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HAEMATOLOGICAL CHANGES INDUCED BY SHORT - TERM EXPOSURE TO COPPER IN THE INDIAN FRESHWATER FISH, *NOTOPTERUS NOTOPTERUS* (PALLAS)

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ABSTRACT
The normal ranges of blood chemistry and the effect of copper sulfate was studied in Indian fresh water fish, *Notopterus notopterus*. The blood serum biochemical components like, hemoglobin, proteins, urea, urea nitrogen, cholesterol, and glucose levels were determined during the phase of reproductive cycle. Toxic level (LC_{50}) of copper sulfate was found to be 30ppm. Under this concentration (30ppm) after exposure to 96hr the haematological parameters such as glucose, protein, urea, urea nitrogen, cholesterol, creatinine and haemoglobin content were estimated in the blood serum. A significant decrease ($p < 0.001$) in the biochemical profile such as protein, glucose, urea, urea nitrogen and creatinine were observed; where as haemoglobin ($p < 0.001$) and cholesterol ($p < 0.01$) levels in the blood of exposed group was significantly ($p < 0.001$) higher than that of the control group. The results suggest that the reduction in the above biochemical levels of blood serum may be because of stress due to copper concentration.

INTRODUCTION

The health of fish can be affected by environmental factors (stress), nutrition as well as by pathogens. Stress in fish may be induced by various abiotic environmental factors such as changes in water temperature, pH, oxygen concentration and water pollutants including pesticides, insecticides (Meier *et al.,* 1983; Lebelo *et al.,* 2001), petroleum products and heavy metals (Witeska, 1999). Biotic interactions such as predator pressure, parasitic invasions or strong competition with other organisms or among the fish in overcrowded areas and by human activities related to fish rearing and harvesting (manipulation, transport) can also be a source of stress to fish (Witeska, 2005). Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms. Stress also induces changes in blood cell numbers and activities. An increase in red blood cell count and volume, and hemoglobin level usually has been reported in fish subjected to stress (Wendelaar Bonga, 1997). The increase in the number of circulating RBC is thought to be associated with the release from reservoirs and even division of circulating cells in fish subjected to low oxygen tension (Murad and Mustafa, 1988). A decrease in white blood cell count, especially of lymphocytes usually occurs in fish subjected to stress (Ellsaesser and Clem, 1986). Heavy metal toxicity invariably reduces white blood cell count particularly lymphocytes (Witeska, 2005) leading to compromised immune responses in the affected fish. The aim of this study is to investigate the alterations of blood levels of hemoglobin, total plasma proteins, glucose and urea, and other hematological parameters in the fresh water fish *N. notopterus* under exposed and normal conditions.

MATERIALS AND METHODS

Blood sample collection

In the laboratory, approximately 1.5-2.0 mL of blood was collected from caudal vein and was then used for estimation of total protein, glucose, urea, and urea nitrogen

Hemoglobin concentration (Hb)

Hemoglobin was measured using the standard cyanmethemoglobin method described by Baker and Silverton (1976). Blood sample (20µmL) was diluted in 5 mL of the diluent in a test tube and thoroughly mixed using a vortex mixer. The absorbance of the solution was measured by spectrophotometer (Cecil, 2000 Spectrophotometer, UK) after 10 minutes of standing time at a wavelength of 540 nm. The absorbance of cyanmethemoglobin was then used to obtain hemoglobin concentration (in g/dL), using a standard Hb estimation chart.

Total serum proteins (TP)

The total plasma proteins were measured by using the modified Biuret method, end point assay as described by Lawrence (1986).The peptide bonds of proteins reacts with

cupric ions in alkaline solution to form a colored chelate, the absorbance was measured at 578 nm. The biuret reagent contains sodium potassium tartrate, which helps in maintaining stability of this complex at alkaline pH. The absorbance of color was proportional to the concentration of total protein in the sample.

Determination of serum glucose

Glucose was determined by GOD-POD end point and assay method. The hydrogen peroxide so generated oxidises the chromogen system consisting of 4-amino antipyrine and phenolic compound to a red quinoaimine dye.

Urea and urea nitrogen

Urea and urea nitrogen were determined by modified Bertholet method.

Serum creatinine

Serum creatinine determined by alkaline picrate method.

Serum cholesterol

Determination of total cholesterol was carried out by CHOD-PAP method using commercial kit available in the market.

The LC_{50} was determined to be 30 ppm (Fig. 3) (Shingadia and Sakthivel, 2003).

Statistical treatment of the data

Standard deviation and probability test i.e.,'t' test were calculated. The student't' test was carried out to know the levels of significance using the standard formula (Mungikar,2003).

RESULTS

The results obtained from the present investigation clearly shows that the fish *N. notopterus* survived well from 15 to 25ppm for 96hr, when 50% mortality was the criterion designation for determining the lethal concentration.

The survival rate of fishes gradually decreased in all media with an increase in the concentration of copper sulfate. The observed lethal concentration (LC_{50}) for 96 hr was 30 ppm.

A significant decrease in the biochemical profile such as protein, glucose, urea and urea nitrogen is observed as shown in (Table 1 and Fig. 1, 2), where as haemoglobin concentration in the blood of exposed group was significantly higher than that of the control group. And the cholesterol levels are fluctuating. Fishes exposed to 20 ppm showed increased level of cholesterol as compare to control in 25 ppm fishes showed decreased levels as compare to 20 ppm and again there is an increased level of cholesterol in 30 ppm.The cholesterol serve as an indicator of propensity towards coronary heart disease (CHD). The results suggest that the increase in cholesterol concentration in fishes subjected to hypercholesterolemia, and it is associated with an increased risk of coronary heart disease. The reduction in the other biochemical levels of blood serum may be because of stress due to copper concentration. Since copper is one of the mineral element needed for the absorption of iron which may leads to increase in the blood cell count. The addition of red blood cells may cause enhancement in the haemoglobin content after exposure to copper sulfate

DISCUSSION

Dallinger and Kautzky (1985) indicated that the uptake of cadmium, copper and zinc from food could be the main route of metal uptake by rainbow trout *Salmo gairdneri*, although high level of elimination through faeces occurs. The teleost kidney functions primarily as a regulator of body fluid and secondarily as an excretory organ and electrolyte regulator. The freshwater fish is hypertonic to its environment and water continually diffuses across the gills into the blood. The kidney eliminates this excess water and reabsorbs most of the body electrolytes. Since copper is one of the element, which is needed for iron absorption, excess of copper may alter the blood biochemical levels. The gill functions not only as a respiratory organ, but also as an excretory and ion-regulating organ. The observations described here indicated that the normal physiology of the kidney was altered.

Domestic waste water did not increase serum enzymes, but did cause histopathological changes characterized by heavy hyaline droplet degeneration of the kidney tubular cells and progressing to necrosis of the kidney tubules and hepatocytes (Bucher and Hofer, 1990). These histopathologic lesions are similar to our observations of copper treated fish. The decreased urea, urea nitrogen levels seen in copper exposed fish may be an indication of compromised renal function as is observed in mammals.

The results of the present study indicate that the blood urea level may reflect renal function and also effect serum

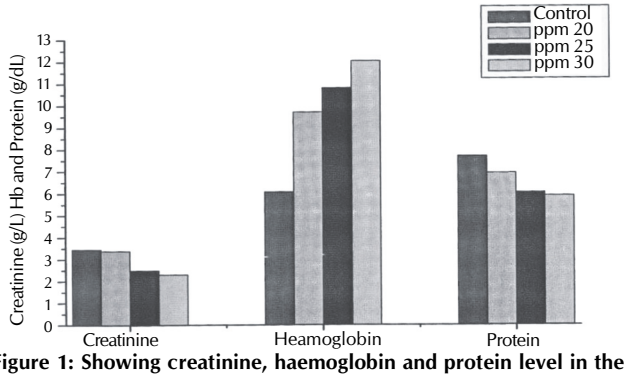

Figure 1: Showing creatinine, haemoglobin and protein level in the fish, *N.notopterus* under different concentrations of Copper sulfate

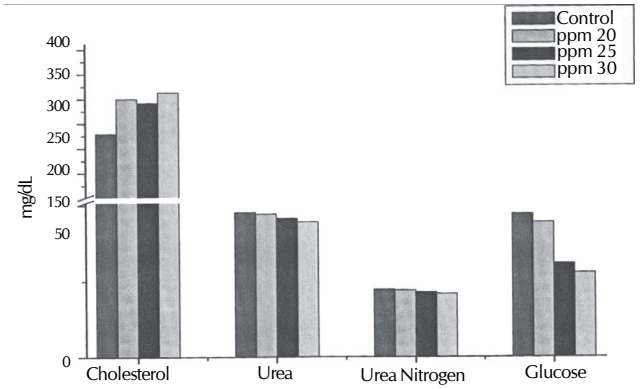

Figure 2: Showing cholesterol, urea, urea nitrogen and glucose level in the fish, *N.notopterus* under different concentrations of copper sulfate

Table 1: Serum biochemical parameters in the fish *N. notopterus* on exposure to copper sulfate different concentrations for 96hrs

Group	Protein	Glucose	Urea	Urea nitrogen	Haemoglobin	Cholesterol	Creatinine
Control	7.70 + 0.20	47.18 ±4.78	47.70 ± 1.54	22.19 ± 0.69	6.07+ 0.2656	229.3 ± 18.40	3.45+ 0.16
20 PPM	6.93 ±1.20**	44.47 ±6.30*	47.14 + 1.65 $^{N\ s'}$	22.00± 0.76$^{N\ 5}$	9.71 ±0.0.79***	299.7 ± 66.49*	3.38±1.27^{NS}
25 PPM	6.05 + 0.31***	30.83 ±5.44***	45.71 ± 2.33**	21.34 + 1.08**	10.80 ± 1.7066***	291.5 ±41.98*	2.49±0.21***
30 PPM	5.90 ±0.55***	27.78 ±4.36 ***	44.51 ±2.60 ***	20.78±1.21***	12.04 ±1.0544***	313.0±80.39**	2.30±0.19***

All the values are in mg/dL; Each value is expressed as mean ± SD, N = 6; NS - Not significant; * - Significant $p < 0.05$; ** - Significant $p < 0.01$; *** - Significant $p < 0.001$; When compared between control and exposed.

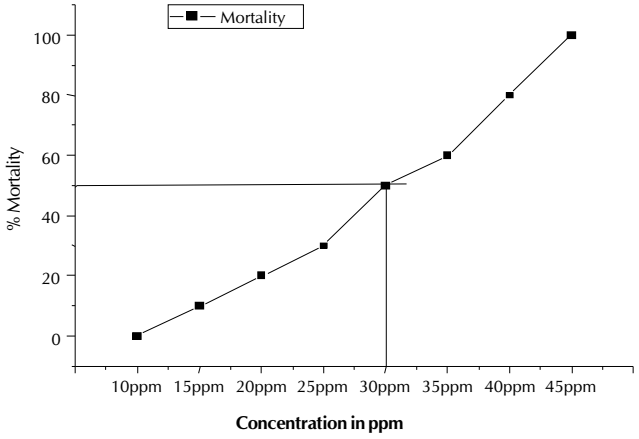

Figure 3: Showing LC_{50} concentration for 96 hour in which 50% of the experimental animals survive

biochemical changes. Diminished levels of serum proteins have been reported in fish under several diseased conditions. (Hunn, 1964 and Mulcahy, 1969). However, with copper exposure a significant reduction in total serum protein was found. A specific alteration in some of the serum proteins was found. (Klontz *et al.,* 1965) Similar to this we found that the serum proteins level was decreased exposed to the concentrations of copper sulfate.

It is reported that the nitrate treated fish had reached a state of exhaustion and were unable to elevate glucose levels of the fish hybrid striped bass *(Morone chrysops X Morone saxatilis).* Although the values were well within the reference interval, and suggested that it is unlikely that the changes seen in the treated fish were solely stress induced (Hrubec *et al.,* 1996). In the present investigation a significant decrease in the glucose level of the fish exposing to copper sulfate has been noticed that the fish is under stress in all the exposures of copper sulfate.

The heavy metal exposure known to induce changes in blood parameters in fish (Heath, 1991). The direct effects of copper on blood parameters are usually associated with increased erythrocytes disintegration or, in the case of more sensitive species, damage of the hemopoietic system (Svobodová *et al*., 1994), such as cell hypertrophy, cell proliferation, and epithelial lifting may represent a defense response, as pointed out by Mallatt (1985), because these changes increase the distance across which copper must diffuse to reach the bloodstream. Since copper is required for hemoglobin synthesis, a mild excess may stimulate erythrocyte formation or release from hemopoietic tissue (Heath, 1995). In our experiment we found that a significant increase in haemoglobin in the blood, increase in the haemoglobin may be because of increase of erythrocytes.

The data presented here support the theory that prolonged exposure to elevated levels of copper sulfate may decrease the immune response, induce hematological and biochemical changes indicative of a pathologic response, and may increase mortality. The pathologic changes are sufficient to affect the normal physiology of the fish and will probably result in decreased growth and increased susceptibility to diseases. The results reported in this study have potential implications for fish in nature.

Still, this approach is a potentially powerful tool for accomplishing the purpose of ecotoxicology; namely investigating the effects of environmental pollution in an ecological context in order to understand the ways in which contaminants alter organizational levels and linkages within ecosystems. It is only with an ecosystem level understanding that we can regulate the discharge of pollutants with a full knowledge of their impacts.

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REFERENCES

Baker, F. J. and Silverton, R. E. 1976. Introduction to Medical Laboratory Technology Butterworth London UK. pp.736.

Bucher, F. and Hofer, R. 1990. Effects of domestic wastewater on serum enzyme activities of brown trout (Salmo trutta). *Comparative Biochemistry and Physiology.* **97C(2):** 381- 385.

Dallinger, D. and Kautzky, H. 1985. The importance of contaminated food for the uptake of heavy metals by rainbow trout *Salmo gairdneri*): a field study. *Oecologia* (Berlin) **67:** 82-89.

Ellsaesser, C. F. and Clem, L. W. 1986. Hematological and immunological changes in channel catfish stressed by handling and transport. *J. Fish Biol.* **28:** 511-521.

Heath, A. G. 1991. Effect of water-borne copper on physiological responses of bluegill (*Lepomis macrochirus*) to acute hypoxic stress and subsequent recovery. *Comp. Biochem. Physiol.* **100(C):** 559-564.

Heath, A. G. 1995. Water Polution and Fish Physiology, 2nd Ed. CRC Press: Boca Raton, FL

Hrubec, T. H., Smith, S. A. and Robertson, J. L. 1996. Blood biochemical reference intervals for sunshine bass (*Morone chasgps x M saxatilis*) in three culture systems. *American J. Veterinary Research.* **57:** 624-627.

Hunn, J. 1964. Some patho-physiologic effects of kidney disease in brook trout. *Proc. Soc. Exp. Biol. Med.* **117:** 383-385.

Klontz, G. W., Yasutake, W. T. and Parisot, T. J. 1965. Virus diseases of Salmonidae in Western U.S. III. Immunopathological Aspects. *Ann. N. Y. Acad. Sci.* **126:** 531-542.

Lawrence, M. S. 1986. Amino acids and Proteins. *In: Textbook of Clinical Chemistry.* Tiezt, N W *(editor)* W B Sounders Company, US

p. 519-618.

Lebelo, S. L., Saunders, D. K. and Crawford, T. G. 2001. Observations on the blood viscosity in striped bass, *Morone saxatilis* (Walbaum) associated with fish hatchery conditions. *Transaction of the Kansas Academy of Science.* **104(3/4):** 183-194.

Mallatt, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Can. J. Fish. Aquat. Sc.* ***42*: 630-**648.

Meier, P. G., Fook, D. C. and Lagler, K. F. 1983. Organochlorine pesticide residues in rice paddies in Malaysia 1981 *Bulletin of Environment Contaminants and Toxicology.* **30:** 351-357.

Mulcahy, M. F. 1969. Serum protein changes in U.D.N. infected atlantic salmon. A possible method of diagnosis. *J. Fish. Biol.* **1:** 333-338.

Mungikar, A. M. 2003. Biostatical analysis, Saraswati Publ. Aurangabad.

Murad, A. and Mustafa, S. 1988. Blood parameters of catfish, *Heteropneustes fossilis* (Bloch), parasitized by metacercariae of *Diplostomulum* sp. *J. Fish Dis.* **11:** 365-368.

Shingadia, H. U. and Sakthivel, V. 2003. Estimation of LC50 values for *Lamellidens marginalis* (Lamarck) with detergent (Wheel) a component of domestic sewage: *J. Aqua Biol.* **18(2):**187-189

Svobodova, Z., Vykusova, B. and Machova, J. 1994. The effects of pollutants on selected haematological and biochemical parameters in fish. *In*: R. Müller and R. Lloyd (Eds.), *Sublethal and Chronic Effects of Pollutants on Freshwater Fish*. Fishing New Books, London.

Wendelaar Bonga, S. E. 1997. The stress response in fish. *Physiological Reviews.* **77(3):** 591- 625.

Witeska, M. 1999. The influence of copper exposure on blood parameters of common carp fingerlings. Heavy metals in environment. An integrated approach. Ed. D. A. Lovejoy. 291-294 pp.

Witeska, M. 2005. Stress in fish – Hematological and immunological effects of heavy metals. *Electronic J. Ichthyology*. **1:** 35-41.