International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 2; March 2018; Page No. 293-298

Effect of lead acetate and recovery response of DMSA on protein metabolism in liver and brain of freshwater fish, *Channa striatus* (BLOCH)

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Abstract
Today man is living in a chemical sphere and these chemicals whether, natural or manmade, has the capacity to degrade the delicately balanced ecosystem by poisoning air, water and land. Heavy metals such as lead 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. Lead is a non-essential metal and is a common historical and contemporary contaminant throughout the world. This element is one of the most ubiquitous and a useful metal known to humans and it is detectable in practically all phases of the inert environment and in all biological systems. The main route of exposure for general population is food and air. 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 cholestrol level in the blood. Moreover, fish are rich in fat-soluble vitamins, iodine and phosphorous. The aim of the present study was to assess the protein and amino acid levels in liver and brain of *Channa striatus* was exposed to sublethal concentration of lead acetate further the fish treated with *meso 2,3-* dimercapto succinic acid (DMSA) 2.5 ppm and 5 ppm in group 3 and group 4 respectively 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 further the fish were treated with dimercaptosuccinic acid showed gradually the protein contents increased and amino acid levels were decreased for the periods of 10, 20 and 30 days in liver and brain. The objective of the present investigation was to observe the reversing ability of dimercaptosuccinic acid reduced the lead acetate induced alterations in protein and amino acid in the liver and brain of freshwater fish, *Channa striatus*.

Keywords: protein, amino acid, lead acetate, dimercaptosuccinic acid, channa *striatus*

Introduction

Today human beings is living in a chemical sphere and these chemicals whether, natural or manmade, has the capacity to degrade the delicately balanced ecosystem by poisoning air, water and land. Hence, while realizing the needs of chemicals, it should be ensured that it will not spoil the environment. Global chemical pollution has been a matter of great concern with increase in public awareness towards environmental problems [1, 2]. The various human activities through which heavy metals reach the environment are smelting or processing of ores of metals, mining, burning of fossil fuels such as coal, petrol and kerosene oil, discharge of agricultural, industrial and domestic waste, auto exhaust and pesticides containing compounds of heavy metals. Heavy metals are also widely used in household appliances, paints, photographic paper, photo chemicals etc., Pollution of ground water and surface water systems through anthropogenic activities is the major environmental problem faced all around the globe [3, 4].

The pollution of aquatic medium with an extensive range of contaminants has become a matter of concerned over the last few decades [5, 6]. The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other anthropogenic activities [7, 8]. Heavy metal contaminations have devastating effects on the ecological balance of the recipient environment passing through altering the diversity of aquatic organisms [9, 10, 11] particularly fish community [12]. These metals reach food chain through a variety of biochemical process and ultimately biomagnified in various trophic levels and ultimately threaten the health of man by aquatic food utilization [13, 14, 15]. The main sources of heavy metal pollution of the agriculture, industry and metropolitan cities, the bioaccumulation of toxic heavy metals in fish species from different aquatic systems is dependent on their foreign polluted substances. The distribution of heavy metals in water, sediments and fish play a key role in detecting sources of heavy metal pollution in aquatic ecosystem [16].

Lead is recognized as one of the most pervasive and elusive environmental health threats throughout the globe. Numbers of epidemiological works have been documented the adverse health effects associated with lead. Acute exposure in children leads to abdominal pain, constipation, cramps, nausea and vomiting. Children who are chronically exposed to high concentrations of lead also have an increase in urinary and aminolevulinic acid and urinary coprotoporphyrin, and an increase in blood zinc erythrocyte protoporphyrin. Low levels of lead exposure have also been strongly linked to learning disabilities such as IQ deficits reading problems, reduced attention span, behavioral changes, developmental defects and language difficulties. Lead is one of the most dangerous pollutants in our environment which accumulates in the body due to its low rate of elimination. Lead enters aquatic systems from urban, mining and agricultural runoff, atmospheric precipitation, plating processes and gasoline containing lead that leaks from fishery boats and a variety of natural sources. Several reports have indicated that Pb can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to Pb [17, 18].

Increases use of metal based fertilizers in agricultural revolution could result in the continued rises in the concentration of metal pollutants in fresh water due to the water run-off [19, 20, 21]. The speedy urbanization and industrialization has led to increased disposal of pollutants like heavy metals, radio nuclides, and various types of organic and inorganic substances into the environment. Thus, the industrial wastes are the main source of metal pollution for aquatic organisms. It has been cited that the heavy metals constitute the major pollutants in the environment. The heavy metals are important pollutants for fishes, because these are not eliminated from aquatic systems by natural methods, such as organic pollutants, and are enriched in mineral organic substances. The metal contaminants are mixed in the aquatic system through smelting process, effluents, sewage and leaching of garbage which cause severe harm to the aquatic system [22]. The aquatic systems deposition of contaminants, including heavy metals, can lead to elevated sediment concentrations that cause potential toxicity of the aquatic biota [23, 24, 25]. Dimercaptosuccinic acid (DMSA) is a drug approved for use in the treatment of lead poisoning. It is a chelating agent that binds to heavy metals such as lead and mercury, which are then eliminated in the urine as the DMSA is excreted. A significant benefit is that it does not remove substantial amounts of other beneficial metals such as iron, calcium, magnesium and zinc [26, 27].

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 cholestrol level in the blood. Moreover, fish are rich in fat-soluble vitamins, iodine and phosphorous [28, 29]. Fish products are widely consumed in throughout the world because it has high protein content, low saturated fatty acids, calcium, phosphorus, iron, and trace elements such as copper as well as a fair proportion of the group B-vitamins to support good health [30]. Fish products now account for 30% of the human protein supply in Asia. Because they are neutrally buoyant, most fishes have less need for a supporting skeleton and consequently have a higher ratio of muscle to bone than land animals. This characteristic as well as having high levels of protein, essential fatty acids, minerals and vitamins makes them a very valuable and healthy alternative to other meats [31, 18]. Hence, the present study has been carried out on changes in the protein and amino acid levels in liver and brain of *Channa striatus* treated with sublethal concentration and recovery role of DMSA.

Materials and Methods

The fish *Channa striatus* having mean weight 17 - 21 g and length 11 – 13 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 cement tank for acclimatization for a period of two weeks. They were fed twice daily i.e. morning and evening on boiled chicken eggs approximately 4% of fish body weight divided into two equal meals daily. The lead acetate was used in this study and stock solutions were prepared. Lead acetate, LC_{50} was found out for 96 h (32 ppm) [32] and 1/10th (3.2 ppm) taken as sublethal concentration 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 for 30 days. The 3rd and 4th groups were reexposed to 2,3 meso dimercapto succinic acid (DMSA) 2.5 ppm and 5 ppm respectively for 10, 20 and 30 days.. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein and amino acid estimation. The protein and amino acid content in liver and brain of *Channa striatus* were estimated by the method of [33] Lowry *et al.*, 1951 and [34] 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 (35 Duncan, 1957).

Results

Protein Content in Liver

The protein contents were observed in the control liver to be 134.63 ± 10.25, 136.24 ± 10.37 and 137.96 ± 10.51 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days showed 111.20 ± 8.47, 105.30 ± 8.02 and 98.57 ± 7.51 respectively. Sublethal concentration of lead acetate exposed fish after 30 days treated with DMSA (2.5 and 5 ppm) showed increased the protein content (118.36 ± 9.01, 120.67 ± 9.19 and 125.75 ± 9.58) and (123.17 ± 9.38, 128.44 ± 9.78 and 135.84 ± 10.34) for 10, 20 and 30 days respectively (Fig 1). However, more liver protein contents were observed in DMSA (5 ppm) treated fish, *Channa striatus.* (Fig 1)

Protein Content in Brain

The protein contents were observed in the control brain to be 85.83 ± 6.54, 86.74 ± 6.61 and 87.93 ± 6.70 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days showed 78.36 ± 5.97, 73.53 ± 5.60 and 65.26 ± 4.97 respectively. Sublethal concentration of lead acetate exposed fish after 30 days treated with DMSA (2.5 and 5 ppm) showed increased the protein content (80.23 ± 6.11, 83.56 ± 6.36 and 85.70 ± 6.53) and (82.19 ± 6.26, 84.75 ± 6.45 and 87.19 ± 6.64) for 10, 20 and 30 days respectively (Fig 1). However, more brain protein contents were observed in DMSA (5 ppm) treated fish, *Channa striatus.* (Fig 2)

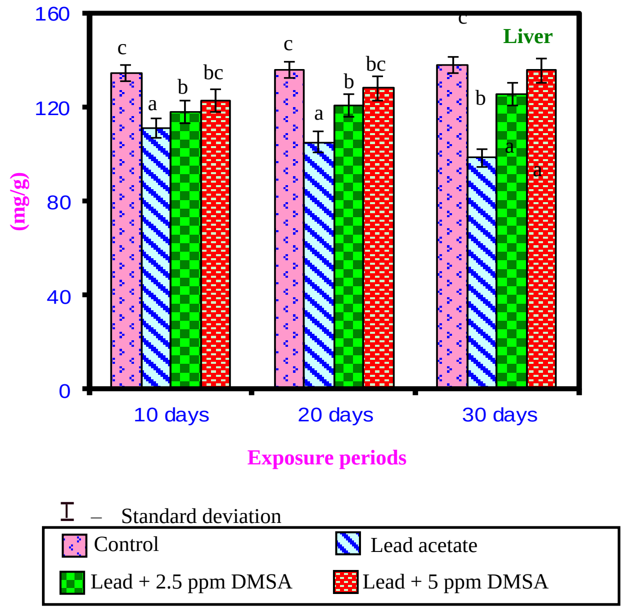

Fig 1: Protein level changes (mg/g) in liver of *Channa striatus* exposed to sublethal concentrations of lead acetate ameliorated by DMSA

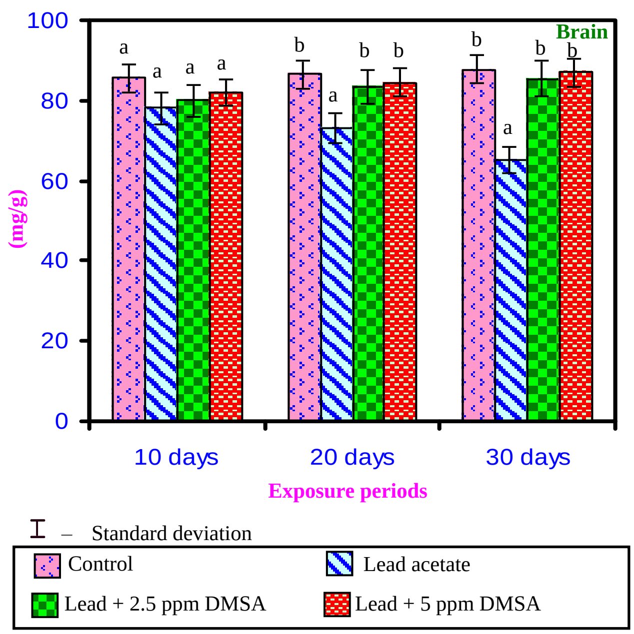

Fig 2: Protein level changes (mg/g) in brain of *Channa striatus* exposed to sublethal concentrations of lead acetate ameliorated by DMSA

Amino Acid Content IN Liver

The amino acid contents were observed in the control liver to be 5.13 ± 0.39, 5.21 ± 0.40 and 5.25 ± 0.40 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly enhanced when the fish *Channa striatus* exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days showed 8.24 ± 0.63, 11.76 ± 0.90 and 14.91 ± 1.14 respectively. Sublethal concentration of lead acetate exposed fish after 30 days treated with DMSA (2.5 and 5 ppm) showed declined the amino acid contents (7.32 ± 0.56, 8.69 ± 0.66 and 9.86 ± 0.75) and (6.45 ± 0.49, 6.53 ± 0.50 and 6.79 ± 0.52) for 10, 20 and 30 days respectively (Fig 1). However, more declined liver amino acid contents were observed in DMSA (5 ppm) treated fish, *Channa striatus.* (Fig 3)

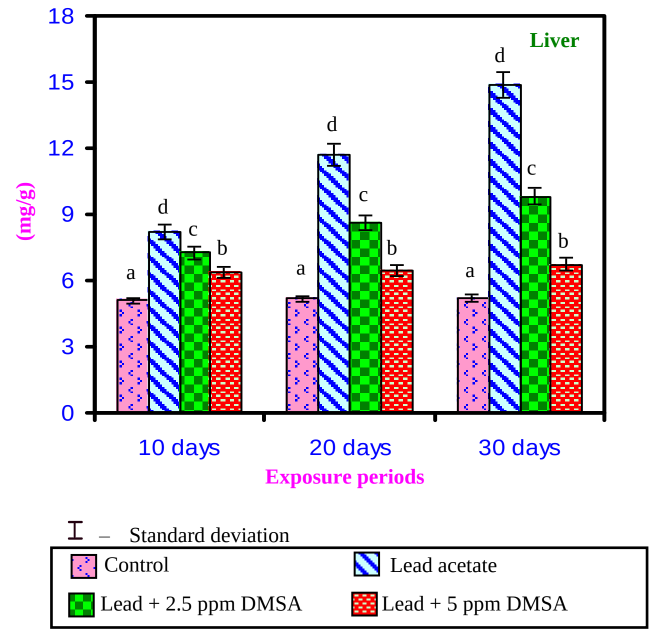

Fig 3: Amino acid level changes (mg/g) in liver of *Channa striatus* exposed to sublethal concentrations of lead acetate ameliorated by DMSA

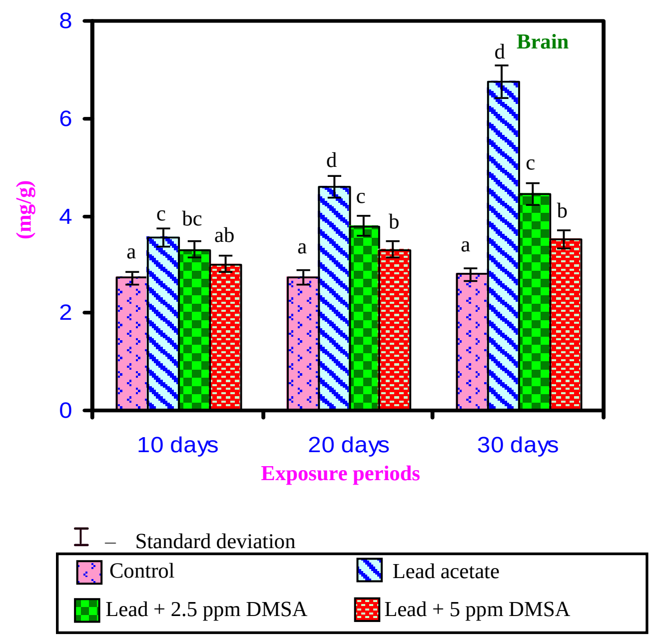

Fig 4: Amino acid level changes (mg/g) in brain of *Channa striatus* exposed to sublethal concentrations of lead acetate ameliorated by DMSA

Amino Acid Content IN Brain

The amino acid contents were observed in the control brain to be 2.74 ± 0.21, 2.76 ± 0.21 and 2.81 ± 0.21 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly enhanced when the fish *Channa striatus* exposed with sublethal concentration of lead acetate for the periods of 10, 20 and 30 days showed 3.58 ± 0.27, 4.62 ± 0.35 and 6.78 ± 0.52 respectively. Sublethal concentration of lead acetate exposed fish after 30 days treated with DMSA (2.5 and 5 ppm) showed declined the amino acid contents (3.32 ± 0.25, 3.81 ± 0.29 and 4.46 ± 0.34) and (3.03 ± 0.23, 3.32 ± 0.25 and 3.54 ± 0.27) for 10, 20 and 30 days respectively (Fig 1). However, more declined brain amino acid contents were observed in DMSA (5 ppm) treated fish, *Channa striatus.* (Fig 4)

Discussion

Aquatic ecosystems are fragile and at risk primarily because the majority of contaminants derived from urban and industrial sources, abandoned military installations and agricultural activities are released into rivers [36, 37]. The aquatic medium polluted with industrial waste water its contains many heavy metals like lead, chromium, zinc and mercury [38, 39]. 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 [40]. Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms [41]. The toxicological profiles in many animal models, it is certainly plausible that waterborne metals could alter physiological and biochemical parameters in fish [42].

Fishes are one of the major sources of protein for human beings and the nutritional value of fish depends on their biochemical composition like protein, amino acids, vitamins and mineral contents [43]. The biochemical parameters in fish are valid for physiopathological evaluation and sensitive for detecting potential adverse effects and relatively early events of pollutant damage [44, 45]. The decreased protein content might also be due to tissue destruction, disturbance of cellular fraction and consequent impairment in protein synthetic machinery [46]. The altered mobility and low content of proteins reflects a change in the rate of synthesis and degradation of protein. Proteins are mainly involved in the architecture of the cell. During stress conditions they are a source of energy as fish need more energy to detoxify the toxicant and to overcome stress. The amount of carbohydrates in fish is less so protein is the alternative source of energy to meet the increased energy demand [47].

The present investigation in the liver and brain protein content had decreased whereas amino acids content had increased at all periods of exposure when *Channa striatus* was exposed with sublethal concentration of lead acetate and the recovery role of DMSA showed the protein contents gradually increased and amino acid contents were decreased for the periods of 10, 20 and 30 days exposure. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis [48]. Simillarly the protein levels were increased in lead with lycopene or vitamin E supplements with diet than lead alone treated fish, *Clarias gariepinus* [45]. The protein levels were decreased and amino acid contents increased significantly in gill, liver and kidney of *Cyprinus carpio* exposed to sublethal concentration of pharmaceutical effluent [49]. The *Lamellidens marginalis* exposed to sublethal concentration of chromium showed decrease the protein and RNA where as amino acid level was elevated [50].

Many investigators have also recorded such a reduction in protein content in fishes exposed to different toxicants [51, 52, 53]. A reduction in the protein content in the present investigation in *Channa striatus* 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 the intoxicated fishes. The recovery phase of DMSA influence in the protein metabolism shows decrease the amino acid content and enhance the protein content in liver and brain of *Channa striatus*. It may be due to the DMSA binds with lead acetate and lead acetate eliminated by the kidney. Protein and lipid contents were increased in tomato paste and vitamin E supplements to atrazine treated fish, *Clarias gariepinus* [54]. Reduction in the protein and enhanced contents of amino acid in liver, kidney and brain of *Labeo rohita* was treated with nickel chloride [55]. Minimize the levels of protein and elevated levels of amino acid in brain and muscle of *Oreochromis mossambicus* when exposed to sublethal concentrations of heavy metal chromium [56]. The protein contents were reduced whereas amino acid contents elevated in gill, liver and kidney of *Hypopthalmichthys molitrix* when exposed to sublethal concentration of cadmium chloride [57]. It is evident that the present investigation, proteins are degraded to meet the energy requirements during sublethal concentrations of lead acetate and the protein contents were increased due to elimination of lead acetate in the liver and brain of *Channa striatus* by the influence of DMSA. The depletion in protein level was due to diversification of energy to meet the impending energy demand when the fish *Channa striatus* exposed to sublethal concentration of heavy metal lead acetate. The reduction in protein content in the present study indicates that the tissue protein undergoes proteolysis resulting in the production of free amino acids leads to disturbances in the physiological activity of the fish *Channa striatus*.

Conclusion

Fish is an integral part of the diet of a most of the population rarely some vegetarian people avoid fish and is one of the sources of protein for the people living in coastal areas. The nutritional value of different species of fish depends on their biochemical components such as protein. The alteration in the proximate component could disturb the metabolic system in fish, affecting the food value of fish. The lead acetate treated fish gradually improved the protein metabolism by reversing role of DMSA.

Acknowledgement

The authors wish to thank the Director, Research and Development Centre, Professor and Head Department of Zoology, Bharathiar University and also Professor and Head, Department of Zoology, Annamalai University for providing the facilities to carry out the research work.

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