Toxicity of Mercuric Chloride ($HgCl_2$) on Total Protein of Liver, Kidney and Gonads of Fish *Heteropneutes fossilis* (Bloch.)

1- Dr. Pratibha Pathak, 2- Dr. Rishikesh Kumar
Department of Zoology, MLSM college, LNM University, Darbhanga.

Abstract

The current study performed the examine toxicity effect of mercuric chloride on fish. The alterations induced by chronic (30 days) exposure of the fish Heteropneutes fossilis to a sublethal concentrations (0.25 ppm conc.) of mercuric chloride on the profile of total protein in the liver, kidney and gonads. The liver showed significant depletion of total protein content. The present study therefore points towards a severe metabolic dysfunction in response to mercuric chloride toxicity in the fish Heteropneutes fossilis (Bloch.)

Keywords:, *Heteropneutes fossilis*, Mercuric chloride , Protein contents, Toxicity.

INTRODUCTION

Aquatic systems are exposed to a number of pollutants hazards that are mainly released from effluents from industries' discharge, sewage treatment plants and drainage from urban and agricultural areas. Aquatic contamination by heavy metals is a widespread phenomenon. Since heavy metals are not destroyed in living organisms through biological degradation, they have the ability to accumulate in various tissues and organs and even be biomagnified in the food chain (Saaba A., 2015).

Mercury is viewed as the most toxic inorganic pollutant available in natural water (Ramdevi & Shrinivasan, 2005). Mercury chloride is danger, may be fatal if swallowed. Harmful if inhaled or absorbed through sin caused severe irritation to eyes, sin and respiratory tract. Mercury chloride is highly toxic not only acute but as a cumulative poison. Mercury is a dangerous pollutant, is one of the worst offenders, mainly after the environmental disaster at Minamata (Japan) and several other poisoning accidents due to the use of Hg pesticides in agriculture (Begam M, Sengupta M, 2015). It is widely known that fish are a great source of Hg in our food and their accumulation could represent a serious risk for human beings (Rice, *et al.,* 2014). Although fish have always been perceived as a healthy and nutritive food, the Environmental Protection Agency (EPA, 2016), has raised public concern as it claimed that the levels of Hg in certain fish species make them unsuitable or be restricted for children and pregnant women consumption (Serra-Majem *et al.,* 2007). This element has a long-biological half-life in humans and it gets accumulated in vital organs-especially in liver and kidney throughout their lives (Sadeghi & Imanpoor , 2015).

The fish, *Heteropneutes fossilis* (Bloch), locally known as "Shinghi", having the presence of suprabranchial accessory respiratory organs, an air-breathing teleost and heavy metal mercuric chloride were selected for present study.

MATERIALS & METHODS

The air-breathing teleost *Heteropneutes fossilis* procured live from the local fish market, Darbhanga were washed with 0.1% $KMnO_4$ solution to remove dermal infection if any. Healthy fish of average length (15–20 cm) and weight (25–30 g) were acclimated for 15 days to laboratory conditions. The fish were fed with chopped goat liver every day adlibitum. Running tap water was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature. No aeration was done.

Static acute bioassays were performed to determine LC_{50} values of mercuric chloride for 24, 48, 72 and 96 hours following the methods of APHA, AWWA & WPCF (1985; 1992). The LC_{50} values for these periods were 2.0 ppm, 1.5 ppm, 1.0 ppm and 0.5 ppm respectively. The sub-lethal concentration was determined following the formula of Hart *et al.* (1945). Twenty acclimated fish were exposed to a sub-lethal concentration (0.25 ppm) of mercuric chloride for 30 days. Side by side same number of fish as that of experimental one was maintained as the control group. At the end of exposure period the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. The liver, kidney, testis and ovary were quickly dissected out, weighed to nearest mg and processed for the quantitative estimation of total protein by the methods of Varley *et al.* (1980).

RESULTS

The protein profiles of liver, kidney, testis and ovary in response to mercuric chloride exposure *H. fossilis* showed a significant decline. Total protein in the control liver, kidney, testis and ovary was estimated to be 40.11±1.87, 50.92±0.1, 30.41±1.47, and 35.93±1.95 respectively. As against there, the total protein profiles in the experimental lots were 25.58±2.57, 40.97±2.53, 1.08±1.26

and 30.93±1.90 respectively (Table-I). The liver showed statistically more significant decline. The liver and testis showed statistically more significant ($P < 0.001$) decline i.e. 36% in , while 30% in liver. The kidney showed decline 19% while ovary 17%.

TABLE – I

Profiles of total protein (mg/g wet tissue) in tissue of *Heteropneutes fossilis* chronically exposed to mercuric chloride(0.25 ppm) for 30 days. Values are mean ± SE 0f 5 observations.

Tissue	Control	Mercuric chloride
Liver	40.11±1.87	25.58±2.57 (-36.22) ***
Kedney	50.92±0.1	40.97±2.53 (-19.54) ***
Testis	30.41±1.47	21.08±1.26 (-30.94) ***
Ovary	35.93±1.95	30.93±1.90 (-13.91) ***

Value indicate %(+), decrease (-) over control value significant at

** =P<0.05

*** =P<0.001

DISCUSSION

The level of tissue protein in control fish recorded in the present study indicates that total proteins are the largest contributors to the wet weight of the tissues after water. Previous workers have also reported decline in tissue protein profiles in a number of fish species exposed to various heavy metal pollutant and mercuric chloride. Ramalingam and Ramalingam (1982) noted a steady decline in the total protein of liver and muscle after 7 and 15 days exposure of the fish, *Sarotherodon mossambicus* to malathion and mercury and correlated it with an intensive proteolysis. Similarly, a significant decrease in the protein content was recorded by Kumar and Ansari (1984) in the Zebra fish, *Brachydanio rerio*, exposed to malathion and suggested inhibition of protein synthesis by the toxicant. Proteins being involved in the architecture and physiology of the cell seem to occupy a key role in the cell metabolism. The observed significant depletion of tissue protein in the present case denotes high catabolic potency of those organs and may be attributed to the intensive proteolysis and utilization of their degradation products for metabolism under the toxic influence of mercuric chloride. The loss of gonadal proteins may also be associated to the direct action of pyrethroids leading to arrest of vitellogenesis in ovary and loss of germ cells in

testis (Jha and Jha, 1995). Moreover, the decreased protein contents might also be attributed to the tissue destruction, necrosis, or disturbance of cellular function and consequent impairment in protein synthetic machinery (Srivastava, *et al*., 1995). The liver of *Clarias gariepinus* exposed to the cypermethrin showed hyperplastic hepatic and necrosis of hepatic cells (Andem A. B. *et al.* 2016). The toxicity was found to increase with mercuric chloride concentration, various structural changes were already induced on the morphology of the vital organs, i.e. gill, liver and kidney even with exposure to low, sublethal mercuric chloride concentration. On the other hand, severe histological lesions in the gonad have been observed in fish after Hg exposure *in vivo*. In testis, exposure to $HgCl_2$ caused the thickening of the tubule walls and disorderly arranged spermatozoa in zebrafish (Zhang, *et al*., 2016) probably resulting in inhibition of spermatogenesis. In ovary, degenerative changes such as atresia (follicular degeneration) were found after $HgCl_2$ exposure in zebrafish in agreement with other studies (Vergilio , *et al*.,2013).

Hg produce an imbalance between the reactive oxygen species (ROS) production and its ROS imbalance leads to cell death by apoptosis (Kim & Sharma, 2004). Hg forms induce apoptosis by inhibiting mitochondrial function. This cell death mechanism has been demonstrated in fish exposed to Hg also reported by Zheng, *et al.*,(2014). The observed significant depletion of tissue protein in the present case denotes this cell death mechanism.

The estimation of total protein was recorded significantly decreases under the experimental concentration and duration of mercuric chloride exposure. Previous workers have similar reported decline in total protein of different tissue profiles in a number of fish species exposed to various heavy metal and mercuric chloride. The decrease might have occurred mainly due to altered protein metabolism and energy demand in fishes under stress of toxicants.

CONCLUSION

The test fish *Heteropneutes fossilis* when exposed to sub lethal concentration of protein (0.25 ppm) for 30 days, significant decrease in total lipid and total protein content in the tissue of all four organs, liver, kidney, testis and ovary. The depletion might have occurred mainly due to altered protein metabolism and energy demand in fishes under stress of toxicants and cell go under apoptosis.

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