings in *Cyprinus carpio* after exposure to Ni. Reduction in these indices was also observed in Pb and Cu toxicity in *Oreochromis mykiss* (Ates et al. 2008). Our findings are also similar to those reported by Mekkawy et al. (2011) who observed that *Oreochromis niloticus* exposed to Cd for 15 and 30 days showed a significant decrease in RBCs, Hb, and Hct content. Shalaby (2007) also found similar results in *Oreochromis niloticus* exposed to Cd metal. Macrocytic anemia was reported by the increase in mean cell volume and mean cell hemoglobin after lethal exposure of crude oil in juvenile Beluga (Rostam and Soltani 2016).

In this study, the histopathological alterations in tissues of *Catla catla* after the exposure of different heavy metal exposure were investigated. Study reveals that fish exposed to heavy metals showed degenerations in vital organs and alterations become prominent as the duration of exposure increased. The present research revealed a strong connection between damage to the liver and toxic substances, vacuolar deterioration, infraction, and necrosis as reported by El-Naggar et al. (2009) in *Oreochromis niloticus* exposed to heavy metals. *Labeo rohita* exposed to Zn, necrosis, hemorrhage, degeneration of hepatocytes, and pyknosis in the liver tissue were observed (Loganathan et al. 2006). *Heteropneustes fossilis* is exposed to heavy metals for 30 days; Kalita et al. (2003) investigated vacuole formation, degeneration of hepatic cells, hemorrhage and necrosis. Our findings also agreed with the observations that intestinal atrophy was recorded in fish collected from contaminated area (Peebua et al. 2008). Necrosis mucosa and submucosal hemorrhage in the intestine of Cd-exposed *O. niloticus* were also observed (Kaoud et al. 2012). Similarly, Hadi and Alwan (2012) recorded hypoxia and congestion of primary gill lamellae, lamellar fusion, and aneurism in Cu-exposed fish in the blood vessels.

Table 3 Severity of different microscopic alterations in the kidney, gills, liver, and intestine of *Catla catla* fish exposed to various concentrations of heavy metals

Histopathological alterations	Cd	Cu	(Cd + Cu)
Kidney			
Melanomacrophage aggregates	++	+++	++
Thyroidization	+++	++++	++
Nuclear hypertrophy	++	++++	+++
Dilation of tubular lumen	+++	+++	++
Deterioration of glomerulus	++++	+++	++++
Cellular necrosis	+++	++++	+++
Liver			
Karyorrhexis	++++	+++	+++
Nuclear hypertrophy	+++	++++	++
Hepatocellular hypertrophy	+++	++++	+++
Eosinophilic infiltration	++	+++	+++
Melanomacrophage aggregates	+	++++	++
Central vein contraction	++	+++	++++
Gills			
Lamellar disorganization	+++	++++	++
Lamellar atrophy	++	++++	+++
Epithelial necrosis	+++	++	++++
Telangiectasia	+++	+++	+++
Epithelial uplifting lamellae	++	++++	+++
Intestine			
Necrosis of intestinal epithelial cells	+++	++++	++++
Sloughing of epithelial villi	++	+++	++
Congestion	+++	++++	++++
Atrophy of villi	++	++++	+++

Mild (+), moderate (++), severe (+++), very severe (++++)

Conclusion

In conclusion, the study indicates that exposure to Cu and Cd causes adverse effects on hematological indices and histopathology of *Catla catla* fish.

Authors' contributions Saima Naz: Designed the study
Riaz Hussain: Performed histological study
Qudrat Ullah: Revised the article
Ahmad Manan Mustafa Chatha and Ansar Shaheen: Conducted, sampled, and analyzed the study
Rifat Ullah Khan: Prepared, edited, and submitted the manuscript

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interest There is no potential competing interest with this study.

Ethical approval This study was approved by the Department Committee on Animal Ethics and Welfare, Government Sadiq College Women University, Bahawalpur Pakistan.

Consent to participate and consent to publish All the authors have equally participated in this study and agreed to publish this work in this journal.

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