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INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

Int. J. Modn. Res. Revs.

Volume 5, Issue 1, pp 1457-1459,January, 2017

ISSN: 2347-8314

ORIGINAL ARTICLE

BIOCHEMICAL CHANGES INDUCED BY HEAVY METAL COPPER ON GILL AND LIVER OF FRESH WATER FISH,*Oreochromis mossambicus (Tilapia)*

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Article History: *Received 5th December,2016, Accepted 31st December,2016, Published 1st January,2017*

ABSTRACT

In the modern world the urbanization and Industrialization have boosted the man kind's economy through various means and ways. But at the same time pollution of aquatic resources has become a huge challenge and a serious threat. Recent years have witnessed significant attention being paid to the problems of environmental contamination by a wide variety of heavy metal pollutants. Heavy metals due to their potential toxicity produce biochemical changes in the organs of animals and continuous exposure may alter genetic composition of aquatic organisms. Heavy metal copper is potentially harmful to most of the organisms, have been reported as hazardous environmental Pollutant able to accumulate along the aquatic food chain with severe risk for animal and human health. Also it has been reported to induce morphological, histological and biochemical alterations in the tissues of the fishes which may critically influence fish quality. The present study is to assess the protein content and Amino acid level in Gill and Liver of the fresh water fish *Oreochromis mossambicus* (Tilapia) exposed to sublethal concentration of copper $1/10^{th}$ (high) and $1/20^{th}$ (low) of LC_{50} , for 96 hr of copper (4.13 and 2.06 mg/L) for the period of 30 days. In the present investigation, gill and liver of protein levels decreased whereas amino acids increased when *O. mossambicus* was exposed to sublethal concentrations of copper. The depletion of protein content in brain and muscle tissue of fresh water fish, *Oreochromis mossambicus* (Tilapia) was due to the utilization of protein to counteract the toxicant stress caused by copper. The increase in amino acid may be due to proteolysis and were routed through gluconeogenesis for increasing the energy supply to cope up with the heavy metal copper stress.

Keywords: *Oreochromis mossambicus,* Copper, Protein,Aminoacid.

1.INTRODUCTION

In last few decades increase in population density, heavy industrialization and agricultural activities have resulted in more and more wastes entering in fresh water resources.Waste water is characterized by low pH, high BOD and COD values and contains a high percentage of organic and inorganic materials (Chauhan, 1991). Heavy metal contamination has been reported in aquatic organisms (Adham *et al*., 2002 and Olojo *et al*., 2005). These pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic organisms (Farkas *et al*., 2002). Metal contamination of aquatic ecosystems has long been recognized as a serious pollution problem. When fish are exposed to elevated levels of metals in a polluted aquatic ecosystem, they tend to take these metals up from their direct environment (Seymore, 1994). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al*., 2007). Heavy metals and chemicals are toxic to animals and many cause death or sublethal pathology of liver, kidneys, reproductive system, respiratory system or nervous system in both invertebrate and vertebrate aquatic animals (Wilbur, 1969). Heavy metals cannot be degraded or destroyed to a small extent they enter our bodies via food, drinking water and air and also known as trace elements. Heavy metals have been recognized as strong biological poisons because of their persistent nature, tendency to accumulate in organisms and undergo food chain amplification (G.S. Dinodia, R. K. Gupta and K. L. Jain. *et al*., 2002) they also damage the aquatic fauna including. Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution (Kock *et al*., 1996). Fish, having great economic importance, are affected immensely by various chemicals including heavy metals directly or indirectly in various ways. Accumulated heavy metals may lead to morphological alterations in the tissues of fish (Monteiro *et al*., 2005). The monitorization of histological, biochemical and enzymological changes on gill and liver of fish is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in field and experimental studies.The fish liver is a vital organ concerned with basic

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metabolism and is the major organ of accumulation, biotransformation and excretion of contaminants in fish (Figueiredo *et al*., 2006). The liver is particularly susceptible to damage from a variety of toxicants. Fish gills on the other hand are critical organs for respiration, osmoregulation and excretion. Gills serve as a good indicator of water quality.

The present study was aimed to evaluate the biochemical changes and estimate the aminoacid level by heavy metals copper on gills and liver of fresh water fish *Oreochromis mossambicus* (Tilapia) and to enhance the knowledge of tissue damage after exposure to heavy metal copper.

2.MATERIALS AND METHODS

The fish *Oreochromis mossambicus* having mean weight of 160-165 gm and length of 22 – 24 cm were collected from KRP Dam located at Krishnagiri, Tamilnadu, India, 10 km away from the Govt. Arts. College (men) campus, Krishnagiri and acclimatized in to laboratory conditions. They were given the treatment of 0.1% KMNO4 solution and then kept in plastic pools for acclimatization for a period of two weeks. They were fed on rice bran and oil cake daily. The copper was used in this study and stock solutions were prepared. Copper LC50 was found out for 96 h (41.36 mg/L) (Sprague, 1971) and 1/10th, and 1/20th of the LC50 values were 4.13 mg/L and 2.06 mg/L respectively taken as sublethal concentrations for this study. Thirty fish were selected and divided into 3 groups of 10 each. The first group was maintained in free from Copper and served as the control (group-I). The other 2 groups (Group-II and Group-III) were exposed to sub lethal concentration of copper (4.13 mg/l and 2.06 mg/l) in 10 litre capacity aquaria for 30 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein and Amino acid estimation. Protein contents in the tissues were estimated by the method of Lowry *et al*. (1951). Total free amino acids and content of the tissue were estimated by the method of Moore and Stein (1954).

The data so obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

Physico-chemical properties of Copper

Table – 1 Physical and Chemical properties of copper

Properties	Copper
Chemical compound	Copper chloride
Empirical formula	Cucl2.2H2O
Molecular weight	170.48 g/mol
Color	Blue-green solid
Physical state	Solid
Melting point	100 °C
Boiling point	993 °C
Density at 20 °C	2.51 g/cm3
Odor	odorless
Solubility	
Water	Soluble
Organic solvents	Acetone, Methanol, Ethanol
CAS number	7447-39-4
Toxicity	More toxic

3.RESULT

Protein level in Gill

When fishes exposed to 2.06 mg/L of copper under low sublethal concentration(Group-II), the total protein level was decreased to 87.19 ± 0.92 mg/G wet weight in the period of 30 days of exposure. Whereas, the gills total protein level significantly decreased under high sublethal concentration (Group-III) over the control was 72.18 ± 0.62 mg/G wet weight at the end of 30 day of exposure.

Protein Level in Liver

The mean values of total protein in the liver of *O. mossambicus* exposed to low sublethal concentration(Group-II) of 2.06 mg/L and high sublethal concentration(Group-III) of 4.13 mg/L of copper under 30 days exposure showed a decline in the significant levels in both the low and high sublethal exposure

Table – 2. Changes of total protein content (mg/g wet wt.) in gill and liver of *O. mossambicus* exposed to sublethal concentration of Cu

Experimental Group	Exposure Duration	Gill	Liver
Group – I (Control)	30 days	117.30 ± 0.56^{a}	176.22 ± 0.72^{a}
Group – II (LSC)	30 days	87.19 ± 0.92^{b}	139.51 ± 0.36^{b}
Group – III (HSC)	30 days	72.18 ± 0.62^{c}	102.14 ± 0.44^{c}

Values are mean ± S.D., Sample Size (N) = 6. Different letter designations denote significant at $p < 0.05$ level between exposure groups.

Amino Acid Level in Gill

When the fish exposed to low sublethal concentration (Group-II) (2.06 mg/L) , the amino acid content in gill is increased to 8.36 ± 0.45 mg/G wet weight of tissue after the period of 30 days of exposure. While in the high sublethal concentration(Group-III) (4.13 mg/L), the amino acid content in gill is significantly increased (13.24 ± 0.77 mg/G) when compared to the control.

Amino acid Level in Liver

When the fish exposed to low sublethal concentration (Group-II) (2.06 mg/L) , the amino acid content in liver is increased to 10.59 ± 0.64 mg/G wet weight of tissue after the period of 30 days of exposure. While in the high sublethal concentration(Group-III) (4.13 mg/L), the amino acid content in liver is significantly increased 17.21 ± 0.89 mg/G when compared to the control.

Table – 3. Changes of amino acid content (mg/g) in gill and liver of *O. mossambicus* exposed to sublethal concentration of Cu

Experimental Group	Exposure Duration	Gill	Liver
Group – I (Control)	30 days	5.12 ± 0.32^{a}	7.03 ± 0.56^{a}
Group – II (LSC)	30 days	8.36 ± 0.45^{b}	10.59 ± 0.64^{b}
Group – III (HSC)	30 days	13.24 ± 0.77^{c}	17.21 ± 0.89^{c}

Values are mean ± S.D., Sample Size (N) = 6.
Different letter designations denote significant at $p < 0.05$ level between exposure groups.

4.DISCUSSION

Protein and Amino acid

Biochemical studies are the best studies to assess the health and functional activity of fish which helps to analyze the effect of heavy metals. Fish are widely used to evaluate the health of aquatic ecosystems and their physiological changes serve as biomarkers of environmental pollution (Kock *et al*., 1996). Gills are the vital organs in fish which have direct contact with the medium through which pollutants enter into the body (Mount, 1962; Holden, 1972; Edwards, 1973). Among gill and liver tissues, liver showed higher protein content which might be due to greater concentration of enzyme. Liver is the site of metabolism (Harper *et al*., 1977). The liver plays an important role in the synthesis of proteins. Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms (Singh and Sharma, 1998). Protein is the most primary biochemical ingredient present in large quantities in the body of fish.

In the present investigation, gill and liver of protein levels decreased whereas amino acids increased at all groups of exposure when *O. mossambicus* was exposed to sublethal concentrations of copper. The depletion of proteins under the stress of copper toxicity observed in different tissues of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* indicates the proteolysis, suggesting that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. The liver gets affected considerably when there is a disturbance in protein metabolism. The accumulation of toxic substance in liver may alter its function (Premdas and Anderson, 1963). Natarajan *et al*., (1983) observed that the Metasystox exposed *Channa striatus* showed increase in amino acid content in the gill and liver and further it had been reported that the increase in amino acid may be due to proteolysis and were routed through gluconeogenesis for increasing the energy supply to cope up with the pesticide stress. Increase in the amino acid level was observed at similar situations (Kabeer Ahamed *et al*., 1978). A reduction in the protein content in the present investigation in *O. mossambicus* suggests that the tissue protein undergoes proteolysis which results in an increase in the production of free amino acids. These amino acids are utilized for energy production during stressful situation in the intoxicated fishes. Many investigators have also recorded such a reduction in protein content in fishes exposed to different toxicants (Karuppasamy, 1990; Rao, 1989). It is evident that proteins are degraded to meet the energy requirements during copper exposure. It can be concluded that *O. mossambicus* exposed to copper at both sublethal concentrations causes energy crisis and alter protein metabolism.

5.ACKNOWLEDGEMENT

The authors wish to thank the authorities of Government Arts College (Men), Krishnagiri for providing the facilities to carry out the work.

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