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RESEARCH ARTICLE

COPPER INDUCED MODULATION ON THE ACTIVITY OF GILL ATPases IN FINGERLINGS OF FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT

Heavy metals occur in aquatic systems from natural sources and anthropogenic activities. The pollution of aquatic environment by heavy metals affects aquatic organisms and poses considerable environmental risks and concerns. The gills are one of the vital organs which come into direct contact with water and are indicative of any environmental stress. Preliminary toxicity tests were carried out to find the median lethal tolerance limit of experimental fishes to copper for 96 hours. The LC_{50} was found to be 12 mg/L. One-tenth (1.2 mg/L) was taken as the sublethal concentration for the study. The fishes were maintained for a period of 14 days in sublethal concentration. Experiment was conducted using sublethal and toxicologically safe concentration. The present study would decipher the effect of heavy metal copper on the modulations in the activity of gill ATPases in fingerlings of freshwater fish *Oreochromis mossambicus.*

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INTRODUCTION

Heavy metals pollutants compared with other types of aquatic pollution are less visible but its effects on the ecosystem and humans are intensive and very extensive due to their toxicity and their ability to accumulate in the aquatic organisms (Rainbow, 2007). Of all types of aquatic pollutants, heavy metals are of great concern. These reach water bodies, decline the life sustaining quality of water and damage both flora and fauna. Copper is highly toxic in aquatic environments and has effects in invertebrates, fishes and amphibians, with all three groups equally sensitive to chronic toxicity (USEPA, 1993). Biomagnifications play critical role in their toxicity (Houne and Dunston, 1995). Copper will bioconcentrate in many different organs in fish and molluscs. Copper is a common pollutant in surface water and its toxicity is largely attributable to its cupric (Cu^{2+}) form which is commonly found in inorganic and organic substances and absorbed into particulate matter (Alabaiter and Llyod, 1984). The gills are one of the vital organs which come into direct contact with water and are indicative of any environmental stress (Evans, 1987). Fish gills are strategically located between the venous and arterial circulation and have an extensive endothelial surface area which is directly exposed to blood and indirectly to the water (Nowak, 1992). Gills are the vital structures of the fish, since they are the main site of gaseous exchange (Hughes, 1992; Lin and Randall, 1993) as well as partially responsible for osmoregulation, acid-base balance and therefore useful for environmental impact and ecotoxicology studies (Rankin *et al*., 1982; Goss *et al*., 1992). Toxic chemicals in water alter the morphology of branchial cells of fishes (Mallat, 1985).

ATPases are membrane bound enzymes and are involved in the transport of sugars and amino acids across membranes. ATPases are lipid dependent as well as thiol dependent membrane bound enzymes. Na^+ K^+ ATPase is a universal membrane bound enzyme that provides a driving force for any transport systems. The lipoproteinaceous nature of Na^+ K^+ ATPase is sensitive to changes either in the lipid or protein component of the membrane. Phospholipid is quite necessary for the activity of Na^+ K^+ ATPase. Alterations in membrane lipids and proteins thereby affect the membrane permeability which in turn alters ATPase activity and cellular function (Davis and Wedemeyer, 1971).

Gills therefore, are potentially useful to monitor the health of fish (Evan *et al.,* 2005). The gills in fish can be a valuable model for assessing the effects of toxicants, it was felt that it would be worthy to study the changes in gills, the structural

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target of fishes to environmental toxicants, and the extent of damage that is caused. The objective of this study is to assess the response of freshwater fish *Oreochromis mossambicus* fingerlings to copper through determination of acute 96 hours LC_{50} value and modulations in gill ATPases activity.

MATERIALS AND METHODS

Experimental Animals and Maintenance

Freshwater fish *Oreochromis mossambicus* fingerlings was used as the experimental model to evaluate the toxicity of copper. The fish used in this experiment were transferred from natural ponds around Arakonam district and bought to the laboratory and acclimated for 7 days to laboratory conditions in glass aquaria each measuring (60cm x 30cm x 30cm) filled with 25 litres of dechlorinated tap water with aerator fitted to the aquaria for continuous oxygen supply. The aquaria were disinfected with potassium permanganate solution and washed thoroughly prior to introduction of fishes to prevent any fungal infection. Feeding was stopped 24 hours before the commencement of the toxicity test to keep the animals more or less in the same metabolic state.

Initial mean weight and length of the fish were 20-28 gms and 8-12 cm respectively. The fishes were maintained in normal light dark period and room temperature.

Metal toxicity and Determination of Lethal Concentration of Copper (LC_{50})

Acute toxicity experiments were conducted for 96 hours using a static bioassay method. Five groups of 10 fishes each were set up in triplicates for the LC_{50} calculation. The LC_{50} bioassay method involved the exposure of the five groups of fishes to a range of five different concentrations of copper sulphate. The concentration at which around 50% survival / mortality occurred after 96 hours was taken as the median lethal concentration. The dead fishes were removed immediately from the aquaria to avoid oxygen depletion. Mortality, behavioural and morphological changes were recorded.

The fishes were maintained in a narrow range concentration of 8, 10, 12, 14, 16 mg/L copper respectively. The 96 hour LC_{50} was determined by Probit analysis method (Finney, 1971). The LC_{50} was found to be 12mg/L. One-tenth (1.2 mg/L) was taken as the sublethal concentration for the study. Experiments were conducted using sublethal and toxicologically safe concentrations of copper for 14 days.

Experimental Design

Group I: Control fishes maintained in dechlorinated toxicant free water.
Group II: Fishes maintained 1.2mg / L of copper for a period of 14 days.

The control and the experimental fishes were fed with minced goat liver. Commercial food pellets with ingredients consisting of fish meal, wheat flour, soyabean meal, corn meal, yeast, vitamins and minerals were also fed. Water was changed daily at 08.00 hours which facilitated the removal of nitrogenous waste excreted by the test fishes and for the removal of unconsumed food. After renewal of water the required quantity of metal was added to maintain the toxic concentration of the water medium.

At the end of 14^{th} day six fishes were sacrificed by cervical decapitation. Gill tissue were dissected out and washed thoroughly with 0.9 N saline solution. Tissues were weighed and homogenized in Tris 0.1 M HCL buffer using Potter Elevenhjem homogenizer. The homogenate of the tissues were centrifuged at 2500 rpm for 15 minutes in a refrigerated high speed centrifuge and clear supernatant was used for protein analysis and assay of ATPase enzyme activity.

Total protein estimation and Activity of ATPase in gills

Total protein was estimated by the method of Lowry *et al.* (1951). Specific activity of Na^+ K^+ ATPase was determined according to the method of Bonting (1969), Ca^{2+} ATPases (Hjerton and Pan, 1983) and Mg^{2+} ATPases activity was assayed according to the method of Ohnishi *et al.* 1982

Statistical Analysis

The data collected on the different parameters of the experimental study were subjected to statistical analysis by Mean ± SD (Snedecor and Cochran, 1989). The statistical significance was tested at 1% and 5% levels.

RESULTS

Gill protein content

The total protein content of the liver in experimental groups was significantly depleted ($P < 0.05$) during 14 days of exposure of the fishes to the copper (Table 1 and Fig 1).

Table 1. Effect of copper on gill protein and ATPase activity of *Oreochromis mossambicus*

Biochemical / Enzyme Parameters	Control	Experimental	t - value	p -value	Significance
Total Protein	24.32 ± 2.76	20.27 ± 0.13	3.270	0.01134	< 0.01
Mg^{2+} ATPase	5.12 ± 0.48	2.44 ± 0.38	9.641	1.114	< 0.05
Ca^{2+} ATPase	1.01 ± 0.04	1.78 ± 0.09	15.757	2.629	NS
Na^+K^+ ATPase	11.29 ± 0.18	7.29 ± 0.22	31.032	1.264	< 0.01

Values are expressed as Mean ± SD (n = 5). All values significant at 1 % and 5% level.
Protein content expressed as mg/g tissue
Activity of enzymes expressed as µ moles of inorganic phosphorous formed min/mg protein.

Activity of ATPases in gills

Activity of gill ATPases showed a significant reduction ($p < 0.05$) in activity of Na^+ K^+ ATPases and Mg^{2+} ATPases and no significant changes were observed in Ca^{2+} ATPases after 14 days of exposure to copper (Table 1 and Fig 1).

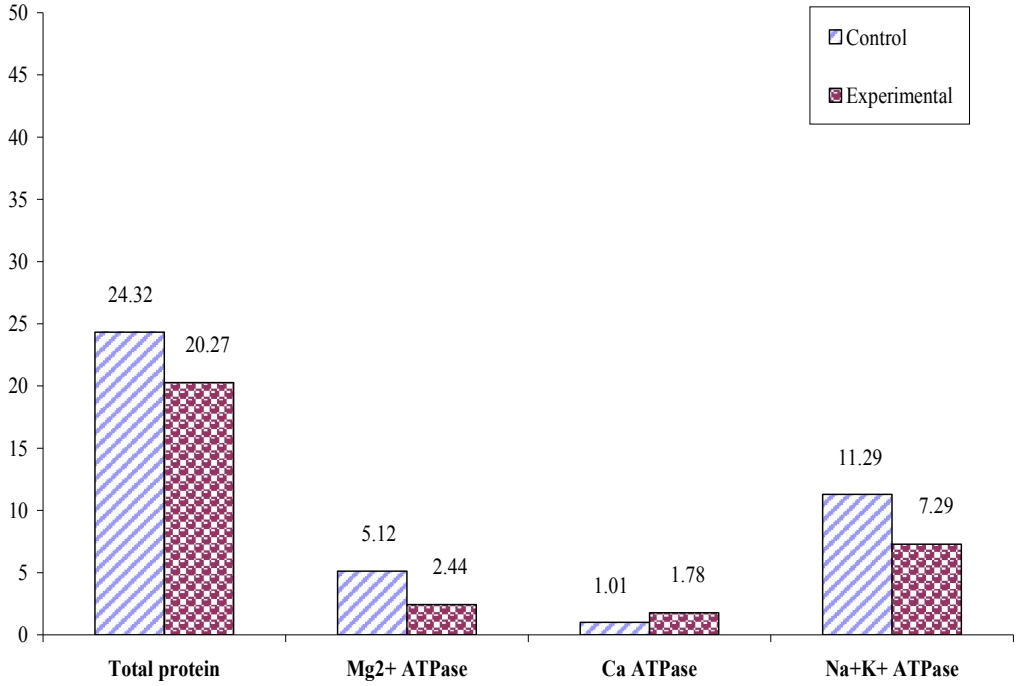

Fig.1. Effect of copper on gill protein and ATPase activity of *Oreochromis mossambicus*

Values are expressed as Mean ± SD (n=5).
Protein content expressed as mg/g tissue
Activity of enzymes expressed as µ moles of inorganic phosphorous formed min/mg protein.

DISCUSSION

Gills are the primary site of osmoregulation and respiration and is the first and probably main target for the aquatic toxicant. It is a well known fact that in any organism the toxic effects are first expected on the oxygen consumption (Vander Oost *et al.,* 2003) followed by biochemical change which serve as a better index (Pandey *et al*., 2008).

Proteins in different tissues and different biological fluids may differ in their composition, properties and functions. They also aid in maintaining cell architecture. Proteins are an important organic constituent of animal cells playing a vital role in the process of interaction between intracellular and extracellular media, being a part of cell membrane (Padmini *et al.,* 2014). In the present study fishes exposed to sublethal concentration of copper for 14 days experienced greater stress during the process of detoxification which could have altered the metabolic status of the animal. The protein depletion in the fishes exposed to copper is a physiological strategy played by the animal to adapt itself to the changed environment (Sanchez and Porcher, 2009). The quantity of protein is dependent on the rate of protein synthesis or on the rate of its degradation.

The quantity of protein may also be affected due to impaired incorporation of amino acids into the polypeptide chain and inhibiting RNA synthesis (Tripathi and Verma, 2004a). Metabolic function in gills may have been hampered due to toxicity of copper. It has also been reported that heavy metal treatment also reduce binding of amino acids to tRNA leading to decreased protein synthesis and hence protein depletion (Frukas, 1975; Dhar and Banerjee, 1983).

ATPase the membrane bound enzyme is a family of catalytic entities that carry out hydrolysis of ATP to ADP and Pi (McCarthy and Shugart, 1990; Jagoe, 1996). In the present study the activity of Na^+K^+ ATPase is decreased in gills of fishes exposed to copper. The enhanced susceptibility of lipids in membranes may lead to loss of protein decreasing the activities of ATPase thereby causing extensive changes in the membrane function in metabolic tissues (Dalela *et al*., 1978; Kako *et al*., 1988). The decrease in ATP production either due to increased utilisation or disrupted oxidative phosphorylation results in decreased / inhibition in activity of ATPase (Reddy and Philip, 1994). Over mobilization of energy rich fuels during oxidative stress depletes ATP concentration in living cells. Decrease in substrate ATP may have decreased the activity of Na^+ K^+ and Mg^{2+} ATPase in the present study (El-Toukhy and Girgis, 1993).

The role of ATPase in energy metabolism and in active transport of ions is well established (Schwartz *et al*., 1975). Davis and Wedemeyer (1971) noted a depression of Na^+ K^+ ATPase activities in brain, gills and kidney of fishes treated with kelthane, thiodon and DDT. Campbell *et al*. (1974) demonstrated that DDT is capable of disturbing the Na^+ K^+ ATPase activity in tissues of rainbow trout. Similar results was observed in vital tissues of *Clarias batrchus* exposed to carbofuran (Mukhopadhyay *et al*., 1982). Chromium induced decrease in activity of ATPase was observed in vital organs of mud skipper *Periopthalmus dipes* (Thaker *et al*., 1999).

Calcium pump present on the plasma membrane and membrane of endoplasmic reticulum help in efflux of Ca^{2+} ions. The present study showed decrease in Na^+ K^+ ATPase and no change in

Ca^{2+} concentration which may interfere with the ionic balance within their cells, since Na^+ and Ca^{2+} are thought to be competitive at a number of sites, it seems likely that a normal concentration of total calcium will compete with sodium specific sites at the inner surface of the membrane leading to a decrease in the sodium content of the cells and an abnormal increase in calcium content culminating in necrosis (Ghosh *et al.,* 2000). The alteration in the lipid and protein content of membranes affect the ATPase activity and in turn the membrane permeability (McKim and Erickson, 1991). The low concentrations of toxicants in the water may be considered within safe limits, still the vital organs are adversely affected which may interfere with growth, development, physiological functioning and reproduction.

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