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Biochemical changes in liver and muscle of the cichlid, *Oreochromis mossambicus* (Peters, 1852) exposed to sub-lethal concentration of mercuric chloride

K. ROY GEORGE, N. A. MALINI AND G. O. SANDHYA RANI
Postgraduate and Research Department of Zoology, St. Thomas College, Kozhencherry -689 641, Kerala, India
e-mail: dr.roygeorgek@gmail.com

ABSTRACT

Heavy metals, as environmental stressors, may alter tissue biochemical parameters in fishes. *Oreochromis mossmabicus,* was exposed to different sub-lethal concentrations of mercuric chloride @ 0.5, 1.0 and 2 ppm to investigate changes in tissue biochemical parameters on 0, 7th and 14th day of exposure. Protein content in the liver and muscle was found depleted due to proteolysis induced by mercury toxicity. The level of liver and muscle glycogen also declined, indicating increased utilisation of glucose to counteract the increased energy demand imposed by severe anaerobic stress of mercury toxicity. The alterations in the alkaline and acid phosphatase activities during exposure period were associated with tissue damage and impaired enzyme activities resulting from toxicity of mercury.

Keywords: Acid phosphatase, Alkaline phosphatase, Mercuric chloride, *Oreochromis mossambicus*

Introduction

The contamination of freshwater bodies with a wide range of pollutants has become a matter of concern over the last few decades (Canli *et al*., 1998; Voegborlo *et al*., 1999; Dirilgen, 2001; Vutukuru, 2005). The natural aquatic systems have been extensively contaminated with heavy metals released from domestic, industrial and other man-made activities (Conacher *et al*., 1993; Velez and Montoro, 1998). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and on the diversity of aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006; Farombi *et al*., 2007). The negative effect of these pollutants are detrimental to the aquatic inhabitants, including fishes **(**Clarkson, 1998; Dickman and Leung, 1998; Olaifa *et al*., 2004).

Mercury is released from chlor-alkali, electrical, pharmaceuticals, pesticides, paper and pulp industries (Schaperclaus, 1991). Some of the industrial effluents release inorganic mercuric compounds like mercuric chloride, which is converted into more toxic organic form *viz*., methyl mercury, through bacterial action (Wiener and Spry, 1996). These compounds are likely to get transferred to the fish from water and enter the food chain. Exposure to mercuric compounds causes damage to fish epidermis and fish starts secreting profuse quantity of mucus (Rajan and Banerjee, 1991). Mercury has also been reported to bind with nucleic acid and inhibit protein synthesis (Mehra and Kanwar, 1980). A wide variety of physiological and biochemical abnormalities have been reported in fish on exposure to sub-lethal concentrations of mercury (Gupta and Sastri, 1981; Naidu *et al*., 1984). Keeping in view of the harmful effects of mercury in fish, the effects of sub-lethal concentrations of mercuric chloride in *O. mossambicus* was investigated in the present study.

Materials and methods

Oreochromis mossambicus (Order: Perciformes and Family: Cichlidae), selected for the present study, was collected from a fish farm near Pannivelichira (a Government of Kerala undertaking), Kozhencherry Taluk, Pathanamthitta District, Kerala, India. Healthy fishes of comparable body weight (8 ± 1.04 g) and length (12 ± 3.55 cm) were selected for the study. The fishes were treated with 0.05% $KMnO_4$ solution for 2 min to clear any external infection. They were then transferred to 100 l capacity glass tanks filled with dechlorinated water, one week prior to the initiation of the experiment for acclimatisation to laboratory conditions. A minimum of four fishes were introduced in each tank. The tanks were provided with continuous aeration and were maintained under normal day-night light duration. Feeding was carried out with oilcake during acclimatisation and stopped 24 h prior to experimentation. The water was exchanged after every 24 h. Every effort was made to provide healthy conditions for fish and no mortality occurred during this period.

Mercuric chloride ($HgCl_2$) stock solution (1 mg ml^{-1}) was prepared by dissolving analytical grade $HgCl_2$ in double distilled water. Test concentrations were prepared by diluting

appropriate aliquots of the stock solution . The 96 h LC_{50} tests were conducted to measure susceptibility and survival potential of fishes to $HgCl_2$ .The 96 h LC_{50} of mercuric chloride was determined following the graphical method of Krouwer and Monti (1995) and confirmed using regression analysis. For this purpose, a preliminary bioassay was performed using increasing concentration of $HgCl_2$ from 0, 10, 20, 30 to 40 ppm for 5 groups, yielding 96 h LC_{50} value below10 ppm. Therefore, five final test concentrations (0, 2, 4, 6 and 8 ppm) were selected and 96 h LC_{50} value for *O. mossambicus* was determined as $HgCl_2$ 4 ppm. Three sub-lethal test concentrations of $HgCl_2$ *viz.,* 0.5, 1, 2 ppm were selected in the experiment based on the 96h LC_{50} value. A control group was also maintained without mercury exposure during the experimental period of 14 days. Water quality characteristics like alkalinity, temperature, dissolved oxygen, pH and CO_2 were recorded daily.

At the start of the experiment (0 day of exposure), one half of the experimental fishes were sacrificed, their liver and muscle tissues were separated, blotted free of blood and processed for analysis of protein, glycogen, acid phosphatase and alkaline phosphatase. The other half of the fish were analysed similarly by the end of the 7th and 14th day of exposure. Control groups were also tested along with the experimental group simultaneously. Twenty fishes per test concentrations were maintained and a minimum of 10 replicates were taken for each parameter. The data were analysed statistically using student's 't' test (Bailey, 1981).

The estimation of protein was done by Folin - Ciocalteu method of Lowry *et al*. (1951). The determination of tissue glycogen was carried out by the method of Seifter *et al*. (1950). The activity of alkaline phosphatase (ALP) **(**EC 3. 1. 3 . 1**)** and acid phosphatase (ACP) (EC 3. 1. 3. 2) in the muscle and liver were estimated by the method of Bessey *et al*. (1946).

Results

Liver protein

Depletion of liver protein was observed at all concentrations and exposure periods which was highly significant ($p<0.01$) in fish exposed to 1 and 2 ppm of $Hg\ Cl_2$ on 14th day, when compared to control (Table 1).

Muscle protein

Depletion of protein content in the muscle of fish exposed to mercury chloride for 7 and 14 days in 0.5, 1.0 and 2.0 ppm concentrations were recorded. On 7th and 14th day, highly significant ($p<0.01$) decrease in the muscle protein was observed in fish species exposed to 1 and 2 ppm concentrations of $HgCl_2$, when compared to control (Table 2).

Liver glycogen

Liver glycogen decreased at all exposure levels of $HgCl_2$ in a dose dependent manner. This depletion of liver glycogen was highly significant ($p<0.01$) at all exposure levels on 14th day when compared to control (Table 3).

Table 1. The level of liver protein (Mean ± SE) in control and experimental fish

Parameter	Experimental group	Day 0	Day 7	Day 14
Liver protein	Control	5.2 ±0.0774	5.2 ±0.0774	5.2 ±0.0774
(mg g wet tissue^{-1})	0.5 ppm	5.2 ±0.0774	4.8 ± 0.102	2.8 ±0.64
	1.0 ppm	5.2 ±0.0774	3.4 ± 0.796	** 1.9 ±0.315
	2.0 ppm	5.2 ±0.0774	2.5 ± 0.065	**1.2 ±0.114

** ($p<0.01$)

Table 2. The level of muscle protein (Mean ± SE) in control and experimental fish

Parameter	Experimental group	Day 0	Day 7	Day 14
Muscle protein	Control	6.5 ± 0.174	6.5 ± 0.174	6.5 ± 0.174
(mg wet tissue^{-1})	0.5 ppm	6.5 ± 0.174	5 ± 0.077	**3.5 ± 0.075
	1.0 ppm	6.5 ± 0.174	**3.6 ± 0.078	** 2.4 ± 0.064
	2.0 ppm	6.5 ± 0.174	**2.1 ± 0.22	**1.78 ± 0.002

** ($p<0.01$)

Table 3. The level of liver glycogen (Mean ± SE) in control and experimental fish

Parameter	Experimental group	Day 0	Day 7	Day 14
Liver glycogen	Control	4.18 ± 0.014	4.18 ± 0.014	4.18 ± 0.14
(mg g wet tissue^{-1})	0.5 ppm	4.18 ± 0.014	2.15 ± 0.016	** 0.856 ± 0.001
	1.0 ppm	4.18 ± 0.014	2.05 ± 0.063	** 0.652 ± 0.152
	2.0 ppm	4.18 ± 0.014	1.91 ± 0.706	** 0.351 ± 0.041

** ($p<0.01$)

Muscle glycogen

Depletion of muscle glycogen was observed at all exposure periods with dose and duration. In 1 and 2 ppm concentrations of $HgCl_2$, highly significant ($p<0.01$) decrease of muscle glycogen was recorded on 7th and 14th day, compared to the control (Table 4).

Liver alkaline phosphatase (ACPase)

The liver ALPase activity showed an increasing trend in all exposure levels except at 2 ppm concentration. Among these, enzyme activity significantly increased ($p<0.01$) at 0.5 and 1 ppm exposure levels on 14th day when compared to control. On the contrary, the enzyme activity significantly ($p<0.01$) decreased at 2 ppm concentration on 7th and 14th day of treatment when compared to control (Table 5).

Muscle alkaline phosphatase

Increased muscle ALPase activity was observed at 1 and 2 ppm exposure levels of $HgCl_2$ on 14th day of treatment when compared to control. However, statistically insignificant decrease was noted in 0.5, 1 and 2 ppm concentrations on 7th day (Table 6).

Liver acid phosphatase (ACPase)

The liver ACPase activity showed changes at all exposure levels. The enzyme activity showed increase on day 7 at 0.5 ppm concentration, but it decreased with increase in dose and duration of exposure. A significantly ($p<0.01$) high enzyme activity was observed at 0.5 ppm level on 7th day when compared to control. The liver ACPase level returned to normalcy by 14th day at 0.5 ppm exposure (Table 7). However, the enzyme activity was found to decrease significantly ($p<0.01$) at 2 ppm concentrations on 14th day (Table 7).

Muscle acid phosphatase

The muscle ACPase activity decreased at all exposure levels of $HgCl_2$ on 7th day. On the contrary, highly significant ($p<0.01$), increase in enzyme activity was

Table 4. The level of muscle glycogen (Mean ± SE) in control and experimental fish

Parameter	Experimental group	Day 0	Day 7	Day 14
Muscle glycogen	Control	0.887 ± 0. 045	0.887 ± 0.085	0.887 ± 0.045
(mg g wet tissue^{-1})	0.5 ppm	0.887 ± 0. 045	0.265 ± 0.072	0.238 ± 0.036
	1.0 ppm	0.887 ± 0. 045	**0.250 ± 0.048	**0.201 ± 0.020
	2.0 ppm	0.887 ± 0.045	**0.191 ± 0.014	**0.156 ± 0.010

** ($p<0.01$)

Table 5. The level of liver ALPase activity (Mean ± SE) in control and experimental fish

Parameter	Experimental group	Day 0	Day 7	Day 14
Liver ALPase	Control	3.90 ± 0. 961	3.90 ± 0.961	3.90 ± 0.961
(µg p–nitrophenol	0.5 ppm	3.90 ± 0. 961	7.56 ± 0.216	**19.51 ± 0.775
liberated^{-1} µg^{-1}) (30 min)	1.0 ppm	3.90 ± 0. 961	6.12 ± 0.024	** 11.17 ± 0.253
	2.0 ppm	3.90 ± 0.961	**1.56 ± 0.342	**2.24 ± 1.105

($p<0.01$)

Table 6. The level of muscle ALPase (Mean ± SE) in control and experimental fish

Parameter	Experimental group	Day 0	Day 7	Day 14
Muscle ALPase	Control	2.72 ± 0. 831	2.72 ± 0.831	2.72 ± 0.831
(µg p–nitrophenol	0.5 ppm	2.72 ± 0. 831	2.56 ± 0.342	2.23 ± 0.493
liberated^{-1}µg^{-1}) (30 min)	1.0 ppm	2.72 ± 0. 831	2.22 ± 0.493	4.81 ± 0.059
	2.0 ppm	2.72 ± 0.831	2.51 ± 0.559	5.67 ± 1.243

Table 7. The level of liver ACPase (Mean ± SE) in control and experimental fish

Parameter	Experimental group	Day 0	Day 7	Day 14
Liver ACPase	Control	5.45 ± 0. 014	5.45 ± 0.014	5.45± 0.014
(µg p – nitrophenol	0.5 ppm	5.45 ± 0. 14	**14.5 ± 0.293	5.41 ± 0.013
liberated^{-1} µg^{-1}) (60 min)	1.0 ppm	5.45 ± 0. 14	10.98 ± 0.154	4.23 ± 0.006
	2.0 ppm	5.45 ± 0. 14	7.89 ± 0.567	**2.71 ± 0.015

** ($p<0.01$)

observed at all exposure levels on 14th day when compared to control (Table 8).

Table 8. The level of muscle ACPase (Mean ± SE) in control and experimental fish

Parameter	Experimental group	Day 0	Day 7	Day 14
Muscle ACPase	Control	7.27 ± 0. 279	7.27 ± 0.279	7.27 ± 0.279
(μg p–nitrophenol	0.5 ppm	7.27 ± 0. 279	3.67 ± 0.025	**14.5 ± 0.300
liberated^{-1} μg^{-1}) (60 min)	1.0 ppm	7.27 ± 0. 279	5.98 ± 0.223	**13.78 ± 0.524
	2.0 ppm	7.27 ± 0. 279	4.81 ± 0.302	**15.17 ± 0.390

* ($p<0.01$)

Discussion

Sub-lethal doses of mercuric chloride produced severe biochemical abnormalities in blood, liver and muscles that were ultimately reflected through decrease in fish growth (Shakoori *et al*., 1994). In the present study also, mercuric chloride induced pronounced biochemical changes in *O. mossambicus* indicating altered metabolism. Mercuric chloride caused drastic depletion in liver and muscle protein at all exposure levels. Proteins are highly sensitive to heavy metal poisoning (Jacobs *et al* ., 1977) Depletion of protein content has been observed in the muscle, intestine and brain of *Catla catla* as a result of mercury chloride toxicity. The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of mercury chloride at the lower exposure period (Shakoori *et al*., 1994). Depletion in protein level in the exposed fish could be either due to arrested metabolism or owing to its utilisation to build up new cells or enzymes in order to combat the stress (Sakar and Al lail, 2005). The rapid depletion in total protein content due to active degradation of proteins under stress is dependnt on the development of resistance towards the pollutant stress. The decrease of total protein might be attributed to the destruction or necrosis of cells and consequent impairment in protein synthetic machinery (Mehra and Kanwar, 1980; David *et al*., 2004). When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted (Neff, 1985). On the contrary, Martin and Arivoli (2008) observed an increase in protein content at increased exposure period of 96 h at 0.1, 0.3 and 0.5 mg l^{-1} of mercuric chloride. These indicate that mercury induces proteolysis in the fish even under sub-lethal toxic stress resulting in elevated levels of protein.

Glycogen is one of the immediate fuel reserves and an important constituent which can be influenced by stress. In the present study, the liver and muscle glycogen levels were depleted at all exposure levels in accordance with earlier reports (Sastry and Rao, 1981). Liver is the vital metabolic and detoxifying organ in the body. The effect on the liver glycogen level observed in the present study, indicated that liver is the most affected organ with great loss of glycogen during stress period. Mercuric chloride induced similar effects on the total carbohydrate in muscle and intestine tissues of *Catla catla* at 0.1, 0.3 and 0.5 mg l^{-1} sub-lethal concentration (Martin and Arivoli, 2008). The disturbance in the carbohydrate metabolism is considered as one of the most outstanding biological lesions due to the action of heavy metal (De Bruin, 1976). The decrease in carbohydrate content in the muscle, intestine and brain may be due to glucose utilisation to meet excess energy demand imposed by severe anaerobic stress of mercury intoxication (Margarat and Jagadeesan, 1999). Another possible reason for depletion in the tissue may be the impairment of glycogen synthesis. Under hypoxic conditions; fish derive the energy by anaerobic breakdown of glucose which is available to the cells with the increased glycogenolysis. The observed depletion of glycogen in the present study explains the increased demand of these molecules to provide energy for the cellular biochemical process under toxic manifestations (Martin and Arivoli, 2008). Similar results were observed in *Thalmile crenata, Anabas testudineus* and *Anabas scandens*, when exposed to copper, lead, nitrate and mercury chloride, respectively (Villalan *et al*., 1988; Candravathy and Reddy, 1991).

In the present study, alkaline phosphatase activity was found to alter in a dose–dependent manner. The enzyme activity was stimulated at lower concentrations and suppressed at higher concentrations especially in liver. Depletion in ALPase activities was reported in *Heteropneustes fossilis,* exposed to 0.3 mg l^{-1} of mercuric chloride for 7, 15 and 30 days (Gupta and Sastry, 1981). Significant decreases in the activities of alkaline phosphatase was also observed in *Sarotherodon mossambicus,* exposed to 1.5 ppm of mercuric chloride (LC_{50}/48 h) (Akhilender *et al*., 1984). Contradictory to these studies, goldfish (*Carassius auratus*), when exposed to 0.25 μg or 0.30 Hg^{2+} l^{-1} for 3 or 6 weeks showed increased alkaline phosohatase activity in liver and muscle (McEwen *et al*., 1989). Decrease in ALPase activity may be taken as an index of hepatic parenchymal damage and hepatocytic necrosis (Onikienko, 1963). Inhibition of ALPase reflects alteration in protein synthesis and uncoupling of oxidative phosphorylation (Verma *et al*., 1984).

Acid phosphatase is a marker enzyme of lysosomes and exists in a latent form. Stimulation or inhibition of this enzyme can result in the disturbance of metabolism. Changes in the activities of acid phosphatase in the present study seem to be characteristic of tissue damage. Increased liver ACPase activity was associated either with the decrease in stability of liver lysosome membrane or with liver damage (Moraes *et al*., 1998). It has been speculated that the acid phosphatase elevation reflects proliferation of lysosomes in an attempt to sequester the toxic chemicals (Gill *et al*., 1992). Increase in ACPase activities in liver tissue of *S. mossambicus* on exposure to sub-lethal concentration of 1.5 ppm of mercuric chloride (LC_{50}/48 h) has been reported (Akhilender *et al*., 1984). From the above discussion, it can be inferred that exposure to sub–lethal concentrations of mercuric chloride adversely affected the metabolic activities in *O. mossambicus*.

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