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Impact Of Heavy Metal, Mercury Chloride On Protein And Aminoacid Levels In Gill, Liver And Kidney Of Edible Exotic FISH *Hypophthalmichthys Molitrix* (Valenciennes)

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Abstract

Environmental pollution is a worldwide problem; heavy metals constitute one of the most important pollutant challenges. The progress of industry has led to increased emission of pollutants into ecosystem. Environmental pollution can cause poisoning, diseases and even death to fish. Heavy metal contamination has gradually become a very much important significant global issue due to its continual existence in the environment and bioaccumulation in the ecosystems, posing deleterious risks to human health. Mercury contamination is amongst the most significant and universal pollution problems in the aquatic medium It primarily occurs in the aquatic environment In industries, huge amounts of effluents containing mercury are discharged as a result of poor industrial operations, fertilizer industry, landfill leaching, and carbon combustion. Dead zones, otherwise termed as zones of oxygen-depleted water, have been reported to be the repository of huge deposits of inorganic mercury. 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 gill, liver and kidney of freshwater fish, *Hypophthalmichthys* molitrix have been studied. The fish were exposed to sublethal concentrations of mewrcury 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 gill, liver and kidney of *Hypophthalmichthys* molitrix The significant alterations showed toxic effect of heavy metal mercury chloride at biochemical levels.

KEYWORDS: Heavy metals, mercury chloride, protein, aminoacids, Hypophthalmichthys molitrix

INTRODUCTION

Expeditious expansion and industrial development near the rivers have led to more stress on the river, and with increased stress, the water becomes polluted, and worsening environmental health (Giri and Singh, 2014). The water-soil interface and the water-atmosphere interface are the medium through which the heavy metals travel (Ali, 2018; Varol, 2011). Both anthropogenic activities and geochemical processes are responsible for heavy metal contamination in ecosystems. Elements that have high density and are less noxious are known as heavy metals (Li et al., 2007).

Pollution of these heavy metals into the river may cause distressing effects on the ecological balance of the aquatic environment, and with the extent of contamination, the diversity of aquatic organisms becomes limited (Ay, 2009).. The fish in the aquatic ecosystem can be used for examining the well-being of biota. Due to pollutants in the food chain of organisms, harmful effects can be seen and the aquaculture can become dead (Sankhla and Kumar, 2020). These heavy metals are neurotoxins for the fish living in the aquatic environment. When these heavy metals enter the fish body, they interact with them to generate biochemical reaction inside the fish, which makes it difficult for fish to communicate with their surroundings (Baatrup, 1991). The presence of these heavy metals leads to diseases like Minamata, which is organic mercury poisoning. When these heavy metals get bioaccumulated, they become a threat to both the human population and animals who uses that water (Sonone, 2020).

MATERIALS AND METHODS

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. 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. 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 gill, liver and kidney 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

PROTEIN AND AMINO ACID

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 gill, liver and kidney 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).

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

Treatments	10 days	20 days	30 days
Gill			
Control	117.61 ± 8.72^{c}	118.66 ± 8.80^{c}	120.8 ± 8.96^{c}
Low concentration	100.14 ± 7.37^{b}	94.02 ± 6.93^{b}	90.51 ± 6.66^{b}
Medium concentration	93.00 ± 6.84^{ab}	85.31 ± 6.27^{a}	79.90 ± 5.85^{a}
High Concentration	90.10 ± 6.63^{a}	81.17 ± 5.95^{a}	74.70 ± 5.46^{a}
Liver			
Control	131.70 ± 9.80^{c}	135.10 ± 10.05^{c}	140.52 ± 10.46^{c}
Low concentration	112.50 ± 8.33^{b}	115.86 ± 8.60^{b}	104.62 ± 7.73^{b}
Medium concentration	103.42 ± 7.64^{ab}	102.70 ± 7.50^{a}	85.67 ± 6.30^{a}
High Concentration	97.64 ± 7.28^{a}	95.04 ± 7.00^{a}	76.63 ± 5.60^{a}
Kidney			
Control	87.07 ± 6.40^{a}	87.93 ± 6.46^{b}	87.31 ± 6.47^{c}
Low concentration	84.97 ± 6.24^{a}	83.20 ± 6.11^{ab}	78.90 ± 5.77^{b}
Medium concentration	81.50 ± 5.97^{a}	78.00 ± 5.71^{a}	74.05 ± 5.41^{ab}
High Concentration	80.71 ± 5.91^{a}	76.57 ± 5.60^{a}	68.60 ± 4.50^{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 and kidney of *Hypophthalmichthys molitrix* exposed to sublethal concentrations of mercury chloride

Treatments	10 days	20 days	30 days
Gill			
Control	4.27 ± 0.31^{a}	4.35 ± 0.31^{a}	4.31 ± 0.32^{a}
Low concentration	5.43 ± 0.40^{b}	6.23 ± 0.45^{b}	7.54 ± 0.55^{b}
Medium concentration	5.95 ± 0.43^{c}	7.07 ± 0.52^{c}	10.11 ± 0.75^{c}
High Concentration	6.41 ± 0.47^{d}	8.61 ± 0.63^{d}	13.24 ± 1.01^{d}
Liver			
Control	5.97 ± 0.43^{a}	6.01 ± 0.45^{a}	5.95 ± 0.43^{a}
Low concentration	7.03 ± 0.52^{b}	7.43 ± 0.54^{b}	9.64 ± 0.71^{b}
Medium concentration	7.95 ± 0.60^{c}	9.74 ± 0.72^{c}	11.53 ± 0.85^{c}
High Concentration	10.11 ± 0.75^{d}	13.55 ± 1.01^{d}	16.27 ± 1.22^{d}
Kidney			
Control	3.73 ± 0.22^{a}	3.80 ± 0.27^{a}	3.77 ± 0.27^{a}
Low concentration	4.27 ± 0.31^{b}	4.88 ± 0.35^{b}	6.09 ± 0.44^{b}
Medium concentration	4.91 ± 0.35^{c}	5.77 ± 0.42^{c}	6.84 ± 0.50^{c}
High Concentration	5.43 ± 0.40^{d}	7.51 ± 0.55^{d}	9.13 ± 0.67^{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)

DISCUSSION

CHANGES IN THE PROTEIN AND AMINO ACID LEVELS IN GILL, LIVER AND KIDNEY

Heavy metals get into the fish through three routes: the first is via the fish gills, the second is through the digestive tract of the fish and the last one is through the body of the fish. The gills of fish are the area that is known for the primary metal intake from the contaminated water (Beijer, 1986). The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. Aquatic animals inhabiting polluted water bodies tend to accumulate many chemicals in high concentrations even when the ambient environmental contamination levels are low potentially hazardous situation for the entire food chain. Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity. The biochemical processes represent the most sensitive and relatively early events of pollutant damage. Thus, it is important that pollutant effects be determined and interpreted in biochemical terms, to delineate mechanisms of pollutant action, and possibly ways to mitigate adverse effects. Contamination of heavy metals in the aquatic environment is very harmful since these elements cannot be degraded and they get accumulated inside the living organisms (Jardim, 1983).

Protein is one of the important biochemical components and plays an important role in metabolic pathways and biochemical reactions. Under extreme stress conditions, protein supply energy in metabolic pathways and biochemical reactions. Therefore, an assessment of the protein content in different tissues could be used as a diagnostic tool for determining the physiological status of an organism (Prasath and Arivoli, 2008; Maharajan *et al.,* 2012). Proteins are long chains of amino acids forming three dimensional structures. Proteins do play both structural and functional role of cellular level. Being an integral part of the cell membrane, intracellular and extra cellular passages are linked through it. Any sort of cellular metabolism occurring in body involves one or many different proteins. The proteins are among the most abundant biological macromolecules and are extremely versatile in their function and interaction during metabolism of proteins, amino acids, enzymes and co-enzymes (Harper *et al.,* 1978; Anilkumar and Meenakshi, 2012).

The liver plays an important role in the synthesis of proteins. 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). The impact of contaminants on aquatic ecosystem can be assessed by measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination (Petrivalsky *et al.,* 1997). Despite their limitation, such as relatively high mobility, fishes are generally considered to be the most feasible organisms for pollution monitoring in aquatic ecosystem. Fish employ several enzyme systems in biotransformation of various xenobiotics (Van der Oost *et al.,* 2003). Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms (Singh and Sharma, 1998).

In the gill, liver and kidney of protein content had decreased, whereas amino acids content had increased at all periods of exposure 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). 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 increased amino acid in Zinc sulphate exposed freshwater fish *Channa striatus* (Reddy and Devi, 2021). The protein content declined gradually in gill, liver and muscle tissues of *O. mossambicus* when exposed to deltamethrin and it was reported that it may be due to the utilization of protein controls to counteract the toxicant stress caused by pesticide (Rao and Rao, 1979; Rath and Mishra, 1980).

The sublethal concentration of mercury chloride caused a significant reduction in the liver protein content of *Hypophthalmichthys molitrix* at all exposure periods. The liver is affected considerably when there is a disturbance in protein metabolism. The protein contents in liver and kidney of *Catla catla* are depleted under the sublethal stress of chromium (Vincent *et al.,* 1995). Palanichamy and Baskaran (1995) have reported a decrease in muscle and liver protein in *Channa striatus* exposed to mercury, cadmium and lead. Increase in amino acid content in liver and muscle were observed in *Mystus vittatus* exposed to cadmium (Rengarajan, 1989) in *Labeo rohita* exposed to median lethal concentration of mercuric chloride (Jagadeesan, 1994) and in *Mystus vittatus* exposed to sublethal and median lethal concentration of copper (Rajamanickam, 1992).

Bhaskaran (1980) and Manoharan and Subbiah (1982) have reported that depletion in protein level was due to diversification of energy to meet the impending energy demand when the animals was under toxic stress. 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 gill, liver and kidney amino acid level rapidly increased at all exposure periods. The elevated amino acid levels in the gill. Liver and kidney 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

A reduction in the protein content in the present investigation in *Hypophthalmichthys molitrix* 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 mercury chloride treated fishes. It is evident that proteins are degraded to meet the energy requirements during mercury chloride exposure. It can be concluded that in *Hypophthalmichthys molitrix* exposed to mercury chloride at sublethal concentrations causes energy crisis and alter protein metabolism.

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