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ORIGINAL ARTICLE

IMPACT OF HEAVY METAL, LEAD ACETATE ON PROTEIN AND AMINO ACID CONTENTS IN KIDNEY AND BRAIN OF EDIBLE EXOTIC FISH, *CYPRINUS CARPIO* (LINN.)

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ABSTRACT

The world populations are suffers from deficiency of protein sources. Fish are considered as an important source of high quality animal protein as they contain large amounts of essential amino acids. Also, fish contain crude lipids, which supply the body with energy and essential fatty acids that are necessary for life and play an important role in regulation of the cardio-vascular system and for reducing cholesterol level in the blood. Moreover, fish are rich in fat-soluble vitamins, iodine and phosphorous. Environmental pollution is a worldwide problem as heavy metals belong to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems. environmental pollution can cause poisoning, diseases and even death for fish and the absorption and the accumulation of different biological tissues on pollutants are different. The absorption of heavy metal elements of various biological tissues on pollutants is an important biomedical problem. The aim of the present study was to assess the protein and amino acid levels in kidney and brain of *Cyprinus carpio* was exposed to sublethal concentrations of lead acetate 1/5th (high), 1/10th (medium) and 1/15th (low) of the 96 hour LC_{50} values for the period of 10, 20 and 30 days. The fish exposed to lead acetate showed a decrease the protein and increase the amino acid levels for 10, 20 and 30 days in kidney and brain. The objective of the present work was to observe the effect of lead acetate on protein and amino acid levels in the kidney and brain of exotic fish, *Cyprinus carpio*.

Keywords: Protein, Amino acid, Lead acetate, Sublethal concentration, *Cyprinus carpio*.

1.INDRODUCTION

Water pollution has a large set of adverse effects upon water bodies such as lakes, rivers, oceans, and underground water caused by human activities (James Salemcity *et al.*, 2014). Water pollution is the addition of something that changes its natural qualities (Coulson and Forbes, 1952). Metals, especially heavy metals, are important pollutants of aquatic environments worldwide. Metal pollution has increased with the technological progress of human society. Industry, mining, advanced agriculture, household waste, and motor traffic is all among the activities considered to be major sources of metal pollution. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments (Luoma and Rainbow, 2008). The growth of human population and increasing activities associated with agriculture, urbanization and industrialization have resulted in a staggering release of anthropogenic sources (Nwani *et al.*, 2015).

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (AS), chromium (Cr), thallium (Ti) and lead (Pb). Heavy metals are natural components of the earth' crust. They can't be degraded or destroyed. Heavy metals are natural trace components of the aquatic environment, but their levels were increased due to industrial wastes, geochemical structure, agricultural and mining activities (Sprocati *et al.*, 2006). All of these sources of pollution affect the physicochemical characteristics of the water, sediments and biological components and thus the quality and quantity of fish (Singh *et al.*, 2006). Although there is no clear definition of what a heavy metal is, density is in most cases taken to be the define factor. Heavy metals are commonly defined as those having a specific density of more than 5 g/cm. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. Heavy metals have been used in many different areas for thousands of years. Lead has been primarily used at least 5000 years ago in building materials, pigments for glazing ceramics and pipes for transporting water. In ancient Rome, lead acetate was used to sweeten old wine and some Romans might have consumed as much as gram of lead a day (Zeitoun and Mehana, 2014).

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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. The increasing worldwide contamination of freshwater systems with thousands of industrial and natural chemical compounds is one of the key environmental problems (Schwarzenbach *et al.*, 2006). Besides numerous organic compounds entering aquatic ecosystems, heavy metal input is still rising (Bopp *et al.*, 2008).

Fish is generally appreciated as one of the healthiest and cheapest source of protein and it has amino acid compositions that are higher in cysteine than most other sources of protein (Akan *et al*., 2012). Fish is a commodity of potential public health concern because it can be contaminated by a range of environmentally persistent chemicals, including heavy metals (Soliman, 2006; El- Morshedi *et al*., 2014). The present investigation was to assess the protein and amino acid content in kidney and brain of *Cyprinus carpio* exposed to three different sublethal concentrations of lead acetate.

2.MATERIALS AND METHODS

The fish *Cyprinus carpio* having mean weight 22-25 g and length 10 – 12 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 two weeks. They were fed on rice bran and oil cake daily. The lead acetate was used in this study and stock solutions were prepared. Lead acetate, LC_{50} was found out for 96 h (27.50 ppm) (Sprague, 1971) and $1/15^{th}$, $1/10^{th}$ and $1/5^{th}$ of the LC_{50} values were 1.83, 2.75 and 5.50 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 lead acetate, and served as the control. The other 3 groups were exposed to sub lethal concentration of lead acetate, 10 litre capacity aquaria. The 2^{nd}, 3^{rd} and 4^{th} groups were exposed to lead acetate, 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 and aminoacid estimation. The protein and amino acid content in kidney and brain of *Cyprinus carpio* were estimated by the method of Lowry *et al.*, 1951 and Moore and Stein (1954) respectively. The data obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

3.RESULTS

The protein and amino acid contents in kidney and brain of *Cyprinus carpio* exposed to low, medium and high sublethal concentrations of lead acetate showed significant decrease in the level of protein whereas increase the levels of amino acids when compared to control fish. The decrease the level of protein and increase the level of amino acid in kidney and brain of *Cyprinus carpio* were more pronounced at 30 days of exposure period (Table 1 and 2).

Table 1. Protein level changes (mg/g) in kidney and brain of *Cyprinus carpio* exposed to sublethal concentrations of lead acetate

Treatments	10 days	20 days	30 days
	Kidney		
Control	85.53 ± 6.63^{a}	86.39 ± 6.69^{b}	85.77 ± 6.65^{c}
Low concentration	83.43 ± 6.47^{a}	81.65 ± 6.34^{ab}	77.36 ± 6.00^{b}
Medium concentration	79.95 ± 6.20^{a}	76.46 ± 5.94^{a}	72.51 ± 5.64^{ab}
High concentration	79.17 ± 6.14^{a}	75.03 ± 5.83^{a}	67.05 ± 5.22^{a}
	Brain		
Control	79.27 ± 6.15^{b}	80.08 ± 6.21^{c}	80.65 ± 6.26^{c}
Low concentration	74.95 ± 8.52^{ab}	70.13 ± 5.46^{b}	65.13 ± 5.08^{b}
Medium concentration	72.09 ± 5.61^{a}	65.55 ± 5.11^{ab}	61.77 ± 4.82^{ab}
High concentration	70.33 ± 5.47^{a}	63.19 ± 4.92^{a}	57.06 ± 4.46^{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 kidney and brain of *Cyprinus carpio* exposed to sublethal concentrations of lead acetate

Treatments	**10 days**	**20 days**	**30 days**
	Kidney		
Control	3.58 ± 0.29^{a}	3.64 ± 0.30^{a}	3.62 ± 0.30^{a}
Low concentration	4.12 ± 0.34^{b}	3.79 ± 0.38^{b}	5.94 ± 0.47^{b}
Medium concentration	4.76 ± 0.38^{c}	5.62 ± 0.45^{c}	6.69 ± 0.53^{c}
High concentration	5.29 ± 0.42^{d}	7.36 ± 0.58^{d}	8.98 ± 0.70^{d}
	Brain		
Control	3.42 ± 0.28^{a}	3.44 ± 0.28^{a}	3.38 ± 0.28^{a}
Low concentration	3.79 ± 0.31^{b}	4.40 ± 0.36^{b}	5.88 ± 0.47^{b}
Medium concentration	4.16 ± 0.34^{c}	4.74 ± 0.38^{c}	6.40 ± 0.51^{c}
High concentration	4.90 ± 0.40^{d}	5.59 ± 0.45^{d}	7.76 ± 0.61^{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)

4.DISCUSSION

Environmental pollution is a worldwide problem as heavy metals belong to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems (Saleh *et al*., 2010). Environmental pollution can cause poisoning, diseases and even death of the fish. Toxicants was absorbed and accumulated in different organs of the organism. The absorption of heavy metals of various biological tissues on pollutants is an important biomedical problem (Wan *et al*., 2013). Pollution describes the introduction of foreign substances into the biosphere. As xenobiotics, some of these pollutants find their way into the human system through the food chain. In the body, pollutants may undergo biotransformation, metabolism and excreted without the risk of toxicity depending on the chemical characteristics of these compounds and their concentration. However, some of the pollutants resist chemical and biological transformation and accumulated in the tissues including, liver, kidney and nerve to cause toxicity (Gabriel *et al*., 2006; Zeitoun and Mehana, 2014).

Fish is a commodity of potential public health concern because it can be contaminated by a range of environmentally persistent chemicals, including heavy metals (EI-Morshedi *et al.*, 2014). Fish represents the higher tropic level in the aquatic food chain. Therefore, persistence of toxic chemical accumulates to a maximum concentration in their body when compared to other organisms in the aquatic environment (Sackmauerova *et al*., 1977). Fish is widely consumed in many parts of the world because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health (Ikem and Egiebor, 2005). Proteins are important organic substances necessary for organisms in tissue building and play a significant role in energy metabolism (Al-Kahtani, 2011). Fish are constantly exposed to chemicals in polluted and contaminated waters. Fish have been found to be good indicators of heavy metal contamination in aquatic systems because they occupy different trophic levels and are of different sizes and ages (Burger *et al*., 2002; Tuzen and Soylak, 2007). The kidney, which is an important organ of excretion and osmoregulation, is indirectly affected by pollutants through blood circulation (Newman and MacLean, 1974). The brain is highly vulnerable to oxidative stress due to its high metabolic rate, the reduced capacity for cellular regeneration, and numerous cellular oxidative stress targets like lipids, nucleic acids, and proteins. Generally, most molecules cannot cross the blood–brain barrier (BBB). But, due to large surface area, the NPs made of certain materials and with varying particle sizes can overcome this physical barrier and enter into the brain (Palaniappan and Pramod, 2011).

Proteins are vital ingredient involved in the architecture of the cell, which is the main source of amino acids for building up of new tissues and for the synthesis of biologically important molecules such as enzymes, hormones, etc as well as the source of energy for fish. Alterations in protein content of various tissues of fish exposed to different concentrations of insecticides are linked through a biochemical metabolic pathway. The increase in the protein level in liver of freshwater fish (*Oreochromis niloticus*) was maybe due to check the influence of thiamethoxam and effort to recover from the stress of insecticide at lower doses. They found when the concentration of thiamethoxam was increased; there was decrease in liver protein level. Thus, reduce a significant portion of protein in different tissues, especially the liver, may have been due to their degradation and possible utilization for metabolic purposes. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis. Since, free amino acids are used in gluconeogentic pathway to glucose production, reduced levels of protein synthesis in fish exposed to insecticides (Banaee, 2013).

In the present investigation freshwater fish, *Cyprinus carpio* exposed to sublethal concentrations of lead acetate for the periods of 10, 20 and 30 days shows decrease the levels of protein and elevated levels of amino acid in kidney and brain. The present work agrees with Tulas and JayanthaRao (2013) addressed that total protein content is decreased and it may be due to breakdown of proteins in the fabrication of some amount of energy for organism. The degree of increase in free amino acids was resulted by the decreased protein level. Bhaskaran (1980) and Manoharan and Subbiah (1982) 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. Protein contents were decreased in gill, liver, muscle and heart of *Oreochromis niloticus* exposed to sublethal concentration of cadmium chloride (Faheem *et al*., 2012). Similar observations were noted when the fish were exposed to pollutants (Lone and Javaid, 1976; Shakoori *et al*., 1976; Rath and Mishra, 1980; Ramalingam and Ramalingam, 1982). The protein contents in gill, liver and kidney of *Cyprinus carpio* weree depleted under the sublethal concentration of pharmaceutical effluent (Muthulingam, 1999). Moorthikumar and Muthulingam (2010) reported that the decrease in liver, kidney and brain protein in *Labeo rohita* exposed to nickel chloride for a period of 30 days. Senthil Elango and Muthulingam (2014) addressed that the protein content of brain and muscle were decreased in *Oreochromis mossambicus* when exposed to sublethal concentrations of heavymetal, chromium. The change in the protein concentration suggest an intestinal proteolysis in the respective tissues which in turn could contribute to the like of free amino acids to be fed into TCA cycle (Tricalboxylic acid cycle) as keto acid, and it support the hypothesis (Kabeer Ahmed Sahid, 1979). The decreased protein and increased amino acid contents in the gill, liver, kidney, heart and muscle of *Catla catla* exposed to cadmium chloride (Sobha et al., 2007). It is evident that proteins are degraded to meet the energy requirements during heavy metal lead acetate exposure. It can be concluded that in *Cyprinus carpio* exposed to sublethal concentrations of lead acetate causes energy crisis and alter protein metabolism.

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