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SUBLETHAL EFFECT OF COPPER ON PLASMA CONSTITUENTS OF *Cirrhinus mrigala* AND *Ctenopharyngodon idella*

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KEYWORDS

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ABSTRACT

The measurement of serum/plasma parameters is of significance in assessing the nutritive value of cultivable fishes, in the light of drawing waters from contaminated sources and employing biocides containing heavy metals in culture ponds. The main objective of this study was to determine the sublethal effects of copper (0.16 mg/l Cu and 0.53 mg/l Cu) on plasma glucose, total protein, albumin, globulin, total cholesterol and triglycerides in an endemic carp *Cirrhinus mrigala* and an exotic carp *Ctenopharyngodon idella* at weekly intervals, over a period of four weeks. Glucose, albumin, total cholesterol and triglycerides were estimated by enzymatic methods and total protein by non-enzymatic method. Glucose level has elevated on day 14 and then returned to levels comparable to those of control fish. The result of this study revealed that total protein, albumin, cholesterol and triglycerides indicated a positive linear relationship with the duration of exposure. Globulin expressed an intermittent rise and fall till the end of the experiment. All parameters, other than glucose and globulin, showed a gradual decrease with increasing exposure period.

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1 Introduction

Studies of the chemical composition of fish blood, in general, are fragmentary. Various pollutants bring changes in the blood biochemistry, as a result of metabolic and biochemical processes of an organism. Metabolic changes are indicated by plasma/serum parameters (Luskova et al., 2002). Plasma reflects the pathological and physiological state of the body, and hence measurement of these parameters gives us an understanding of the health status of toxicant stressed fish (Adhikari et al., 2004; Asadi et al., 2006; Bahmani et al., 2010; Kataria et al., 2010; Muñoz et al., 2010). This facilitates an aquaculturist to make sure that the health condition of the animals is unaltered, in order to get a good yield (Farahi et al., 2010; Csep et al., 2010). Various studies revealed that metals can either increase or decrease the blood protein, glucose, cholesterol and serum enzyme activity, depending on the type of the metal, fish species, quality of water and exposure period (Monteiro et al., 2005; Jee et al., 2005).

Glucose is an instant source of energy for the heart and muscles. Unfavourable environmental conditions cause marked elevations in plasma glucose levels. Maintaining normal concentration of glucose is one of the finest homeostatic mechanisms which can be estimated quickly, employing a cost-efficient method (Dobšikova et al., 2009; Nordlie, 2009; Patriche et al., 2010; Shahsavani et al., 2010; Velisek & Svobodova, 2004). Serum/plasma analysis for total protein, albumin and globulin is significant from a diagnostic point of view, as it not only indicates the general nutritional status of fish, but also a healthy liver and vascular system (Abdel-Tawwab et al., 2008). Total plasma protein content decreases due to impairment of protein synthesis, reduced absorption, or loss of protein (Bernet et al., 2001; Yang & Chen, 2003). Plasma proteins comprise a mixture of approximately 100 components with different physico-chemical properties and functions, some of which are directly involved in fish homeostasis (Petrescu-Mag et al., 2007a; Petrescu-Mag et al., 2007b; Petrescu-Mag et al., 2007c; Petrescu-Mag & Petrescu-Mag, 2010). Hence, a measure of plasma proteins is the most important indicator of the nutritional status of fish. Cholesterol, a structural component of cell membranes, forms the outer layer of plasma lipoproteins, and the precursor of all steroid hormones. The negative effect of stress on fish may be observed by a decrease in body lipid content (Svobodova et al., 2006). Reduction in cholesterol and triglyceride content (the most important function of which is to store and provide cellular energy), reflects an increased physiological discomfort or impairment of lipid metabolism (Patriche et al., 2011).

Oner et al. (2008) reported changes in serum parameters of *Oreochromis niloticus* on exposure to Ag, Cd, Cr, Cu and Zn for a prolonged duration. Heydarnejad et al. (2013), investigated the sublethal effects of copper on serum biochemical parameters in

rainbow trout. Similarly, Mutlu et al. (2015), worked on alterations in blood chemistry of *Nile tilapia*, due to affect of long-term exposure to copper sulphate. Various researchers studied the acute effect of copper on blood/plasma biochemical parameters in carps (Khabbazi et al., 2015; Javed et al., 2017; Osman et al., 2019). However, information regarding the chronic effect of copper on blood biochemistry is in paucity. Therefore, present study was carried out to investigate the sublethal effect of copper on the plasma constituents of carps.

2 Materials and Methods

Fingerlings, both male and female, of *C. mrigala* and *C. idella*, measuring 3½'' - 4'' and weighing $8 \pm 0.5\text{g}$ and $8.5 \pm 1\text{g}$ respectively, have been procured from a private fish farmer in Kaikaluru, Andhra Pradesh, India. The fingerlings after prior conditioning, were packed and transported in polythene bags filled with water and oxygen. Immediately after arrival, they were emptied into separate, rectangular fibre glass tubs (200 litres) filled with potable water and pond water in the ratio 1:1, with continuous aeration. The fingerlings were allowed to stay overnight. The next day, water in the tanks was exchanged with fresh potable water, and from thereon, every alternate day, tanks were neatly cleaned and water exchange carried for one week. During the week-long acclimation at temperature of $28 \pm 2^{\circ}\text{C}$, fingerlings were fed with rice bran and oil-cake. They were also observed for parasites, disease and recovery from collection and transport stress. Dead and abnormal individuals were removed periodically. After acclimation, experiments were carried out with both species, by exposing them to sublethal concentrations of copper (0.16 mg/l Cu and 0.53 mg/l Cu), calculated for *C. mrigala* and *C. idella* respectively. Aeration was ceased, to avoid alteration in results. Temperature was maintained at $28 \pm 2^{\circ}\text{C}$. Fish were fed once a week, for the duration of the experiment. Control fish were maintained under similar conditions in water without copper. Groups of 25 fish were exposed to 1/5 of LC50 of copper, in 500 litre fibre-glass tanks, not exceeding 1g fish / l, using static test method.

Every week, control fish and fish from copper concentration were sampled, anaesthetized with 1.5 ppm Quinaldine for 30 minutes, and blood withdrawn from the heart into K3 EDTA vials by piercing 1 ml insulin syringe (disposable). Blood was centrifuged for 15 minutes at 3000 rpm, for further analysis. Supernatant plasma was aspirated into 2 ml Eppendorf vials, and stored in a refrigerator at $2-8^{\circ}\text{C}$, and analyzed for total protein, albumin, globulin, glucose, cholesterol and triglycerides, in triplicate. Analysis for glucose was carried out within 24 hrs, for cholesterol and triglycerides within 48 hrs, and for total protein and albumin within 72 hrs of centrifugation. The intensity of colour for all the parameters has been recorded on GENESYS 10 UV Spectrophotometer.

2.1. Glucose

Plasma glucose was determined by Eco Pak Glucose kit (Accurex Biomedical Pvt. Ltd., India, by enzymatic method (using glucose oxidase and peroxidase) (Young et al., 1976). Glucose oxidase converts glucose to gluconic acid. Hydrogen peroxide formed in the presence of peroxidase, oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. This dye has absorbance maximum of 505 nm (500-550 nm). The intensity of the colour complex is directly proportional to the concentration of glucose.

2.2. Total Plasma Protein

Total plasma protein was determined by Lowry method (Lowry et al., 1973). The phenolic group of tyrosine and tryptophan residues (aminoacid) in a protein will produce a blue – purple colour complex, which shows maximum absorption of 660 nm wavelength, with Folin-Ciocalteu reagent and consists of sodium tungstate molybdate and phosphate. The intensity of colour depends on the amount of these aromatic amino acids present, and will thus vary for different proteins.

2.3. Albumin

Albumin was determined by Autozyme Albumin kit (Accurex Biomedical Pvt. Ltd., India), based on BCG method (Dumas et al., 1971). Plasma albumin in the presence of Bromocresol-green, under acidic condition, forms a green coloured complex which has maximum absorption at 600 nm (600-650 nm). The absorbance of this complex is proportional to the albumin concentration in plasma.

2.4 Globulin

Globulin content was obtained by subtracting plasma albumin in g / dl from plasma total protein in g / dl.

2.5. Total Cholesterol

Total cholesterol was determined by Infinite Cholesterol kit (Accurex Biomedical Pvt. Ltd., India), based on enzymatic method (Allain et al., 1974). Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. Cholesterol oxidase converts cholesterol to cholest-4-n-3-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye which has absorbance maximum at 510 nm (500 – 530). The intensity of red colour is proportional to the amount of total cholesterol in the specimen.

2.6. Triglycerides

Triglycerides were determined by Infinite Triglycerides kit (Accurex Biomedical Pvt. Ltd., India), based on enzymatic method (Tietz, 1994). Glycerol released from hydrolysis of triglycerides by lipoprotein lipase is converted by glycerol kinase into glycerol-3-phosphate which is oxidised by glycerol phosphate oxidase to dihydroxy-acetone-phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidises phenolic chromogen to a red-coloured compound which has a maximum absorption at 510 nm (500-530 nm).

The comparison of the control and treatment groups was statistically analyzed by student's 't' test, and the validity of the investigation was expressed as probability (p) values. Values of $p < 0.05$ were considered significant and $p > 0.05$ not significant.

3 Results and Discussion

The plasma glucose, total plasma proteins, albumin, globulin, total cholesterol and triglyceride concentrations of the control and treatment groups of *C.mrigala* and *C.idella*, are shown in figures 1- 6.

In the present study, it was observed that in copper treatment groups of *C.mrigala* and *C.idella*, the selected parameters exhibited a varying trend (compared to those of control), with increase in exposure period. Among the different constituents, a wide range of variation was exhibited by glucose followed by cholesterol and triglycerides, and a narrow range of variation by total plasma proteins (TPP), albumin and globulin. The chronic sublethal copper exposure from 7th to 28th day caused an increase in glucose level, gradual decrease in total protein, albumin, cholesterol and triglycerides, and an intermittent rise and fall in globulin content (compared to those of control), in both the species.

The plasma glucose values of the control and treatment groups of *C.mrigala* and *C.idella* recorded least values 46.55 mg/dl and 60.19, mg/dl and 42.78 mg/dl and 25.57 mg/dl on the 28th day respectively. The glucose level of both species has shown a significant increase ($p < 0.05$) of 249% and 81% respectively, on the 14th day (Figure 1).

The initial elevation and subsequent reduction of glucose level is in accordance with Heydarnejad et al. (2013), who has reported elevated glucose on exposure to sublethal concentrations of copper in rainbow trout *Oncorhynchus mykiss*. The higher level of glucose on the 14th day of exposure, may probably be due to glycogenolysis. Glycogenolysis results in high glucose levels under the influence of stress related hormones in organisms

Figure 1 Sublethal effect of copper on plasma glucose levels of *C. mrigala* and *C. idella* during an exposure period of four weeks

Figure 2 Sublethal effect of copper on total plasma protein of *C. mrigala* and *C. idella* during an exposure period of four weeks

Figure 3 Sublethal effect of copper on plasma albumin content of *C. mrigala* and *C. idella* during an exposure period of four weeks

Figure 4 Sublethal effect of copper on plasma globulin content of *C. mrigala* and *C. idella* during an exposure period of four weeks

Figure 5 Sublethal effect of copper on plasma cholesterol levels of *C. mrigala* and *C. idella* during an exposure period of four weeks.

Figure 6 Sublethal effect of copper on plasma triglycerides of *C. mrigala* and *C. idella* during an exposure period of four weeks

(Heydarnejad et al., 2013). Drop in glucose level on the 21st and 28th days ($p < 0.05$) is probably due to depletion of energy reserves. Similar response has been observed by Oner et al. (2008), in copper exposed *O. niloticus*. Alterations in the glucose level might also be related to renal injury, liver damage and lack of nutrition (Oner et al., 2008).

Total plasma proteins, albumin and globulin of the control groups of *C.mrigala* and *C.idella* recorded least values of 7.28 and 8.48 g/dl, 3.44 and 4.02 g / dl, and 3.84 and 4.46 g / dl, while those of the treatment groups recorded 6.87 and 7.89 g/dl, 2.27 and 2.86 g / dl, and 4.6 and 5.03 g/dl respectively, on the 28th day (Figures 2,3,4).

Total protein concentration in the present study decreased significantly in exposed fish, compared to the control value. Oner et al. (2008) reported significant reduction in protein contents of *O. niloticus*, after 30 days of copper exposure and attributed this to differences in exposure concentration and species, or due to changes in the level of serum total protein synthesized mainly in the liver. Stressed condition of fish might probably be the reason for low albumin level in the present study. Similar response has been reported by Mutlu et al. (2015) in *O. niloticus* and Javed et al. (2017) in *Channa punctatus*. While hypoalbuminaemia in *O. niloticus* has been explained to be due to stress situation, that in *C. punctatus* was said to be due to liver damage (as albumin is produced by liver). Comparable result has also been reported by Eldeen & Hamid (2002). However, stress induced *Nile tilapia* exposed to individual and combined mixtures of heavy metals, expressed significant increase in total protein and albumin (Firat & Kargin, 2010). Previous studies revealed that changes in haematological indices of fish caused by heavy metals and their mixtures are different. Globulin level of *C.mrigala* and *C.idella* showed intermittent elevation and reduction ($p > 0.05$) with increase in exposure period. Similar result has been recorded by Gopal et al. (1997) in *C. carpio* exposed to sublethal concentration of copper.

Total cholesterol and triglycerides of the control groups of *C.mrigala* and *C.idella* recorded least values 193.28 and 215.51 mg/dl, and 165.12 and 147.82 mg/dl respectively, while those of the treatment groups recorded 101.98 and 134.57 mg /dl, and 104.13 and 119.71 mg/dl respectively, on the 28th day (Figures 5,6).

Cholesterol values showed a significant increase ($p < 0.05$) on the 14th day, and then decreased towards the end of the exposure period. It has been revealed by earlier studies that cholesterol concentrations in serum of copper exposed fish generally increase. Similar trend in cholesterol values of copper stressed fish, was described by Oner et al. (2008) in *O. niloticus*, Heydarnejad et al. (2013) in *O. mykiss* and Yang & Chen (2003) in gallium stressed *Cyprinus carpio*. The probable reason for high cholesterol levels

may be liver and kidney failure causing the release of cholesterol into the bloodstream (Oner et al., 2008). The increase in cholesterol concentrations is due to hazardous effects of copper, particularly on cell membrane (Heydarnejad et al., 2013).

Triglyceride concentration in plasma of copper-exposed fishes showed significant decrease. Oner et al. (2008) reported a similar effect in *O. niloticus* exposed to copper, and a severe reduction in triglyceride levels after 20 days of exposure. Similarly, reduction in serum triglyceride concentrations of *O. mykiss*, was observed by Heydarnejad et al. (2013) after 15 days of exposure. Fishes exposed to copper show inconsistent triglyceride concentrations, because lipid metabolism and glycogen storage are different for different fish species, in addition to variation in exposure period (Heydarnejad et al., 2013). It can be concluded that the studied blood parameters can not only be employed as ideal biomarkers to monitor the well being of the farmed fishes, but the responses of these biomarkers can also be related to population parameters, enabling comparison of predictions from computer-based models in risk assessment of aquatic environments.

Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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