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ASSESSMENT OF ACUTE METALS TOXICITY IN *Catla catla* THROUGH HEMATOLOGICAL AND BIOCHEMICAL BLOOD MARKERS

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The objective of the current study was to determine the toxicological effects of acute cadmium (Cd) and copper (Cu) exposure on hematological and serum biochemical parameters of major carp, *Catla catla*. The experimental group of fish were exposed to Cd and Cu for 24-, 48-, 72- and 96-hr. The hematological analysis of exposed fish at all exposure durations exhibited significant decrease in red blood cells count, hemoglobin and hematocrit content while marked elevation in white blood cells count, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration was recorded in comparison to control. Among serum biochemical parameters, the level of sodium, chloride, albumin and total protein was lower at all exposure durations of metals. However, potassium, urea, glucose, aspartate aminotransferase and alanine aminotransferase levels were noticeably higher in serum of treated fish. Results also indicated that Cu has more toxic effects on hematological and serum biochemical parameters than Cd. This study proposed that the occurrence of toxic metals in aquatic environment has significant impact on the hematological and serum biochemical parameters in *C. catla*. The observed changes in these parameters may provide valuable information for further concerning environmental conditions and risk assessment of aquatic organisms.

Keywords: Hematology, serum biochemistry, metals, *Catla catla,* toxicological effects.

INTRODUCTION

Heavy metals constitute a major group of aquatic contaminants and their large amount accumulates in aquatic ecosystems as a consequence of land based activities (Vutukuru, 2003). Among metals, cadmium (Cd), one of the most dangerous environmental pollutant because of its tendency to bio-accumulate in living organisms raises environmental concern (Liao *et al*., 2011; Sfakianakis *et al*., 2015). The use of Cd containing agricultural chemicals, pesticides, fertilizers and sewage sludge in farm land, might also enhance to the water contamination (ATSDR, 2003). Survival rate of aquatic organisms is affected due to the exposure to Cd and leads to a gradual extinction of their generations in polluted water (Sridhara *et al*., 2008). Although copper (Cu) is essential to the health of all living organisms as it is involved in several fundamental biological processes however, it proves disastrous to aquatic organisms when it surpasses the normal limits. Its toxicity to aquatic organisms had previously been described by several workers (Paquin *et al*., 2002; Dhanapakiam *et al*., 2006; Ketpadung and Tangkrock-Olan, 2006; Isani *et al*., 2013). Copper sulphate ($CuSO_4$) is generally used as an algaecide as well as an herbicide in aquatic weed control (Carbonell and Tarazona, 1993).

Fishes are the animals that cannot escape from the negative effects of these contaminants and prove as good bio-indicators of aquatic pollution (Pandey, 2013). Fish blood is being studied increasingly in environmental monitoring and toxicological research as a potential indicator of pathological and physiological changes in disease investigations and fishery management (Remyla *et al*., 2008). Numerous studies have showed that metals, for instance cadmium and copper induce changes in blood parameters of fish (Kotsanis *et al*., 2000; Mazon *et al*., 2003; Ajani and Akpoilih, 2010; Al-Ghanim, 2011; Sayed and Shokr, 2015).

Numerous hematological indices, for instance hemoglobin, hematocrit, white blood cells, red blood cells, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration have been used as indicators of metal contamination in the aquatic ecosystem (Kavitha *et al*., 2010). Measurement of serum biochemical parameters such as aspartate aminotransferase, alanine aminotransferase, ions, total protein and glucose is valuable to ascertain the toxicity of target organs along with the overall health status of animals (Kori-Siakpere *et al*., 2012).

In fisheries management program hematology and serum biochemistry data are of great importance to monitor the health status of aquatic animals (Skjervold *et al*., 2001). Accordingly, the objective of this study was to investigate the effects of acute cadmium and copper exposure for varying durations on hematological and serum biochemical parameters as sensitive indices for the evaluation of fish

physiology under metallic stress in economically important fish (*Catla catla*) of Pakistan.

MATERIALS AND METHODS

The present experiment was conducted in the Wet Laboratory of Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. *Catla catla* of desired weight (30 g) were obtained from the Fish Seed Hatchery, Faisalabad. They were brought to the Wet Laboratory and acclimatized to the laboratory conditions for 14 days.

Pure chloride compounds of cadmium ($CdCl_2$. H_2O) and copper ($CuCl_2$. $2H_2O$) were used in this experiment and stock solutions were prepared for required metal dilution. Fish were exposed for 96-hr to waterborne lethal concentrations (LC_{50}) of metals which were already determined by Yaqub and Javed (2012), and Kousar and Javed (2015). The water temperature (30°C), pH (7) and total hardness (225 mg/L) of the test media were kept constant. Total ammonia, sodium, potassium, magnesium, carbon dioxide and calcium contents of the test media were monitored on daily basis by following the methods of A. P. H. A. (1998).

***Hematological parameters*:** The blood samples were taken at different time intervals (24-, 48-, 72- and 96-hr) from metals exposed and control fishes to study the hematological and serum biochemical parameters. Specimens with an average body weight of 30 g (ranged from 27 to 34 g) were used for sample collection. After anaesthetizing the fish with MS-222 (100 mg/L), blood samples were collected under sterile conditions by the puncture of caudal vein with a heparin-coated 23-gauge needle attached to a 2.5 mL syringe. The hematological parameters i.e. red blood cells, hemoglobin, hematocrit, white blood cells, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration were determined by using automated cell counter (Sysmex KX 21).

***Serum biochemical parameters*:** Blood samples for serum biochemical analysis were collected without an anticoagulant. The blood samples were left for 1-hr on ice and then centrifuged at 3000 rpm for 10 minutes to isolate the serum. Samples were stored at -80°C before the further analysis. Serum biochemical parameters (sodium, potassium, chloride, albumin, total protein, urea, urea, aspartate aminotransferase, alanine aminotransferase) were estimated following standard methods using commercially available kits by BioMed Company.

***Statistical analysis*:** Data were reported as mean ± S.D. (n=3). Significance of samples means ($p<0.05$) between control and metals exposed fish were measured using Sudent's T-Test by SPSS.

RESULTS AND DISCUSSION

***Hematological parameters*:** Clinical blood indices have been extensively used as effective bioindicators in aquatic toxicology (Singh and Srivastava, 2010). These indices are very crucial for the assessment of fish physiological status under metal stress. The hematological parameters in *C. catla* exposed to 96-hr LC_{50} of cadmium (Cd) and copper (Cu) are given in Table 1. RBCs, Hb and Hct contents of the fish exposed to either Cd or Cu were significantly decreased as compared to control at the end of all the exposure periods. However, the percent decrease in the RBCs, Hb and Hct content was relatively high i.e. -45.90, -26.95 and -40.26 %, respectively in Cu exposed fish and 39.75, -17.73 and -31.02 %, respectively in Cd exposed fish after 96-hr exposure.

Similar result was also detected in *O. mykiss* exposed to Pb and Cu (Ates *et al*., 2008). The authors reported that decrease in these parameters indicates the anemic condition of the fish which inhibit the blood formation in blood forming organs. Production of reactive oxygen species caused by metals may be another potential cause for the noticed decline in Hb content (Larsson *et al*., 1985). Failure of red blood cell production, impaired osmoregulation or internal bleeding under stress condition may lead to decrease in number of RBCs (Kavitha *et al*., 2010). Furthermore, lower Hct content also designates contraction of cell as a result of toxicant stress on erythropoietic tissue (Saravanan *et al*., 2011). The high concentrations of metals for short term exposure usually declines the above mentioned parameters. Previous studies also reported a decline in RBCs, Hb and Hct content in freshwater fishes exposed to Cd and Ni showing anemia, leucopoiesis and erythropenia (Vincent *et al*., 1996; Nanda and Behera, 1996).

White blood cells (WBCs) increased significantly ($p<0.05$) in both Cd and Cu treated fish as compared to control. The MCV increased gradually from 24-hr to 96-hr exposure duration. A significant ($p<0.05$) increase in MCH and MCHC was also observed in metal treated fish as compared to control. The percent increase in WBCs, MCV, MCH and MCHC over control was relatively higher in Cu exposed fish as compared to Cd exposed.

In current study, the increase in WBCs indicates a defensive response to the metals exposure (Abhijith *et al*., 2012). Nevertheless, the detected rise in MCV and MCH during lethal exposure designates the macrocytic anemia (Saravanan *et al*., 2011). Furthermore, high number of smaller undeveloped RBCs in blood due to hyperplasia in the erythropoietic sites also leads to higher MCV (Ferrando and Andreu-Moliner, 1991). However, the detected increase of MCHC during lethal exposure may be owing to genetic sphaerocytosis (Sobecka, 2001).

***Biochemical parameters*:** The effect of metals on serum biochemical parameters of *C. catla* exposed to the Cd and Cu for 24-, 48-, 72- and 96-hr is illustrated in Figure 1. In eco-toxicology serum ion levels are considered as good biomarkers because these can easily alter as a result of reduced intestinal fluid absorption, reduced branchial ion

Table 1. Hematological parameters of *Catla catla* in control and metal exposed fish.

Hematological parameters	Exposure duration	Control	Cu exposure	Percent Change	Cd exposure	Percent Change
RBCs (10^6/µL)	24-hr	2.43±0.39	1.75±0.49*	(-27.98)	1.98±0.47*	(-18.52)
	48-hr	2.43±0.50	1.59±0.58*	(-34.57)	1.78±0.38*	(-26.75)
	72-hr	2.44±0.59	1.47±0.36*	(-39.75)	1.70±0.56*	(-30.33)
	96-hr	2.44±0.61	1.32±0.47*	(-45.90)	1.47±0.32*	(-39.75)
Hb (g/dL)	24-hr	5.63±0.41	4.67±0.78*	(-17.05)	5.03±0.72*	(-10.66)
	48-hr	5.63±0.43	4.52±0.95*	(-19.72)	4.95±0.87*	(-12.08)
	72-hr	5.64±0.69	4.32±0.61*	(-23.40)	4.70±0.61*	(-16.67)
	96-hr	5.64±0.77	4.12±1.18*	(-26.95)	4.64±0.61*	(-17.73)
Hct (%)	24-hr	25.11±1.06	19.00±1.44*	(-24.33)	21.00±1.08*	(-16.37)
	48-hr	25.11±1.44	17.35±1.27*	(-30.90)	19.76±1.08*	(-21.31)
	72-hr	25.11±1.06	16.67±1.12*	(-33.61)	18.65±1.47*	(-25.73)
	96-hr	25.11±1.02	15.00±1.05*	(-40.26)	17.32±1.22*	(-31.02)
WBCs (10^3/µL)	24-hr	29.98±1.54	39.98±1.39*	(+33.36)	35.54±1.40*	(+18.55)
	48-hr	29.16±1.48	41.78±1.77*	(+43.28)	37.55±1.32*	(+28.77)
	72-hr	29.67±1.60	45.12±1.31*	(+52.07)	39.67±1.50*	(+33.70)
	96-hr	29.67±1.97	48.26±1.98*	(+62.66)	42.59±1.56*	(+43.55)
MCV (fL)	24-hr	104.44±11.72	113.67±28.71*	(+8.84)	109.26±19.28	(+4.62)
	48-hr	105.30±14.37	116.83±31.87*	(+10.95)	113.92±18.82*	(+8.19)
	72-hr	106.45±20.99	120.33±40.67*	(+13.04)	116.81±31.53*	(+9.73)
	96-hr	106.43±20.34	122.13±39.41*	(+14.75)	120.90±21.78*	(+13.60)
MCH (pg)	24-hr	23.36±2.19	27.42±4.27*	(+17.38)	25.79±2.32*	(+10.40)
	48-hr	23.54±2.78	29.42±4.32*	(+24.98)	28.00±1.13*	(+18.95)
	72-hr	23.66±3.28	30.08±3.81*	(+27.13)	29.08±6.28*	(+22.91)
	96-hr	23.65±3.60	31.64±2.16*	(+33.78)	32.05±3.46*	(+35.52)
MCHC (g/dL)	24-hr	22.41±0.76	24.48±2.27	(+9.24)	23.86±2.20	(+6.47)
	48-hr	22.40±0.46	25.88±3.86*	(+15.54)	24.92±3.08*	(+11.25)
	72-hr	22.43±1.90	26.16±5.30*	(+16.63)	25.13±1.31*	(+12.04)
	96-hr	22.42±2.22	27.29±6.45*	(+21.72)	26.72±1.79*	(+19.18)

Data represents mean ± S.D. (n=3), * Significant, $p<0.05$ (Based on t test).
(-) Denotes percent decrease over control, (+) Denotes percent increase over control.

extrusion and alterations in the morphological structure of the cells (Oner *et al*., 2008). The present study exhibited significantly lower level of Na in serum of metal exposed fish in comparison to control. The rainbow trout exposed to Cu showed significant decrease in serum Na level than the control (Shaw *et al*., 2012). In addition, Oner *et al*. (2008) documented reduced serum Na level in Ag exposed freshwater fish, *Oreochromis niloticus*.

In the present study, increase in serum K level of exposed *C. catla* was noted. The K level in rainbow trout showed a significant increase with metal treatment (Ramsden *et al*., 2009). Chloride level in serum of exposed fish decreased as compared to control. Logaswamy *et al*. (2007) reported that the decline in electrolytes, including Na and Cl, might be caused by histological changes in the gills or instabilities in membrane permeability owing to toxicity.

A time dependent decrease in albumin and total protein content of serum was observed in metal treated fish as compared to control. The exposure of Cu resulted in more drastic effects as compared to Cd. Hypoproteinemia and hypoalbuminemia observed in metal treated fish could be due to liver and kidney damage. Oronsaye (1989) reported kidney damage in *Gasterosteus aculeatus* exposed to Cd. In contrast to our finding Oner *et al*. (2008) observed insignificant alteration in TP in *O. niloticus* intoxicated with Cd while significant decrease under Cu exposure.

The high urea level in metal exposed fish was noted in our study. Urea in fish is produced by liver, it is defecated mainly by the gills rather than kidney (Stoskoph, 1993). The higher urea level in our study may be ascribed to gills dysfunction. Gills damage as a result of Cd intoxication was reported in *Gasterosteus aculeatus* by Oronsaye (1989). Similarly, Oner *et al*. (2008) documented increased blood urea in Cd exposed *O. niloticus*.

Glucose is one of the most sensitive parameter for assessing the stressed state of an organism; its high concentration in the blood indicates that a fish is in stress and is using its energy reserves (Vosyliene, 1999). In our study, the glucose level in the metal treated fish significantly increased by the end of the exposure duration. An increase in serum glucose level under

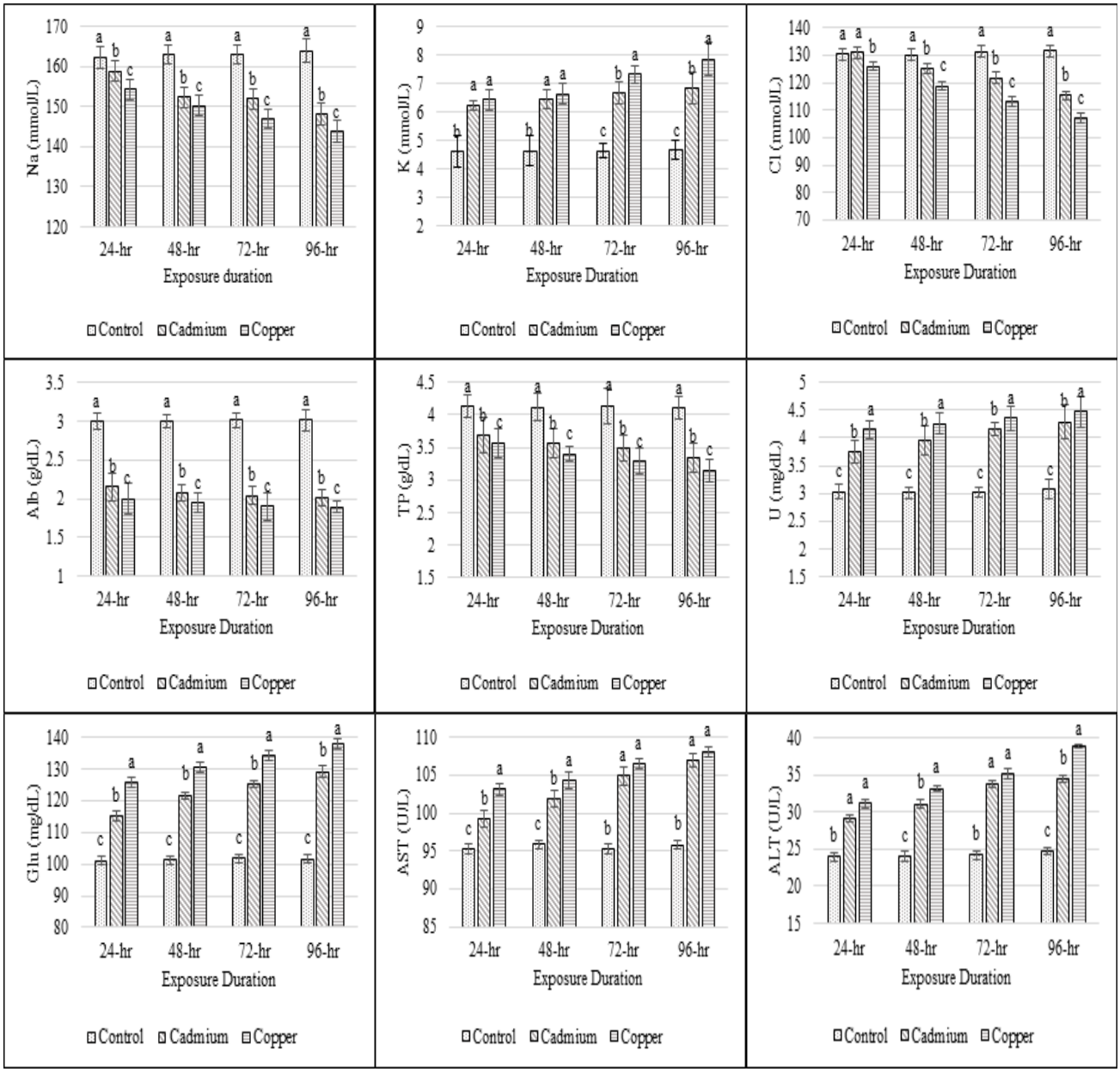

Figure 1. Changes in serum biochemical parameters i.e. sodium (Na), potassium (K), chloride (Cl), albumin (Alb), total protein (TP), urea (U), glucose (Glu), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of *Catla catla* after exposure to cadmium and copper.

The bars represent the mean values (±SEM). Treatments followed by the same letters are not significantly different (P>0.05).

the influence of Cd was linked to a decrease in liver glycogen reserves (Cicik and Engin, 2005).

The ALT and AST activities are often used in the diagnosis of fish diseases and detection of tissue damage caused by metal pollutants (Firat *et al*., 2011). In this study higher level of AST and ALT was noted in serum of metal exposed fish at all exposure durations. The increase in enzymes levels might be as a result of activation of gluconeogenesis. Increase in ALT and AST after toxicant exposure in this study is in agreement with previous studies (Abdel-Tawwab *et al*., 2007; Firat *et al*., 2011).

The present study accomplishes that exposure of fish, *C. catla* to cadmium and copper alters the hematological and serum biochemical parameters. The changes in these parameters can be used as biomarkers or as a sensitive tool to determine the toxicity of metals in aquatic environment. The results of this

study also provide a better understanding of the toxicological endpoint of aquatic pollutants and to establish a safer level of these metals in the aquatic environment in order to protect aquatic organisms.

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