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 biochemical 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$_2$O) and copper (CuCl$_2$, 2H$_2$O) 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 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$_2$H$_8$O$_4$ and Javel (2011). The effect of metals on serum biochemical parameters of *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.

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

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Exposure duration</th>
<th>Control</th>
<th>Cu exposure</th>
<th>Percent Change</th>
<th>Cd exposure</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (10^6/µL)</td>
<td>24-hr</td>
<td>2.43±0.39</td>
<td>1.75±0.49*</td>
<td>(-27.98)</td>
<td>1.98±0.47*</td>
<td>(-18.52)</td>
</tr>
<tr>
<td></td>
<td>48-hr</td>
<td>2.43±0.50</td>
<td>1.59±0.58*</td>
<td>(-34.57)</td>
<td>1.78±0.38*</td>
<td>(-26.75)</td>
</tr>
<tr>
<td></td>
<td>72-hr</td>
<td>2.44±0.59</td>
<td>1.47±0.36*</td>
<td>(-39.75)</td>
<td>1.70±0.56*</td>
<td>(-30.33)</td>
</tr>
<tr>
<td></td>
<td>96-hr</td>
<td>2.44±0.61</td>
<td>1.32±0.47*</td>
<td>(-45.90)</td>
<td>1.47±0.32*</td>
<td>(-39.75)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>24-hr</td>
<td>5.63±0.41</td>
<td>4.67±0.78*</td>
<td>(-17.05)</td>
<td>5.03±0.72*</td>
<td>(-10.66)</td>
</tr>
<tr>
<td></td>
<td>48-hr</td>
<td>5.63±0.43</td>
<td>4.52±0.95*</td>
<td>(-19.72)</td>
<td>4.95±0.87*</td>
<td>(-12.08)</td>
</tr>
<tr>
<td></td>
<td>72-hr</td>
<td>5.64±0.69</td>
<td>4.32±0.61*</td>
<td>(-23.40)</td>
<td>4.70±0.61*</td>
<td>(-16.67)</td>
</tr>
<tr>
<td></td>
<td>96-hr</td>
<td>5.64±0.77</td>
<td>4.12±1.18*</td>
<td>(-26.95)</td>
<td>4.64±0.61*</td>
<td>(-17.73)</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>24-hr</td>
<td>25.11±1.06</td>
<td>19.00±1.44*</td>
<td>(-24.33)</td>
<td>21.00±1.08*</td>
<td>(-16.37)</td>
</tr>
<tr>
<td></td>
<td>48-hr</td>
<td>25.11±1.44</td>
<td>17.35±1.27*</td>
<td>(-30.90)</td>
<td>19.76±1.08*</td>
<td>(-21.31)</td>
</tr>
<tr>
<td></td>
<td>72-hr</td>
<td>25.11±1.06</td>
<td>16.67±1.12*</td>
<td>(-33.61)</td>
<td>18.65±1.47*</td>
<td>(-25.73)</td>
</tr>
<tr>
<td></td>
<td>96-hr</td>
<td>25.11±1.02</td>
<td>15.00±1.05*</td>
<td>(-40.26)</td>
<td>17.32±1.22*</td>
<td>(-31.02)</td>
</tr>
<tr>
<td>WBCs (10^3/µL)</td>
<td>24-hr</td>
<td>29.98±1.54</td>
<td>39.98±1.39*</td>
<td>(+33.36)</td>
<td>35.54±1.40*</td>
<td>(+18.55)</td>
</tr>
<tr>
<td></td>
<td>48-hr</td>
<td>29.16±1.48</td>
<td>41.78±1.77*</td>
<td>(+43.28)</td>
<td>37.55±1.32*</td>
<td>(+28.77)</td>
</tr>
<tr>
<td></td>
<td>72-hr</td>
<td>29.67±1.60</td>
<td>45.12±1.31*</td>
<td>(+52.07)</td>
<td>39.67±1.50*</td>
<td>(+33.70)</td>
</tr>
<tr>
<td></td>
<td>96-hr</td>
<td>29.67±1.97</td>
<td>48.26±1.98*</td>
<td>(+62.66)</td>
<td>42.59±1.56*</td>
<td>(+43.55)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>24-hr</td>
<td>104.44±1.72</td>
<td>113.67±28.71*</td>
<td>(+8.84)</td>
<td>109.26±19.28</td>
<td>(+4.62)</td>
</tr>
<tr>
<td></td>
<td>48-hr</td>
<td>105.30±1.43</td>
<td>116.83±31.87*</td>
<td>(+10.95)</td>
<td>113.92±18.82</td>
<td>(+8.19)</td>
</tr>
<tr>
<td></td>
<td>72-hr</td>
<td>106.45±20.99</td>
<td>120.33±40.67*</td>
<td>(+13.04)</td>
<td>116.81±31.53*</td>
<td>(+9.73)</td>
</tr>
<tr>
<td></td>
<td>96-hr</td>
<td>106.43±20.34</td>
<td>122.13±39.41*</td>
<td>(+14.75)</td>
<td>120.90±21.78*</td>
<td>(+13.60)</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>24-hr</td>
<td>23.36±2.19</td>
<td>27.42±4.27*</td>
<td>(+17.38)</td>
<td>25.79±2.32*</td>
<td>(+10.40)</td>
</tr>
<tr>
<td></td>
<td>48-hr</td>
<td>23.54±2.78</td>
<td>29.42±4.32*</td>
<td>(+24.98)</td>
<td>28.00±1.13*</td>
<td>(+18.95)</td>
</tr>
<tr>
<td></td>
<td>72-hr</td>
<td>23.66±3.28</td>
<td>30.08±3.81*</td>
<td>(+27.13)</td>
<td>29.08±2.8*</td>
<td>(+22.91)</td>
</tr>
<tr>
<td></td>
<td>96-hr</td>
<td>23.65±3.60</td>
<td>31.64±2.16*</td>
<td>(+33.78)</td>
<td>32.05±4.6*</td>
<td>(+35.52)</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>24-hr</td>
<td>22.41±1.76</td>
<td>24.48±2.27*</td>
<td>(+9.24)</td>
<td>23.86±2.20*</td>
<td>(+6.47)</td>
</tr>
<tr>
<td></td>
<td>48-hr</td>
<td>22.40±1.46</td>
<td>25.88±3.86*</td>
<td>(+15.54)</td>
<td>24.92±4.08*</td>
<td>(+11.25)</td>
</tr>
<tr>
<td></td>
<td>72-hr</td>
<td>22.43±1.90</td>
<td>26.16±5.30*</td>
<td>(+16.63)</td>
<td>25.13±1.31*</td>
<td>(+12.04)</td>
</tr>
<tr>
<td></td>
<td>96-hr</td>
<td>22.42±2.22</td>
<td>27.29±6.45*</td>
<td>(+21.72)</td>
<td>26.72±1.79*</td>
<td>(+19.18)</td>
</tr>
</tbody>
</table>

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 (Stoskopf, 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...
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.

REFERENCES


