Biochemical changes of mercuric chloride on blood metabolite levels of freshwater Fish *Heteropneutes fossilis* (Bloch.)

1-Dr. Pratibha Pathak, 2-Ajay K. Anand
Department of Zoology, MLSM college, LNM University, Darbhanga.

Abstract

The current study performed the examine 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 blood metabolites. The experimental fish become hyperglyceric as evident by a highly significant ($p < 0.001$) elevated level of glucose in the blood, which counts to an increase of 28.53% and the serum cholesterol has been found to significantly increase by 13.07% showed hypercholestrolemic response as evident by significant ($p< 0.05$) increase while protein level depleted 37.93%. 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 , blood metabolites, total serum protein.

INTRODUCTION:

Rapid industrialization and urbanization have led to the utilization of heavy metals including mercury on a larger scale. These metals ultimately enter the aquatic ecosystems directly as effluents or indirectly by precipitation, thereby causing deleterious effects to aquatic life at an alarming level. 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). They are also harmful to humans who are relay on aquatic products as food sources. 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 (Ye J, *et al.* 2007).

Mercury (Hg) is considered a devastating environmental pollutant, 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). The toxicity of mercury depends greatly on the forms of the mercury compounds

(inorganic and organic). Both inorganic and organic mercury in waters pose considerable risk to aquatic biota since mercury in both forms is cumulatively toxic (Skubal and Meshkov 2002).

The fish, *Heteropneutes fossilis* (Bloch), locally known as "Singhi", having the presence of suprabranchial accessory respiratory organs, an air-breathing teleost and 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). 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. On 30^{th} day blood samples was extracted from the caudal dorsal of the test fish and were then processed for quantitative estimation of blood glucose (Sinha, 1990), serum protein ('Biuret method' of Varley *et. al.*,1980) and serum cholesterol (Kabara's method,1966).

RESULTS:

FISH BEHAVIORAL RESPONSE :

The control fish shows a tendency to remain at the bottom of the aquarium with little disturbance. However, mortalities were removed immediately, and behavioural abnormalities were assessed at these regular intervals using a modified behavioural protocol checklist (Klesius *et al*. 2000). Scores were assigned daily to individual fish in the experiment and were based on the following scoring system: 0, no observed changes in behaviour; 1, swimming abnormally, lethargic or unresponsive, changes in skin coloration; 2, hyperactive or excitable, rapid operculation; 3, death. Mean behaviour scores were calculated per replicate treatment.

Just after introduction to test solution fishes showed increased swimming, surfacing and hyperactivity. Restlessness, rapid surfacing, peeling of skin and colour fading were prominent after 24 hrs exposure. After 48hr exposure the fishes showed slightly reduced activity and gradual increase in colour fading. Gill adhesion and a thin film of mucous were noticed on gills, operculum and general body surface at this stage. After 72h

exposure increased surfacing and gulping of air was observed. At this stage fishes showed loss of balance and jerky movements during swimming. The school formation, a characteristic of this fish, was found weakened in test animals as compared to controls at this stage. After 96h ulceration on trunk, base of caudal and pectoral fins were prominent in 95% of the animals. A thick film of mucous on whole body and gills was observed in almost all test fishes. Test fishes lost their natural colouration and. Loss of equilibrium before death is a symptom shown all the test fish. Similar steps of behavioral response also found in sublethal dose of mercuric chloride i.e. 0.25 ppm for 30 days of duration of experiment.

BLOOD BIOCHEMISTRY STUDIES

The carbohydrates, proteins and lipids are the three principal constituents of the blood. These are also known as metabolites and play a major role as energy precursors for fishes exposed to stress conditions. The optimum concentrations of these biomolecules are required for the proper and normal functioning of a cell. Any alteration in their organization or concentration may therefore, disturb the normal physiology of the cell. Therefore, the ultimate search for any disruptive process brought about by the chemicals must be made within the cell. One of the probable and logical ways which may eventually lead to an understanding of the events that follows fertilizers treatment could be an examination at biochemical level.

BLOOD METABOLITE LEVELS:

It was thought worthwhile to identify the metabolic dysfunctions in the non-target species, the *H. fossilis* in the present case challenged with a chronic sub-lethal concentration (0.25 mg/l) of mercuric chloride. The variables monitored at blood metabolites levels are blood glucose, serum protein and serum cholesterol of experimental fish *H. fossilis.* On exposure to a sub-lethal concentration (0.25 ppm) of mercuric chloride the fish reveals following changes in the biochemical parameters.

BLOOD GLUCOSE:

The blood glucose level in the control fish group is assessed to be 70.66±0.83 mg/100ml of blood. The experimental fish become hyperglyceric as evident by a highly significant ($p < 0.001$) elevated level of glucose in the blood 90.82± 1.41 mg/100ml which counts to an increase of 28.53% (Table-1) the present values of the present observations are expressed as meant S.E. of 5 fish in each group.

SERUM PROTEIN:

Contrary to the elevated blood glucose level the experimental fish group show highly significant ($P< 0.001$) depletion in the level of serum protein in control group estimated to be 6.01 ± 0.37 g/100 ml as against 3.73 ± 0.15 g/100 ml in the treated group (Table-1).

SERUM CHOLESTROL:

The serum cholesterol level in the fish of control group has been analysed 205±2.08 mg/100 ml. The treated fish show hypercholestrolemic response as evident by significant ($p < 0.05$) increase (231.8±1.96 mg/100 ml) in its level (Table-1) the serum cholesterol has been found to significantly increase by 13.07% in the present case.

Tables:-1

Changes in the blood / serum metabolite levels in *H. fossilis* exposed to mercuric chloride **(0.25 ppm) for 30 days. Values are mean ± SE of 5 observations.**

Parameters	**Control**	**Mercuric chloride exposed**
Blood glucose (mg/100 ml)	70.66±0.83	90.82± 1.41(+28.53) ***
Serum protein (mg/100ml)	6.01 ± 0.37	3.73 ± 0.15 (- 37.93) ***
Serum cholesterol (mg/100 ml)	205±2.08	231.8±1.96 (+13.07) *

Values indicate percent increase (+)

Or decrease (-) over control values significant at

* *P<0.05*

*** *p<0.001*

DISCUSSION :

BLOOD GLUCOSE:

Blood glucose level in serum of *H. fossilis* after treated with different concentration of mercuric chloride showed highly significant increases (P<0.001) (90.82± 1.41 mg/100ml), in all groups compared to the control fish group (70.66±0.83 mg/100ml). Mercuric chloride caused an effect is known to increase the levels of activating glycogenolysis and glyconeogenesis with a net result of increasing plasma glucose levels. These results confirm the corticosteroid response to high ammonia observed by Tomasso *et al.* (1981). Blood

glucose increased due to stimulation of glucocorticoids in stressed catfish. Davis *et al.* (2003) reported that High NH_3-N concentration caused a significant increase in plasma glucose concentrations in both PC and isoeugenol treated catfish. Recently Jha (2009) and Poonam *et al.* (2010), Singh and Chaudhary (2011), Koley and Kumar (2012) and Pratibha & Kumar, (2013) has observed similar result under the exposure of Nuvan, fenvalerate and Eklaux in various fresh water fishes.

However, the hyperglycaemic response in mercuric chloride treated fish *H. fossilis* indicated an imbalance of glucose homeostasis which might be due to decreased utilization of glucose (glycolysis) or increased glucose formation (gluco-neogenesis). Blood glucose concentration of fish can be decreased by starvation or increased in response to acute and chronic stressors. Changes in blood glucose concentration is the most widely recognized and consistent response to stressor. An increase in the O:N ratio represents an increase in the catabolism of lipids and carbohydrates (*Zhang et al*., 2017).

The present study also suggests the involvement of adrenal pituitary glucocortcoied axis and the depletion of energy stores and resultant debility under chronic mercuric chloride exposure. Again the tissue acidosis might have favoured glycogenolysis by changing the pH of the blood thus, disturbing the buffering system of organs. It is further assumed that the activation of glycogen phosphorylase and depression of glycogen transferase might have been caused by the toxic stress as the former is known to accelerate glyconesis and the latter limits the glycogen storage.

PLASMA PROTEIN:

In the present investigation decreased levels of plasma proteins was observed. Serum proteins appear to be very sensitive to mercuric chloride exposure as is evident by the apparent fall (37.93%) in its content. The decrease in serum protein as observed during the present study suggests that the detoxification/ degradation of the toxicant either took place partially in the blood itself or involved the serum protein. This fall further suggests a progressive protein degradation or biochemical transformation of the protein Nitrogen into other nitrogenous products as suggested by Rao *et al.* (1983). Recently Jha (2009) and Poonam *et al.* (2010), Singh and Chaudhary (2011), Koley and Kumar (2012), Pratibha & Kumar, (2013) and Ahsan (2016) has observed similar result under the exposure of Nuvan, fenvalerate, Atrazine and Eklaux in various fresh water fishes.

We know that the plasma protein provide colloid, osmotic pressure, transport hormone and also act as buffer. It is likely that the metal could have caused drastic changes in the permeability of the membrane of the fish body surface thereby greatly altering water and ionic balance. Rao *et al.* (1983) found significant fall in Na^+, K^+ and Ca^{2+} in *Channa punctatus* exposed to parathion.

Again we know that the serum proteins of the fish are of hepatic origin. A depletion in the total serum protein level may be due to possible reduction or stoppage of their synthesis by liver cells due to direct toxic actions of the ammonia on the hepatocytes. It is possible that in *H. fossilis* part of the depletion may be due to the

utilization of serum protein as energy reserves to meet the higher energy demands of fish under mercuric chloride exposure.

Significant decrease in total plasma protein was recorded and it was found that the percent decrease was higher in 120 days exposure period (68.2%) then 96 hour (46.4%) in fish *Channa punctatus* exposed to 11.12 ppm sublethal concentration of cadmium (Sastry & Shukla 1990). In agreement with Sastry & Shukla (1990) decrease in 30 days of exposure of cadmium chloride at sublethal concentration of 1.32 ppm to *C. punctatus* was recorded.

Apoptosis seems the probable cause as another explanation also in which "decision" for apoptosis can come from the cell itself, from the surrounding tissue or from a cell that is part of immune system. In these cases apoptosis were functioning to remove the damaged cell, preventing it from sapping further nutrients from the organism.

SERUM CHOLESTEROL:

The present investigation exhibited hypercholesterolemia (Table-1) under the toxic influence of endosulfan and in agreement with the findings of Verma *et al.*(2002); Ayyaduri, (2004); Das *et al.* (2004); Prakash & Maheshwari, (2005); Prakash *et al.*,(2006); Kalaivani *et al.*,(2008) and Jha & Jha, (2010); Jha (2009) and Poonam *et al.* (2010); Singh and Chaudhary (2011), Koley and Kumar (2012) and Pratibha & Kumar, (2013), Sunita Rani, *et al*.b (2015), and Dilip, M. and Vidya B. (2016) has observed similar result under the exposure of Nuvan, fenvalerate and Eklaux in various fresh water fishes.

The elevated blood cholesterol level may be due to the hypermetabolic state of the fish or due to the impaired liver function as suggested by Holmberg *et al.* (1972). Again the increase in cholesterol may be due to its increased synthesis in the liver as the precursors for interval hormones caused by the toxicant. Part of this increased cholesterol store might have found its way into the blood as suggested by Ahsan & Ahsan (1988); and Madhavan P, Elumalai K. (2016).

The carbohydrate (glucose) also is converted by adipose tissue into fatty acids and stored as triglyceride. These two process- up take of fatty acids and glucose from blood and their synthesis into triglyceride are enhanced by the action of the hormone "insulin". Several hormones such as epinephrine, norepinephrine, glucagons, ACTH, GH and thyrotropic hormone stimulates the release of fatty acids from adipose tissue (Popjak and Grant, 1963).

Triglycride synthesis & Lipolysis do not follow the same path way in the adipose tissue. Adipose tissue does not possess 'glycerokinase enzyme', hence it has to depend on the production of de- hydroxyacetone phosphate by glycolysis.

This interrelationship between FFA and glucose level (as we have seen in the case of diabetes in the present experiment) in plasma and their up take & utilization by adipose tissue & other tissues is referred as "Glucose-Fatty – Acid-Cycle". Lipolysis in adipose tissue is brought about by "hormone-sensitie-lipase" mediated by C. AMP in that tissue increasing FFA in plasma. Adipokinin secreted by anterior pituitary also enhance the FFA levels by increasing lipolysis of fat depot. Glucose or insulin decreases free fatty acid level as in diabetes mellitus where as EN or NEP from adrenal or ACTH and TH all increases FFA level in plasma.

CONCLUSION:

It could be concluded that *H. fossilis* with average weight 25.0± 5.0 g, were more suitable to culture at water mercuric chloride concentration of < 0.25 mg/l for optimum growth performance and survival rate than other water conditions. Therefore, it can be recommended to be carried out under the similar experimental conditions.

ACKNOWLEDGEMENT:

The authors are thankful to the Department of Zoology, MLSM, LNM University, Darbhanga, for the provision of laboratory facilities used in this study.

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