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Research Article

IMPACT OF MERCURY CHLORIDE ON PROTEIN AND AMINO ACID PROFILES IN BRAIN AND MUSCLE OF FRESHWATER FISH *HYPOPHTHALMICHTHYS MOLITRIX* (VALENCIENNES)

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ABSTRACT

Pollution is the negative feedback of the environment which affects living organisms. In recent times interest has been focused on rivers and estuaries as these are considered major sources of pollutants of aquatic medium. Human beings have been responsible for marine pollution, as they have introduced directly or indirectly, harmful waste substances into the marine environment. Estuaries and rivers have not been spared with the result the deleterious effects have paved the way for health hazards to human beings. Fish are one of the most widely distributed organisms in the aquatic environment and, being susceptible to metal contamination, may reflect the extent of the biological effects of metal pollution in waters. The effect of mercury chloride on protein and amino acid contents of brain and muscle of freshwater fish, *Hypophthalmichthys molitrix* has been studied. The fish were exposed to sublethal concentrations of mercury chloride 1/5th (high), 1/10th (medium) and 1/15th (low) of the 96-hour LC50 for the period of 10, 20 and 30 days. All the sublethal concentrations of mercury chloride exposed fish for the period of 10. 20 and 30 days showed decrease the protein and increase the amino acid content in brain and muscle of *Hypophthalmichthys molitrix*. The significant alterations showed toxic effect of heavy metal mercury chloride at biochemical levels.

Keywords: Brain, Muscle, Mercury chloride, Protein, Amino acids, *Hypophthalmichthys molitrix.*

INTRODUCTION

Pollution is the changes that occur in physical, chemical and biological characteristics of the environmental system. Environmental contamination of air, water, soil and food have been still the most important subject in recent years because it causes threat extend to many plants and animals and may ultimately threaten the survival of humanity and results from direct and indirect human activities (Salem, 2003). Since intensive farming practices are essential to produce enough food for the increasing population, farmers have been using more inorganic fertilizers, pesticides and herbicides (Tong and Naramngam, 2007). Many of heavy metals such as mercury, lead, nickel, chromium and cadmium have no nutritional importance and their presence in relatively high concentration in body tissues can result in health problems in human as well as in animals (Goldfrank *et al*., 2001). The over accumulation of these heavy metals in tissues of animals has received considerable attention, partly because lethal and sub-lethal effect of such accumulation (Klaverkamp *et al*., 1984). The levels of these metals in water may not be lethal to these organisms but the concentration of such metals in their tissues creates hazards when used as food for human consumption (Carbonell *et al*., 1998).

Industrial development, agriculture and increasing urbanization are associated with environmental pollution. This leads to changes in ecosystems and the deterioration of food products of plant and animal origin. One of the most common environmental pollutants is mercury. The presence of mercury in the environment is partly due to its natural occurrence, but mainly due to anthropogenic activities. Its relatively high persistence in the environment, as well as its mobility and toxic effects, mean that mercury can pose a risk to human health. An important source of

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human exposure to mercury is food, mainly of aquatic origin. Mercury is a metal with toxic effects on the environment, including living organisms. Organic Hg derivatives readily penetrate biological membranes and pose a particular health risk. Food of aquatic origin is the main source of human exposure to methyl-mercury (Barbara and Agnieszka, 2023). Mercury is a naturally occurring element that humans have discharged into the environment from geologically stable forms of actions. In addition to having the ability to directly harm lower tropic levels, such as benthic invertebrates, mercury biomagnifies via the food web comparing the chemical concentrations in sediment to sediment is frequently the first step in screening for hazards that chemicals in sediment pose to benthic invertebrates. Every environment on this planet is contaminated with heavy metals. Heavy metals like mercury, cadmium, copper, zinc, nickel, chromium and lead, which are dispersed globally in the atmosphere, are present in even the most isolated places, like Antarctic, in amounts higher than those predicted for the area. Therefore, environmental organizations increasingly focus on determining the extent of pollution rather than whether an environment is contaminated (Paloma de Almeida Rodrigues *et al*., 2019) Generally, there are two major categories of contaminants that harm to the ecosystems. The compounds found in the wastewater generated by major cities, particularly those connected to the inappropriate disposal of solid wastes and the poor sanitary wastewater treatment. These places were most severely impacted by the pollutants in the rivers, estuaries and oceans (Luma *et al*., 2025). Even at sub lethal concentrations, mercury is exceedingly toxic to fish and changes the physiology, biochemistry and structure of the nervous system. It accumulates in fish neural tissues because it can cross the blood-brain barrier (Luma *et al*., 2025).

MATERIALS AND METHODS

Sample Collection

The fish *Hypophthalmichthys molitrix* having mean weight 14 -16 gm and length 12-14 cm were collected from PSP fish farm, at Puthur and acclimatized 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 seven days. They were fed on rice bran and oil cake daily. The mercury chloride was used in this study and stock solutions were prepared.

Study plan

Mercury chloride LC50 was found out for 96 h (2.60 ppm) (Sprague, 1971) and 1/5th ,1/10th and 1/15th of the LC50 values were 0.13, 0.26 and 0.39 ppm respectively taken as sublethal concentrations for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from mercury chloride and served as the control. The other 3 groups were exposed to sub lethal concentrations of mercury chloride in 10-liter capacity aquaria. The 2nd, 3rd and 4th groups were exposed to mercury chloride for 10, 20 and 30 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein estimation.

LC50 determination

The mercury chloride was used in this study and stock solutions were prepared. Mercury chloride LC50 was found out for 96 h (2.60 ppm) and 1/5th ,1/10th and 1/15th of the LC50 values were 0.13, 0.26 and 0.39 ppm respectively taken as sublethal concentrations for this study. Fishes were exposed to sublethal concentrations of mercury chloride separately in plastic troughs and control fishes were also maintained separately. They were fed on ad libitum diet of rice bran and oil cake. The medium was renewed daily with sublethal concentrations of the mercury chloride. After the exposure period, *Hypophthalmichthys molitrix* were sacrificed and the brain and muscle were removed for biochemical assays. The protein and amino acid content of the tissues were estimated by the method of Lowry *et al.* (1951) and Moore and Stein (1954) respectively.

Statistical analysis

The values are expressed as mean ± SE. Data were statistically analysed by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) (Duncan, 1957) which was applied to find out significant difference between various treatment means and control means for the observed parameters.

RESULTS AND DISCUSSION

The present results raveled that mercury chloride induced alterations are time dependent, tissue-specific, and they point to altered protein metabolism has shown significant elevation of amino acid and decrease the levels of protein in brain and muscle of *Hypophthalmichthys molitrix* exposed to low, medium and high sublethal concentrations of mercury chloride for the period of 10, 20 and 30 days (Table 1 and 2). Mercury pollution in aquatic ecosystems has received a great deal of attention since the discovery of mercury as the cause of Minimata disease in Japan in the 1950s (Allen *et al*., 1988). The fate of mercury in the environment depends on the chemical form of mercury released and the environmental conditions. The elemental mercury, the inorganic mercury and the methyl-mercury are the three most important forms of mercury in natural aquatic environments. Most mercury is released into the environment as inorganic mercury, which is primarily bound to particulates and organic substances and might not be available for direct uptake by aquatic organisms (Beckvar *et al*., 1996).

Toxicity is influenced by the form of mercury, environmental media, environmental conditions, sensitivity or tolerance of the organism, and its life history stage. The inorganic mercury is less acutely toxic to aquatic organisms than methyl-mercury, but the range in sensitivity among individual species for either compound is large. Fish is an essential part of a balanced human diet. They are a source of healthy protein, polyunsaturated fatty acids, minerals and vitamins (Barbara and Agnieszka, 2023). Fish is a food rich in proteins of high nutritional value, lipids, minerals and vitamins. Proteins are used to synthesize digestive enzymes, hormones and to repair and maintain tissues, such as skin, muscles and bones (Manz *et al*., 2023). Proteins are basic molecules to any living system. In cells they function as enzymes, structural materials, lubricants and carrier molecules (Muneesh kumar *et al*., 2017). In recent decades, the consumption of fish worldwide has been growing rapidly due to their nutritional benefits and high-quality proteins. However, heavy metals contamination of fish has caused a great global concern, which also poses a health threat to human health. Fish can absorb heavy metals from surrounding water, sediment and their diet, large or improper consumption is likely to cause adverse effects on human body, therefore, it is important and necessary to determine the accumulation of heavy metals contents in the widely consumed economical fish species (Huang *et al*., 2022). Fish have ability to accumulate toxic metals and it is of absolute importance that the content of undesirable metals is low. Heavy metals and trace elements have certain effect on ecosystem as well as human beings (Murthy *et al*., 2016).

Table 1. Protein level changes (mg/g) in gill, liver, kidney, brain and muscle of *Hypophthalmichthys molitrix* exposed to sublethal concentrations of mercury chloride.

Treatments	10 days	20 days	30 days
		Brain	
Control	80.81 ± 5.92^{b}	81.64 ± 6.00^{c}	82.21 ± 6.03^{c}
Low concentration	76.51 ± 5.60^{ab}	71.67 ± 5.23^{b}	66.67 ± 4.85^{b}
Medium concentration	73.63 ± 5.40^{a}	67.11 ± 4.90^{ab}	63.31 ± 4.60^{ab}
High Concentration	71.87 ± 5.24^{a}	64.73 ± 4.71^{a}	57.61 ± 4.23^{a}
		Muscle	
Control	74.71 ± 5.46^{a}	75.61 ± 5.52^{b}	75.77 ± 5.54^{c}
Low concentration	72.67 ± 5.30^{a}	69.81 ± 5.08^{ab}	64.61 ± 4.70^{b}
Medium concentration	69.10 ± 5.20^{a}	65.65 ± 4.90^{a}	61.67 ± 4.54^{ab}
High Concentration	67.85 ± 5.01^{a}	63.70 ± 4.77^{a}	58.57 ± 4.23^{a}

All the values mean ± SD of six observations. Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$). Duncan multiple range test (DMRT).

Table 2. Amino acid (mg/g) in gill, liver, kidney, brain and muscle of *Hypophthalmichthys molitrix* exposed to sublethal concentrations of mercury chloride.

Treatments	10 days	20 days	30 days
		Brain	
Control	3.57 ± 0.25^{a}	3.59 ± 0.25^{a}	3.54 ± 0.25^{a}
Low concentration	3.94 ± 0.27^{b}	4.55 ± 0.33^{b}	6.03 ± 0.44^{b}
Medium concentration	4.31 ± 0.31^{c}	4.89 ± 0.35^{c}	6.55 ± 0.47^{c}
High Concentration	5.04 ± 0.37^{d}	5.75 ± 0.42^{d}	7.91 ± 0.60^{d}
		Muscle	
Control	3.39 ± 0.24^{a}	3.37 ± 0.24^{a}	3.40 ± 0.24^{a}
Low concentration	3.93 ± 0.27^{b}	4.41 ± 0.32^{b}	4.95 ± 0.36^{b}
Medium concentration	4.37 ± 0.31^{c}	4.74 ± 0.34^{c}	5.31 ± 0.38^{c}
High Concentration	4.54 ± 0.30^{d}	5.47 ± 0.40^{d}	6.63 ± 0.50^{d}

All the values mean ± SD of six observations. Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$). Duncan multiple range test (DMRT).

In the present investigation brain and muscle of protein content had decreased, whereas amino acids content had increased at 10, 20 and 30 days when *Hypophthalmichthys molitrix* was exposed with sublethal concentrations of mercury chloride. Similarly, protein levels were decreased and amino acid contents were increased significantly in gill, liver and kidney of *Hypophthalmichthys molitrix exposed* to sublethal concentration of cadmium chloride (Kamaraju and Ramasamy, 2018). Satyaparameshwar *et al*., (2006) suggested that *Lamellidens marginalis* exposed to sublethal concentration of chromium showed decrease the protein and RNA whereas amino acid level was elevated. The decreased protein level was observed in the kidney tissue of *Catla catla* at sublethal concentration of chromium (Vincent *et al*., 1995). Muscle rich in proteins, forms mechanical tissue intended for mobility and do not participate in metabolism. Liver being the centre for various metabolisms is also rich in proteins. In the gill, liver, kidney, heart and brain tissues of the exposed fish, the total protein content was found to be reduced (Sobha *et al*., 2007). The decreases in the total protein level and increases in the total free amino acid level in liver and muscle suggest the high protein hydrolytic activity due to elevation of protease activity (Zodape, 2011).

Similarly, Senthil Elango and Muthulingam (2014) suggested that declining trends of protein and elevated levels of amino acid in brain and muscle of *Oreochromis mossambicus* was exposed to sublethal concentrations of chromium. The protein content decreased in the liver, brain and kidney tissues of *Channa punctatus* during lihocin treatment (Abdul *et al*., 2010). Protein and glycogen contents were decreased in gill, muscle and intestine of Zebra fish, Danio rerio exposed to sublethal concentration of mercuric chloride (Vutukuru and Kalpana, 2013). The decreased in the protein content in the liver and muscle of *Channa punctatus* when exposed to distillery effluent (Maruthi and Subba Rao, 2000). The protein content decreased in the gill, liver and kidney tissues of *Oreochromis mossambicus* during nickel chloride treatment (Muthulingam *et al.,* 2015). A reduction in the protein content in the muscle and liver could possibly be due to protein breakdown leading to increase amino acid in Zinc sulphate exposed freshwater fish *Channa striatus* (Reddy and Devi, 2021). The reduction in protein content in the present study indicates that the tissue protein undergoes proteolysis resulting in the production of free amino acids. When the fish, *Hypophthalmichthys molitrix* exposed to sublethal concentration of mercury chloride, the brain and muscle amino acid level rapidly increased at all exposure periods. The elevated amino acid levels in the brain and muscle of *Hypophthalmichthys molitrix* exposed to sublethal concentration of mercury chloride indicate a high turnover of amino acids, which should normally lead to increased deamination and oxidation of amino acids

CONCLUSION

Protein levels were decreased and amino acid levels were enhanced due to proteolysis means break down of proteins and over production of amino acids utilized for energy production during stressful situation due to sublethal concentrations of mercury chloride exposed freshwater fish *Hypophthalmichthys molitrix*.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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