

TOXIC EFFECTS OF COPPER ON HEMATOLOGICAL AND ENZYMATIC RESPONSES OF FRESH WATER CATFISH *CLARIAS BATRACHUS* (LINN.)

Muneesh kumar^{*1}, Anchal Raj²

1. Department of Zoology Govt. Degree College Doda, University of Jammu, J&K, India.
2. Department of Zoology G. G. M. Science College, Jammu, University of Jammu, J&K, India

ABSTRACT: - The aim of this study was investigating hematological and enzymatic effects of Cu on *C. batrachus*. Freshwater fish *Clarias batrachus* were exposed to sublethal concentrations of copper for short and long terms. Responses of hematological parameters which include red blood cells count (RBC) and hemoglobin content (Hb) were investigated. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were also determined. The aquaria were divided into two groups: the first group was exposed for one week to 2, 4 and 6 mg/L of CuSO₄ which represent 0.3, 0.5 and 0.7 of Cu LC₅₀, respectively with three replicates for each concentration, in addition to 0.00 LC₅₀ as the control; while the second group was exposed to the same concentrations of CuSO₄ with the same replicates for 4 weeks. The results of hematological parameters indicated that RBC and HB were significantly increased with increasing the concentration of CuSO₄ sequentially for both short and long exposure periods. The enzymatic estimation showed that the activity of AST and ALT also significantly increased sequentially in copper treated fish at both exposure periods. Results of the present study suggest that sublethal concentrations of copper affect the hematological changes and impaired liver functions. These parameters can be useful in environmental biomonitoring of copper contamination.

KEYWORDS: *Clarias batrachus*, liver enzymes, hematological parameters, LC₅₀ of copper.

INTRODUCTION

The aquatic environment is continuously being contaminated with chemicals from agriculture and urban activities. In many aquatic systems, metal concentrations are elevated over natural background levels due to a continuous release of metals from industrial and agricultural sources. Frequently, metals are present as mixtures in the environment because of their concomitant release from mining activities or industrial uses. Copper become toxic when elevated concentrations are introduced into the environment (Marr *et al.*, 1996; Karan *et al.*, 1998). Studies on the toxicity of metals for

fish have been focused on the effects of short term exposures to single metal at relatively high concentrations; rather than investigating the toxic impact of long-term exposures to metal mixtures at environmentally realistic concentrations. Under conditions of acute, high-dose metal exposure, the maintenance of branchial osmoregulation and gas exchange is of prime importance for the survival of the fish; whereas under conditions of sublethal, chronic metal intoxication, the adaptive capacity of internal metal accumulating organs such as the liver may gain importance (McDonald and Wood, 1993; Schlenk *et al.*, 1999; Stubblefield *et al.*, 1999). Several biochemical and physiological responses can occur when a toxicant is absorbed by aquatic organisms which may be a compensatory response or a toxicity mechanism (Begum, 2004). Chronic exposure of fish to ions, Cu, Cd or Zn, has been shown to cause a variety of behavioral, biochemical and physiological changes including loss of appetite, reduced growth, decreased aerobic scope and mortality (McGeer *et al.*, 2000; Sloman *et al.*, 2003).

Hematological parameters are used as an index to detect physiological changes and to assess structural and functional status of health during stress conditions in a number of fish species (Adhikari *et al.*, 2004; Suvetha *et al.*, 2010). Fish blood is sensitive to pollution-induced stress, and changes on the hematological parameters, such as hemoglobin content, hematocrit and number of erythrocytes can be used to monitor stress caused by pollutants such as heavy metals (Romani *et al.*, 2003; Barcellos *et al.*, 2004). Enzymes activity are considered as sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water for the presence of toxicants (El-Demerdash and Elagamy, 1999; Gul *et al.*, 2004). Estimation of enzymes like aspartate and alanine aminotransferase (AST, ALT) and lactate dehydrogenase (LDH) are considered useful biomarkers to determine pollution level during chronic exposure (Asztalos *et al.*, 1990; Basaglia, 2000; Ozman *et al.*, 2006).

MATERIALS AND METHODS

Experimental fish

Adult and live fish *Clarias batrachus* were collected from the fish farm Patra and Bhadbhada Bhopal M.P.) brought to the laboratory, cleaned by using 0.1% KMnO₄ to avoid dermal infection. Only healthy fishes (Length: 12-15cm, Weight: 50-60g) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h.

Estimation of Cu LC₅₀

The 96 h LC₅₀ values for various fishes depending on the environmental conditions, in case of copper, as a rule, vary from 0.07 to 21.5 mg/L (Scott and Sloman, 2004). To determine the 96 h LC₅₀ of exposure, seventy fish were randomly distributed in seven small aquaria (40 L). The fish were exposed to various concentrations (2, 4, 6, 8, 10, 12 and 14 mg/L) of copper sulphate (CuSO₄) which were previously dissolved in distilled water and added to the aquaria. Fish were exposed to the aforementioned concentrations for 96 h. Mortality in each aquarium were removed and recorded daily. To find out the survival time in each concentration of CuSO₄, observations were recorded and then the LC₅₀ value was calculated from the regression line drawn according to Finney (1964). The 96h LC₅₀ was 8 mg /L.

Experimental design

Two hundred and forty acclimated fish were divided into two groups; the first group was exposed to 2, 4 and 6 mg/L of CuSO₄ which represent 0.3, 0.5 and 0.7 of 96 h Cu LC₅₀, respectively for one week (short exposure period) in addition to the aquaria at the same conditions without CuSO₄ (0.00 LC₅₀) as control. Eighty litre glass aquaria (100 × 50 × 40 cm) were used with three replicates for each concentration. The second group was exposed to the same concentrations of CuSO₄ with the same replicates for 4 weeks (long exposure period). Fish were fed twice daily at a rate of 2% of the body weight with 32% crude protein diet. Temperature was maintained at 26 ± 1°C, and the water quality parameters were monitored biweekly and were kept within the optimal ranges as described earlier by Ali *et al.* (2008). At the end of the period for the two groups, fish blood samples were collected from the caudal vein by sterile syringe containing EDTA solution as anticoagulant. These blood samples were divided into two parts, the first part was used for determining the count of red blood cells (RBC) method of Dacie and Lewis (1984), and hemoglobin content (Hb) method described by Van Kampen and Zijlstra (1961). The second part of the blood samples was used to estimate the plasma AST and ALT activity using a colorimetric method of Reitman and Frankel (1957).

RESULTS

Short term exposure

Hematological parameters of the fish exposed to Cu for one week were significantly different compared to the control group (Table 1). There were significant increase ($p < 0.05$) in both red blood cells count (RBC) and hemoglobin content (Hb) for fish exposed to 0.3, 0.5 and 0.7 of LC₅₀, respectively. According to the results of the enzymes activity estimation, AST and ALT activities of the control *Clarias batrachus* were 18.62 ± 0.87 and 14.36 ± 0.51 IU/L, respectively. Exposure of fish to Cu for one week resulted to increased AST and ALT activity (Table 1). The average levels of AST activities of Cu treated fish, 0.3, 0.5 and 0.7, were 28.14 ± 0.56, 28.43 ± 0.67, and 32.95 ± 0.71 IU/L, respectively which were significantly higher in relation to the average level of the control group. Similarly, the ALT activity was significantly increased compared to untreated fish, and the average levels of ALT activities of individuals exposed to Cu 0.3, 0.5 and 0.7 of LC₅₀ were 21.70 ± 0.42, 22.16 ± 0.39, and 26.81 ± 0.54 IU/L, respectively. However, there were significant differences among treatments of AST and ALT for fish exposed to 0.3 and 0.5 compared to fish exposed to 0.7 of LC₅₀ (Table 1).

Long term exposure

Hematological and liver enzymatic parameters for *Clarias batrachus* exposed to Cu for four weeks are shown in Table 2. There was significant increase ($p < 0.05$) in RBC compared with the control group. The results obtained were 1.72 ± 0.06, 2.37 ± 0.08, 2.50 ± 0.10 and 2.93 ± 0.11 cell/μl for the control group, and fish exposed to 0.3, 0.5 and 0.7 of LC₅₀, respectively. In the same trend, hemoglobin content also increased significantly ($p < 0.05$) after fish exposed to long term copper sulphate concentrations. The results of the enzyme activity determinations, AST activity of control, *Clarias batrachus* were 18.55 ± 0.91; whereas, the average levels of Cu treated fish, 0.3, 0.5 and 0.7 of LC₅₀, were 31.64 ± 0.49, 35.22 ± 0.81, and 40.19 ± 0.71 IU/L, respectively which is significantly higher compared to the control group.

Similarly, the average levels of ALT activities of individuals exposed to Cu 0.3, 0.5 and 0.7 of LC₅₀ were 25.32 ± 0.57, 29.67 ± 0.38, and 33.95 ± 0.73 IU/L, respectively which also showed significant increase compared to untreated fish (14.43 ± 0.51 IU/L).

TABLE 1. Change in the hematological parameters red blood cell (RBC), hemoglobin (Hb) and serum enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT) of *Clarias batrachus* exposed to sublethal concentration of copper sulphate for short term (one week).

Parameter	Control	0.3 LC ₅₀	0.5 LC ₅₀	0.7 LC ₅₀
RBC (cell/ μ l)	1.67 \pm 0.05 ^b	2.30 \pm 0.08 ^a	2.45 \pm 0.09 ^a	2.89 \pm 0.11 ^a
Hb (g/L)	82.13 \pm 0.68 ^b	97.95 \pm 0.79 ^a	100.30 \pm 0.84 ^a	103.41 \pm 0.83 ^a
AST(IU/L)	18.62 \pm 0.87 ^c	28.14 \pm 0.56 ^b	28.43 \pm 0.67 ^b	32.95 \pm 0.71 ^a
ALT(IU/L)	14.36 \pm 0.51 ^c	21.70 \pm 0.42 ^b	22.16 \pm 0.39 ^b	26.81 \pm 0.54 ^a

Means with the same superscript in the same row are not significantly different at ($p < 0.05$).

TABLE 2. Change in the hematological parameters red blood cell (RBC), hemoglobin (Hb) and serum enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT) of *Clarias batrachus* exposed to sublethal concentration of copper sulphate for long term (four weeks).

Parameter	Control	0.3 LC ₅₀	0.5 LC ₅₀	0.7 LC ₅₀
RBC (cell/ μ l)	1.72 \pm 0.06 ^b	2.37 \pm 0.08 ^a	2.50 \pm 0.10 ^a	2.93 \pm 0.11 ^a
Hb (g/L)	83.21 \pm 0.69 ^b	98.97 \pm 0.76 ^a	101.38 \pm 0.77 ^a	105.45 \pm 0.89 ^a
AST(IU/L)	18.55 \pm 0.91 ^d	31.64 \pm 0.49 ^c	35.22 \pm 0.81 ^b	40.19 \pm 0.71 ^a
ALT(IU/L)	14.43 \pm 0.51 ^d	25.32 \pm 0.57 ^c	29.67 \pm 0.38 ^b	33.95 \pm 0.73 ^a

Means with the same superscript in the same row are not significantly different at ($p < 0.05$).

DISCUSSION:

The effect of pollution on the blood picture of fishes the study reported that there was significant increase in RBC count and Hb content with increase in the concentration of copper in both short and long term exposure. These results are in agreement with the findings of Lavanya et al., (2011) who stated that there was significant increase in Hb and hematocrit (Hct) content of arsenic trioxide treated Indian major carp Catla catla compared to the control group. The significant increase in these hematological parameters indicated impaired respiratory capacity of the fish due to damage in the gill caused by the toxicant (Lavanya et al., 2011). The increases in the number of red blood cells, due to the short and long term sublethal exposure to copper can be attributed to several factors. During the exposure of *Clarias batrachus* to copper, it seemed that the fish developed an oxygen deficiency. This decrease in the availability of dissolved oxygen causes the build-up of oxygen debt, hypoxia in the fish. Fish also compensate for a poor oxygen intake in prevailing hypoxic conditions, which may be caused by epithelial lifting of the gill lamellae, by an increase in the number of red blood cells (Wepener et al., 1992a, b).

O'Connor and Fromm (1975) concluded that metal ions are known to stimulate erythropoiesis. Short and long term sublethal exposure to copper induced increases in red blood cells, hemoglobin and haematocrit values, and reflects an attempt by *Clarias batrachus* to survive in an environment with an increased demand for oxygen, resulting from the destruction of gill membranes, causing an impaired gaseous exchange (Buckley, 1976). The significant increase in the hemoglobin content in copper treated fish was accompanied by an increase in the number of red blood cells. Fish were subjected to an oxygen tension (due to internal haemorrhage) and the increase in hemoglobin could well have been to elevate the oxygen capacity of the blood in order to supply more oxygen to the tissues. This is, therefore, a mechanism by which the body attempts to absorb more oxygen from the surrounding medium to meet the increased oxygen demand (Cyriac et al., 1989). During long term exposure, toxicants may accumulate in various tissues which lead to changes in the enzymatic activities. In fish, the liver act as an important organ for uptake, accumulation, biotransformation and excretion of toxicant during sublethal treatment (Maher et al., 1999;

Pedlar and Klaverkamp, 2002). The liver function tests like analysis of serum, AST and ALT, are widely used to demonstrate liver function or toxicant-induced hepatotoxicity (Yang and Chen, 2003; Roy and Bhattacharya, 2005; Datta et al., 2007). In the present study, the significant increase in AST and ALT levels in copper treated fish with short and long term sublethal exposure indicates hepatic damage due to copper accumulation which in turn releases these enzymes into the bloodstream. The damage and severity of the organ is dependent on the type of toxicant and duration of exposure (Casillas et al., 1983; Jacobson-Kram and Keller, 2001). Roy and Bhattacharya (2005) noted significant changes in serum GOT (AST) and GPT (ALT) in *Channa punctatus* exposed to As_2O_3 , and indicated that the changes may be due to histopathological lesions in liver. Therefore, Abdel-Warith et al. (2011) reported that Nile tilapia exposed to zinc cause histopathological injury in the liver. Aspartate aminotransferase (AST) catalyzes an important reaction of the molecular rearrangement involving amino acids linked to the citric acid cycle at two points (oxaloacetic and ketoglutaric acids), being the most important mechanism for introducing reduction equivalents into mitochondria (Urich, 1994). Alanine aminotransferase (ALT) predominates in organs with intensive glycogenesis, such as the liver (Urich, 1994; Torre et al., 2000). Serum AST and ALT are important diagnostic tools in medicine and clinics, and are used to detect the toxic effects of various pollutants (Nelson and Cox, 2000).

The effects of heavy metals on serum or liver enzyme activities including AST and ALT activities of several teleost species have recently been studied (Vaglio and Landriscina, 1999; Torre et al., 2000; Kim and Kang, 2004). After sub-chronic dietary, Cu exposure for 40 days increased serum AST and ALT concentrations with increasing time and dose were observed in the rockfish, *Sebastes schlegeli* (Kim and Kang, 2004). The authors suggest that the liver is rich in AST and ALT, therefore damage to it can result in the liberation of large quantities of these enzymes into the blood. Therefore, increases in AST and ALT activities in the serum of heavy metal treated fish are assumed to be a result of liver damage by the heavy metals. These findings were in agreement with the results in the current study which found that the activity of AST and ALT increased with the increase of copper sulphate concentration for both short and long term exposure of fish. In addition, it was also reported in another teleost (*Sparus aurata*) that Cd exposure may decrease AST and ALT activities in liver cells (Vaglio and Landriscina, 1999). It is very interesting that Cu, Cd and Zn showed different effects on the AST and ALT activities (Wu et al., 2008).

CONCLUSION

The results of our study demonstrated that a short and long term exposure of *Clarias batrachus* to sublethal concentrations of copper could have hematological changes and impaired liver functions. It was evident that the increase in RBC, hemoglobin, AST and ALT related to the increase of exposure period and concentration of copper sulphate sequentially. It can therefore be concluded that hematological parameters and the activity of liver enzymes of exposed fish allow the blood and liver of *Clarias batrachus* to be used as a biomarker of prior exposure to copper. Hematological and liver function changes were mainly observed in fish exposed over the short-term exposure periods while regenerative responses were noted in fish exposed over the long-term period.

ACKNOWLEDGEMENT

Authors are beholden to Principal Dr. A.K. Ganguly S.L.L. Jain P.G. College Vidisha, Bhopal M.P. India for providing laboratory facilities for this research work. This paper forms the part of Ph.D. thesis submitted by author to Barkatullah University, Bhopal M.P. (India).

REFERENCES

1. Abdel-Warith AA, Younis EM, Al-Asgah NA, Wahbi OM 2011. Effect of zinc toxicity on liver histology of Nile tilapia, *Oreochromis niloticus*. *Sci. Res. Essays*, 6(17): 3760-3769.
2. Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S 2004. Effects of cypermethrin and carbofuran haematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). *Ecotoxicol. Environ. Saf.* 58: 220-226.
3. Ali A, Al-Ogaily SM, Al-Asgah NA, Goddard JS, Ahmed SI 2008. Effect of feeding different protein to energy (P/E) ratios on the growth performance and body composition of *Clarias batrachus* fingerlings. *J. Appl. Ichthyol.* 24: 31-37.
4. Asztalos B, Nemcsok J, Benedeczky I, Gabriel R, Szabo A, Refaie OJ 1990. The effects of pesticides on some biochemical parameters of common carp *Cyprinus carpio*. *Arch. Environ. Contamin. Toxicol.* 19:275-282.
5. Barcellos LJG, Kreutz LC, de Souza C, Rodrigues LB, Fioreze I, Quevedo RM, Cericato L, Soso AB, Fagundes M, Conrad J, Lacerda LA, Terra S 2004. Hematological changes in *Jundia* (*Rhamdia quelen* Quoy and Gaimard Pimelodidae) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. *Aquaculture*, 237: 229-236.
6. Basaglia F 2000. Isozyme distribution of ten enzymes and their loci in South American lung fish,

- Lepidosiren paradoxa (Osteichthyes, Dipnoi). Comp. Biochem. Physiol. B 126: 503-510.
7. Begum G 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the *Clarias batrachus* (linn) and recovery response. *Aquat. Toxicol.* 66: 83-92.
 8. Buckley JA 1976. Heinz bodies anemia in coho salmon (*Clarias batrachus*) exposed to chlorinated waste water. *J. Fish. Res. Bd. Can.* 34: 215-224.
 9. Casillas E, Meyers M, Ames W 1983. Relationship of serum chemistry values to liver and kidney histopathology in English sole (*Parophrys vetulus*) after acute exposure to carbon tetrachloride. *Aquat. Toxicol.* 3: 61-78.
 10. Cyriac PJ, Antony A, Nambison PNK 1989. Hemoglobin and hematocrit values in the fish *Oreochromis mossambicus* (Peters) after short term exposure to copper and mercury. *Bull. Environ. Contamin. Toxicol.* 43: 315-320.
 11. Dacie JV, Lewis SM 1984. *Practical Hematology* 6th edition. Churchill.
 12. Datta S, Saha DR, Ghosh D, Majumdar T, Bhattacharya S, Mazumder S 2007. Sub-lethal concentration of arsenic interferes with the proliferation of hepatocytes and induces in vivo apoptosis in *Clarias batrachus* L. *Comp. Biochem. Physiol. C* 145: 339-349.
 13. *Ecophysiol.*, Chapman Hall, London, pp. 297-315.
 14. El-Demerdash FM, Elagamy EI 1999. Biological effects in *Tilapia nilotica* fish as indicators of pollution by cadmium and mercury. *Int. J. Environ. Health Res.* 9: 173-186.
 15. Finney DJ 1964. Probit analysis: A statistical treatment of the sigmoid response curve, pp. 20-31.
 16. Gül S, Belge-Kurutas E, Yıldız E, Sahan A, Doran F 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake, Turkey. *Environ. Int.* 30: 605-609.
 17. Jacobson-Kram D, Keller KA 2001. *Toxicology Testing Handbook*. Marcel Decker, New York.
 18. Karan V, Vitorovic S, Tutundzic V, Poleksic V 1998. Functional enzymes activity and gill histology of carp after copper sulphate exposure and recovery. *Ecotoxicol. Environ. Saf.* 40: 49-55.
 19. Kim SG, Kang JC 2004. Effect of dietary copper exposure on accumulation, growth and hematological parameters of the juvenile rockfish, *Sebastes schlegelii*. *Mar. Environ. Res.* 58: 65-82.
 20. Lavanya S, Ramesh M, Kavitha C, Malarvizhi A 2011. Hematological, biochemical and ionoregulatory responses of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. *Chemosphere*, 82: 977-985.
 21. Maher W, Goessler W, Kirby J, Raber G 1999. Arsenic concentrations and speciation in the tissues and blood of sea mullet *Mugil cephalus* from Lake Macquarie NSW. *Aust. Mar. Chem.* 68: 169-182.
 22. Marr JCA, Lipton J, Cacela D, Hansen JA, Bergman HL, Meyer JS, Hogstrand C 1996. Relationship between copper exposure duration, tissue copper concentration, and rainbow trout growth. *Aquat. Toxicol.* 36: 17-30.
 23. McDonald DG, Wood CM 1993. Branchial mechanisms of acclimation to metals in freshwater fish. In: Rankin JC, Jensen FB (Eds.). *Fish*
 24. McGeer JC, Szebedinszky C, McDonald DG, Wood CM 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout. 1: Ionoregulatory disturbance and metabolic costs. *Aquat. Toxicol.* 50: 231-243.
 25. Nelson DL, Cox MM 2000. *Lehninger Principles of Biochemistry*. Worth Publishers, New York, pp. 623-658. O'Connor DV, Fromm PO 1975. The effect of methyl mercury on gill metabolism and blood parameters of rainbow trout. *Bull. Environ. Contamin. Toxicol.* 13(4): 406-411.
 26. Ozman M, Gungordu A, Kucukbay FZ, Guler RE 2006. Monitoring the effects of water pollution on *Cyprinus carpio* in Karakaya Dam Lake, Turkey. *Ecotoxicol.* 15: 157-169.
 27. Pedlar RM, Klaverkamp JF 2002. Accumulation and distribution of dietary arsenic in Lake Whitefish (*Coregonus clupeaformis*). *Aquat. Toxicol.* 57: 153-166.
 28. Reitmen S, Frankel S 1957. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28: 56-63.
 29. Romani R, Antognelli C, Baldracchini F, De Santis A, Isani G, Giovannini E, Rosi G 2003. Increased acetylcholinesterase activities in specimens of *Sparus auratus* exposed to sublethal copper concentrations. *Chemico-Biological Interactions*, 145: 321- 329.
 30. Roy S, Bhattacharya S 2005. Arsenic induced histopathology and synthesis of stress proteins in liver and kidney of *Channa punctatus*. *Ecotoxicol. Environ. Saf.* 65: 218-229.
 31. Schlenk D, Davis KB, Griffin BR 1999. Relationship between expression of hepatic metallothionein and sublethal stress in channel catfish following acute exposure to copper sulphate. *Aquaculture*, 177: 367-379.