Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
2(4):1387-1391
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

Alteration in some haematobiochemicl parameters of *Oreochromis mossambicus* under the stress of acute copper toxicity

Karunakar Baghel[1*], Rajesh Behera[2] and Milan Kumar Behera[3]

[1,3] *Fisheries and Toxicology Research Laboratory, P.G. Department of Zoology; G.M. (Autonomous) College, Sambalpur-768004, INDIA*
[2] *Department of Zoology, Stewart Science College, Cuttack-753001, INDIA.*

E-mail: kkbaghel.baghel@gmail.com

ABSTRACT

Alterations in some haemato – biochemical parameters of *Oreochromis mossambicus* (Peters) was studied under the stress of different concentrations of Copper (5, 10, 15 and 20ppm) for 30 days. Copper as $Cu(NO_3)^2$ was used to prepare the stock solution. The RBC, haemoglobin percentage, PCV, MCH, MCV and MCHC significantly decreased with an increase in the concentration of copper, except that the RBC, haemoglobin and PCV exhibited an increase in 5ppm and MCHC showed an increase in 5 and 10ppm copper concentration. Whereas the WBC count increased significantly with an increase in the concentration of copper. The plasma protein level decreased significantly, whereas the cholesterol, triglyceride, glucose and VLDL levels increased significantly with an increase in the concentration of copper, which indicates a stressful condition in the fish.

Keywords : *Copper, acute toxicity, hamato-biochemical parameters, Oreochromis mossambicus*.

INTRODUCTION

At least 11 heavy metals are known to be essential for living organisms. Biologically copper is an essential micronutrient and act as a metal activator in many enzymes, such as, tyrosinase, ascorbic acid oxidase etc. Copper though essential in diet can be harmful when large single or daily intake occurs. Metals usually function in a combination with proteins, either as tightly bound metalloproteins or more loosely bound metal-protein complex. Copper compounds are used for prophylactic purposes to control fish diseases and parasites. Copper compounds are also used to control algae, kill slugs and snails in irrigation water systems and municipal water treatment systems.

Metallic copper causes no undesirable changes in fish. However, its salts (chlorides and nitrates) are toxic even at a concentration of 0.01 to 0.02mg/1. At low concentration copper is an essential element for both plants and animals. It carries oxygen (haemocyanin) in the crustaceans like lobster and shrimps [(1)]. Higher concentration of copper have however been introduced into the environment due to anthropogenic activities, such as, mining, electroplating, paint and pigment industries, textile factory effluents and pesticides. The elevated level of copper may become acutely or chronically toxic to aquatic life. The harmful effects increase with an increase in the concentration as well as the physical and chemical conditions of the water**.** Carvalho and Fernandes [(2)] have reported that temperature has a profound effect on the toxicity of copper in the Neotropical fish, *Prochilodus scrofa*.

Haematological studies are essential as it plays an important role to monitor fish health, pollution load, stress and disease. Hence this piece of investigation was undertaken to find out the toxic effects of copper on some hamato-biochemical parameters of *O*reochromis *mossambicus.*

MATERIALS AND METHODS

Healthy specimens of *Oreochromis mossambicus* were procured from the local fish farm. The fishes were washed with 0.1% $KMnO_4$ and then acclimatized to laboratory condition for 15 days. The fishes were fed with rice bran and oil cake (1:1) during the entire period of this experiment. The water of the test medium was changed every alternate day. The physico-chemical characteristics of the water used was analyzed according to APHA [3].

The experimental fishes (length 12-15cm and weight 30-35g) were divided into several batches of 20 each, irrespective of their sex. Four batches of fishes were subjected to acute doses of 5, 10, 15 and 20ppm copper concentration in separate glass aquaria, for 30 days. At the end of 30 days the fishes were sacrificed and their blood was processed for the analysis of different parameters. The alterations in different blood parameters after exposure from that of the control fishes were subjected to paired 't' test[9] to ascertain, whether the alterations were significant or not. Controls were maintained throughout the course of this investigation. Results so obtained have been depicted in the Table No.1.

Blood was collected directly from the heart with the help of heparinised syringe and stored in 2 vials, one coated with EDTA and the other uncoated. The blood from EDTA coated vial was used for estimation of hemoglobin, R.B.C and W.B.C. counts as per the methods of Shah and Altindag [4,5]. The PCV was calculated by microhaematocrit or Wintrobe's tube method [6]. The mean hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and mean cell volume (MCV) are absolute values and were calculated from the RBC, PVC and Hb values as described by Jain [7].

The bio-chemical parameters like protein, cholesterol, triglyceride and VLDL were estimated using the instrument (analyser) Kodak ektachem DT System [8] . This system uses dry chemistry technology and dry chemical layer coated slides. All required reactions take place within the slide. The test results are automatically printed by a integral printer. Plasma glucose was estimated by O-Toluidine method of Cooper and Mc Daniel [9].

RESULTS AND DISCUSSION

The TEC, haemoglobin percentage and PCV showed an increase at 5ppm concentration and thereafter exhibited a steady and significant decrease on exposure to 10, 15 and 20ppm concentration of copper. Similar results have been obtained in lead treated *Clarias gariepinus*[10]; zinc treated *Clarias gariepinus*[11] and mercury treated *Clarias batrachuss*[12]. In contrast to the above findings Ishikawa *et.al*. [13] and Alwan *et.al*, [14] observed an increase in TEC, Hb% and PCV of fishes exposed to mercury and aluminum respectively. Information exists that the TEC, Hb% and PCV decreased in different fishes treated with atrazine (herbicide) [15] and monocrotophos (pesticide)[16] . This decrease in the blood parameters might be due to haemolysis of the blood forming cells, which fail to absorb required nutrients for normal erythropoisis.

The TLC exhibited a gradual and significant increase with an increase in the concentration of copper. Several workers have reported a similar increase in the TLC of fishes treated with zinc [11], lead [10], mercury [12], atrazine[15] and monocrotophos[16]. Ishikawa *et.al.*[13] did not notice any change in TLC of *Oreochromis niloticus* treated with a sub-lethal concentration of mercury. This increase in TLC may be due to tissue damage and subsequent removal of debris formed. The increase in TLC under different metallic stress, however, has been considered as an adaptation to meet the stressful condition.

The MCH and MCV decrease steadily with an increase in the concentration of copper. Vutukuru[17] has reported a decrease in MCH in *Labeo rohita* treated with chromium, whereas Alwan *et.al*[14] have reported that the MCH increase with an increase in aluminum concentration in *Tilapia zilli*. Adeyemo[10] also reported an increase in MCV in *Clarias gariepinus* treated with lead. The MCHC increased in 5 and 10ppm copper concentrations but decrease significantly in 15 and 20ppm copper concentrations. Alwan *et.al.*[14] have reported an increase in MCHC in Tilapia zilli treated with aluminium. Adeyemo[10] also reported increase in MCHC in *Clarias gariepinus* treated with lead. These alterations in MCH, MCHC and MCV may be due to effect of copper on the haemopoitic organs of the fish [18].

The serum protein content showed a gradual but significant decrease with an increase in the concentration of copper. Vutukuru[17] has also reported similar decrease in the serum protein in *Labeo rohita* exposed to chromium. Various workers, while working on the toxicity of mercury [12], lead [19], and zinc [11] on fish also noted decrease in protein. This decrease in serum protein may be correlated to the malfunction of the liver and reduce protein synthesis or protein breakdown.

The serum cholesterol, triglyceride and VLDL exhibited a significant increase with an increase in the concentration of copper Similar results were also obtained when the fishes were treated with lead [19], zinc [11] etc.

Parameters	Control	5 ppm	10 ppm	15 ppm	20 ppm
RBC (10^6 × mm^3)	3.696 ± 0.28	3.919 ± 0.18	3.061 ± 0.215	2.98 ±0 .106	2.696 ± 0.262
% Change		+ 6.0 [b]	-17.18 [a]	-19.37 [a]	-27.05 [a]
WBC (10^3 ×mm^3)	11.16 + 0.11	11.38 ± 0.17	11.59 ± 0.13	12.17 ± 0.26	14.11 ± 0.26
% Change		+ 1.96 [NS]	+3.91 [c]	+9.05 [a]	+ 26.44 [a]
Haemoglobin (%)	8.84 + 0.75	9021 ± 0.67	7.26 ± 0.55	6.69 ± 0.34	6.24 ± 0.41
% Change		+ 4.1 [b]	-17.94 [a]	-24.38 [a]	-29.46 [a]
PCV value	38.14 + 1.64	38.69 ± 1.41	35.54 ± 0.69	34.89 ± 0.53	34.41 ± 0.54
% Change		+ 1.43 [NS]	-6.81 [a]	-8.52 [a]	-9.79 [a]
MCH (pg)	23.92 + 0.86	23.76 ± 0.72	23.74 ± 0.68	22.44 ± 0.58	23.13 ± 0.64
% Change		-1.78 [NS]	-0.75 [NS]	-6.18 [a]	-3.30 [c]
MCHC (%)	23.24 + 0.58	23.76 ± 0.46	24.16 ± 0.64	19.22 ± 0.64	18.12 ± 0.38
% Change		+ 2.23 [c]	+ 3.96 [c]	-17.30 [a]	-22.03 [a]
MCV (δm3)	131.93 + 3.72	98.76 ± 2.48	116.16 ± 3.28	117.41 ± 3.12	127.63 ± 3.56
% Change		-25.17 [a]	-11.87 [a]	-17.30 [a]	-3.26 [c]
Serum Protein (g/dl)	7.26 + 0.25	6.86 ± 0.31	6.01 ± 0.28	5.73 ± 0.25	4.87 ± 0.26
% Change		-5.64 [b]	-17.21 [a]	-21.07 [a]	-32.92 [a]
Cholesterol (mg/dl)	53.26 + 1.44	58.14 ± 0.59	59.95 ± 1.19	64.73 ± 0.71	70.23 ±1 .36
% Change		+9.16 [a]	+12.56 [a]	+21.53 [a]	+31.86 [a]
Triglyceride (mg/dl)	33.03 + 0.27	36.07 ± 0.37	40.05 ± 0.44	43.10 ± 0.28	45.06 ± 0.42
% Change		+ 9.23 [a]	+ 21.25 [a]	+ 30.48 [a]	+ 36.42 [a]
Glucose (mg/dl)	96.82 + 2.46	102.12 ± 3.12	105.66 ± 2.96	108.44 ± 3.26	112.52 ±3 .44
% Change		+ 5.48 [b]	+ 9.13 [a]	+ 12.02 [a]	+ 16.22 [a]
VLDL (mg/dl)	6.07 + 0.18	6.87 ± 0.13	7.48 ± 0.15	8.25 ± 0.09	8.97 ± 0.08
% Change		+ 13.17 [a]	+ 23.22 [a]	+ 35.91 [a]	+ 47.77 [a]

Table 1. Changes in some haematological parameters of Oreochromis mossambicus on exposure to various concentration of copper for 30 days. n = 20. NS = Non Significant, a = P < 0.001; b = P <0.01; c = P < 0.05

However Martinez *et.al,*[20] noted a decrease in the cholesterol and triglyceride levels in lead treated fishes on 96 hours exposure. This decrease in cholesterol and triglyceride levels in fishes exposed to 96 hour LC_{50} dose of a metal may be due to hypocholesterolemia. This may also be due to malfunctioning of the liver which esterifies it and excretes a part of it after its conversion into cholic acid and bile.

The serum glucose content increased steadily and significantly with an increase in the concentration of copper. Other workers in this field have also reported similar increase in the serum glucose content in lead treated *Prochilodus lineatus*[20]; toxic heavy metal treated *Cyprinus carpio*[21]and chloropyrifos treated *Cyprinus carpio*[22]. This change in serum glucose level has been suggested as a useful general indicator of stress in fishes.

The results indicate that exposure to heavy metals induced stress reaction in fish. The changes in RBC reflected a transient stress induced osmotic imbalance. The changes in W.B.C. showed that stress reduced the immune potential of the fish. Thus, it seems that metallic stress may result in a considerable increase in susceptibility of fish to infections.

REFERENCES

1. Copperinfo, 2004: "Copper and aquatic life"; International Copper Association, U.S.A.
2. Carvalho, C.S. and Fernandes.M.N. 2006: Effect of temperature on Copper toxicity and haematological responses in Neotropical fish, *Prochilodus scrofa* at a low and high pH; Aquaculture; 251(1): 109-117.
3. APHA, 2005: Standard methods for the examination of water and waste water; 21st. Ed.; Amer. Pub. Health Asson.; Newyork, U.S.A.
4. Shah, S.L. and Altindag A. 2004: Haematological parameters of tench *(Tinca tinca)* after acute and chronic exposure to lethal and sub-lethal mercury treatment; Bull. Environ. Contam. Toxicol.; 73: 911-918.
5. Shah, S.L. and Altindag A 2005: Alteration in the immunological parameters of tench *(Tinca tinca)* after acute and chronic exposure to lethal and sub-lethal treatment of mercury, cadmium and lead; Turk. J. Vet. Anim. Sci.; 29: 1163-1168.
6. Ochei, J. and Kolhatkar A. 2005: "Medical Laboratory Science- Theory and Practical"; Tata Mc Graw Hill Publishing Company, Nev~ Delhi, Pp. 281-283.
7. Jain, N.C. 1986:"Schalm's Veterinary Haematology"; 4th Ed.; Lea and Febiger, Philadelphia, U.S.A.; Pp. 1221.
8. CCIS(R),1994:Computerized Clinical Information System; Denver, CO, Micromedex Inc.
9. Cooper, G.R. and Mc Daniel V. 1970: "Standard methods of Clinical Chemistry"; Pp. 159.
10. Adeyemo, O.K. 2007: Haematological profile of *Clarias gariepinus* (Burchell) exposed to lead; Turk, J. Fish and Aqua. Sci.; 7: 163-169.
11. Ololade, I.A. and Ogini O. 2009: Behavioural and haematological effects of zinc on African catfish, *Clarias gariepinus,* Intl. J. Fish. Aquat.; 1(2) :22-27.
12. Maheswaran R; Devapaul A; Muralidharan S; Velmurugan B and Ignacimuthu S.2008: Haematological studies of a freshwater fish, *Clarias batrachus* (L) exposed to mercuric chloride; Intl. J. Integrative Biol.; 2(1): 49-54.
13. Ishikawa, N.M.; Rajanipaiva M.T.T; Lombardi J.V. and Ferreira C.M.2007: Haematological parameters in Nile tilapia, *Oreochromis niloticus* exposed to sub-lethal concentrations of mercury, Braz. Arch. Biol. Technol.; 50(4): 619-626.
14. Alwan S.F.; Haidi A.A. and. Shokr A.E. 2009: Alteration in haematological parameters of freshwater fish, *Tilapia zilli,* exposed to aluminum; J. Sc. & Its Appln.; 3(1): 12-19.
15. Ramesh, M.; Srinivasan R and Saravanan M.2009: Effect of atrazine (herbicide) on blood parameters of common carp, *cyprinus carpio* (Actinopterygii: Cypriniformes); African J. Environ. Sci. Technol.; 3(12): 453-458.
16. Yaji A.J. and Auta J.2007; Sub-lethal effects of monocrotophus on some haematological indices of African Catfish Clarias gariepinus (Teugels) J.Fish Intl.2 (1)115-11.
17. Vutukuru, S.S. 2005: Acute effect of hexavalent chromium on survival, oxygen consumption, haematological parameters and some biochemical profiles of Indian major carp, *Labeo rohita;* Int. J. Environ. Res. Public Health; 2(3): 456-462.
18. Machalinska, A; Wiszniewska B; Tarasiuk J. and Machalinska D.2002: Morphological effect of sodium fluoride on haemopoitic organs of mice, Fluoride; 35: 231-238.
19 Zaki, M.S.; Moustafa S; Rashad H and Sharaf N. 2008: Assessment of the hazardous effect of lead

Pollution in *Oreochromis niloticus*, including haematological, biochemical and immunological parameters; Amer-Eurasian J. Agric. & Environ. Sci.; 3(1): 91-95.

20. Martinez, C.B.R; Nagae M.Y; Zaia C.T.B and. Zaia D.A.M 2014: Acute morphological and physicological effects of lead in the Neotropical fish, *prochilodus lineatus*; Braz. J. Biol.; 64(4): 797-807.
21. Vinodini, R. and Narayanan M. 2009: The impact of toxic heavy metals on the haematological parameters in common carp, *Cyprinus carpio*, L.; Iran J. Environ. Helth Sci.Enng; 6(1):23- 28.
22. Ramesh M and Saravanan M.2008: Haematological and biochemical responses in freshwater fish, *Cyprinus carpio*, exposed to chloropyrifos; Intl.J.Integr.Biol; 3(1):80-83.

*****