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The heavy metal lead nitrate toxicity effect on biochemical alteration in fresh water fingerlings *Labeo rohita*, (Hamilton, 1882)

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Abstract
The character of the environment is degenerating due to the accumulation of several pollutants either direct or in indirect way, which at last causes several unwanted effects on organisms in general and particularly on human beings. The heavy metal lead nitrate is one of the important aquatic toxic pollutant and is ubiquitous trace metal. In the present investigation, an attempt was made to assess the effect of lead nitrate on biochemical parameters in the muscles and liver tissue of *Labeo rohita* fingerlings. The fish were exposed to a sub-lethal dose of 1.56 mg L^{-1} (1/25th of 96 hr LC_{50} value of lead nitrate) for a period 60 days. At the end of each exposure period, the level of protein, lipid and glycogen were analyzed in selected tissues. A significant decrease ($P<0.01$ and $P<0.05$) was noticed in all the biochemical parameters studied in treated liver and muscles tissues groups when compared to the control.

Keywords: *Labeo rohita*, lead nitrate, biochemical parameters, liver, muscle

Introduction

The most common cause of water pollution in developing and developed countries is domestic and industrial waste that is directly released into streams or ponds without treatment. Adversely human activities are directly or indirectly affect the environment. Due to development activities such as construction, transportation and manufacturing not only deplete the nature resources but also produce large amount of wastes that leads to pollution of air, water and soil (Tamizhazhagan, 2015, 2016) [39, 40]. The most important source of irrigate pollution are household, agricultural and industrialized waste which are discharge into ordinary water body (Tamizhazhagan *et al*., 2017) [41]. These wastes mostly contain various types of pollutants such as heavy metals, radioactive substances, pesticides, herbicides and corrosive substances like acids and bases (Mhadhbi *et al.,* 2012) [26] and these could alter physiological and biochemical parameters in fish. The survival of many aquatic species depends not only on the health status of the ecosystem, but also on the type/length of exposure to and inherent toxicities of the metal toxicants (Brungs *et al.*, 1977) [4]. Heavy metals are among the most dangerous substances in the aquatic environment because; they persist and are harmful to aquatic organisms(Tamizhazhagan et al.,2016).

Lead is a naturally occurring heavy metal which has been used in various ways including mining, smelting, refining, gasoline, battery manufacturing, electrical wiring, soldering, painting, ceramic glazing and making of stained glass. Due to its non-degradable nature, it gets into the environment and eventually enters the human and animal's blood stream. Fish accumulate toxic metals directly through the water or indirectly through the food chain. It is accumulated in soft tissues such as gill, liver, kidneys, nervous system, and brain. (Spokas, *et al.*, 2006; Schmitt *et al.*, 2007; Has-Schon *et al.*, 2008) [38, 35, 8] and could alter the physiological and biochemical parameters in fish. The body components like protein, carbohydrate and lipid play a significant role in body construction and energy production. They are involved in major physiological events and the assessment can be considered as biomarker to determine the physiological phases of organism (Martein and Arivoli, 2008). Fish are considered as a suitable biomonitors for environmental pollution and they are exposed to the heavy metals invitro and the effect of metals on fish is studied (Padmini *et al.,* 2004) [27]. As freshwater aquaculture constitutes one-third of the total fish production in India and major carps being the dominant species (*Labeo rohita, Catla catla and Cirrhinus mrigala*). Hence, the aim of our present study is to examine the effect of lead nitrate on biochemical parameters of Indian carp *rohu* (*Labeo rohita*) fingerlings that forms an important candidate species in carp poly-culture systems.

Materials and Methods

Test Chemical and Stock solution

The toxicant being studied was lead nitrate. Stock solution was prepared using analytical grade Lead Nitrate [$Pb(NO_3)_2$]. Since nitrate also cause toxicity, Lead Nitrate as such was used as a test chemical. 10gm of Lead Nitrate was dissolved in 1lit of distilled water and the required concentrations of the test media were prepared using chlorine free tap water as diluents. Stock solution was prepared every day.

Experimental design

The fingerlings of the freshwater fish *Labeo rohita*, weighing 10 ± 2gms and length 7 ± 0.2cm were procured from Venkatesh fish farm, at Pinnalur and transferred to the fibrous tank in the Department of Zoology Annamalai University. The water in the tank was aerated twice a day, the fish were fed daily with ground nut cake and rice bran. The physico-chemical properties of water used for experiments had pH 7.4± 0.2, dissolved oxygen 6-7 ml / lt, hardness 160 ppm and temperature 28±1˚C. Before experimentation has been executed, the fish were acclimated to the laboratory conditions for a period of 15 days. Later, a groups of 50 fish were exposed to the sub-lethal concentration of 1/25th of 96hrs LC_{50} value (ie; 1.56 mg/lit) of lead nitrate and a control group was also maintained for a period of 60 days. After the periods of 15, 30, 45 and 60 days of exposure, six individuals from control and treated groups were sacrificed, muscle and liver tissues were removed for biochemical analysis.

Total Carbohydrate estimation
The total carbohydrate content was estimated by the technique of Roe (1955). A 10% homogenate of tissue was prepared using 5% TCA and this was centrifuged at 3000 rpm for 10 minutes. Samples were cooled in the dark at room temperature for 30 minutes. The supernatant was collect and the optical density was measured in a spectrophotometer (Hitachi 2205) at a wavelength of 620 nm a blank reading. Blank was prepared by mixing 1 ml of distilled water with 4 ml of Biuret reagent. The total carbohydrate content in mg/g of tissue.

Total Protein Estimation
Protein was estimated by the method of Lowry *et al*., (1951) [22]. 1% tissue homogenate were prepared in 10% TCA and centrifuged at 3000 rpm for 15 minutes. The gal set was dissolved in 1 ml of 1N NaOH to the above 5 ml of alkaline copper reagent was added and after 10 minutes, 0.5 ml of folin phenol reagent was measured after was added and rapidly The moisture content was estimated by subtracting the dry weight (dried in a hot air oven) of the muscle tissue from the known wet of the muscle tissue.

Lipid Estimation
The total lipids were extracted by the method of Floch *et al*.,(1957) [7] to find out total lipid, known volume of experiment samples were homogenized with 1 ml of methonal and 2 ml of chloroform to which again 2ml of chloroform : methanol (2:1 v/v) was added and mixed thoroughly. To this, 0.2 ml- 0.09 % sodium chloride solution was added. The above mixture was poured into separately funnel, mixed and allowed to stand for few hours.

Statistical analysis
The data is expressed as mean ± Standard deviation (SD). Statistical comparisons were performed by one-way analysis of variance followed by Duncan's Multiple Range Test. The results were considered statistically significant if the P values were less than 0.01and 0.05.

Results and Discussion
In the present investigation, the levels of total proteins, glycogen and total lipids were estimated in the muscle and liver of control and in exposed fish for a period of 60 days and the results were presented in the graphs (fig-1-3). The differences between control and exposure period days were found to be statisticaly significant ($P<0.05$; $P<0.01$). A significant decrease of $P<0.01$ level was observed in all the studied biochemical parameters on 15 days of exposure period and a significant decrease of $P<0.05$ level was noticed during 30, 45 and 60 days of exposure periods.

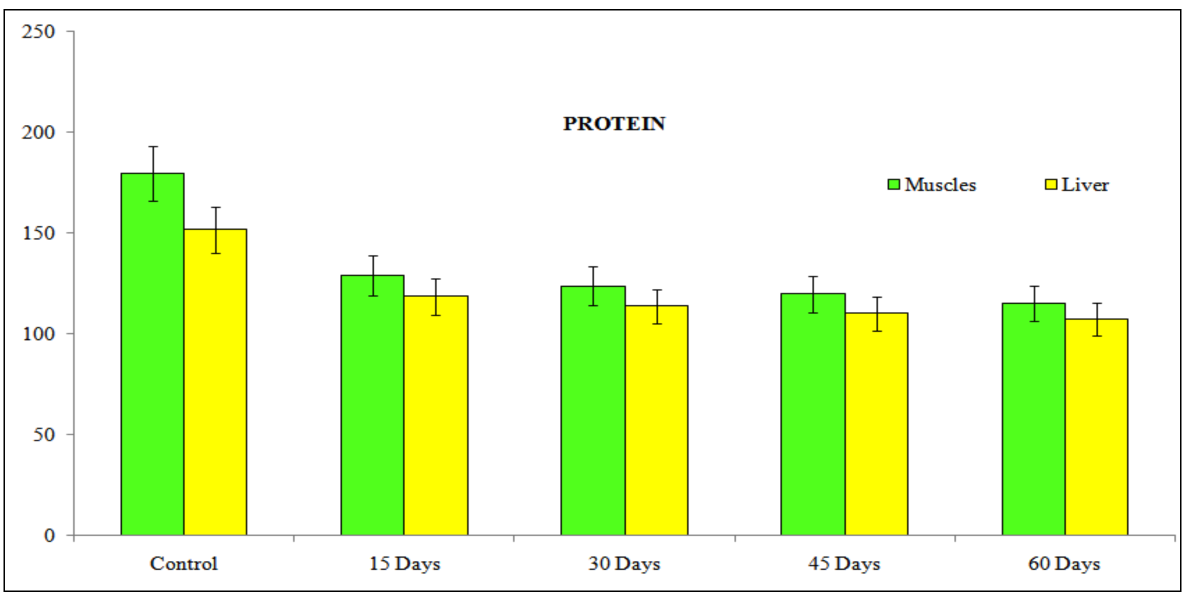

Fig 1: Variations in the level of protein in liver and muscle tissues of fresh water fish *Labeo rohita* fingerlings after 15, 30, 45 and 60 days exposure to lead nitrate.

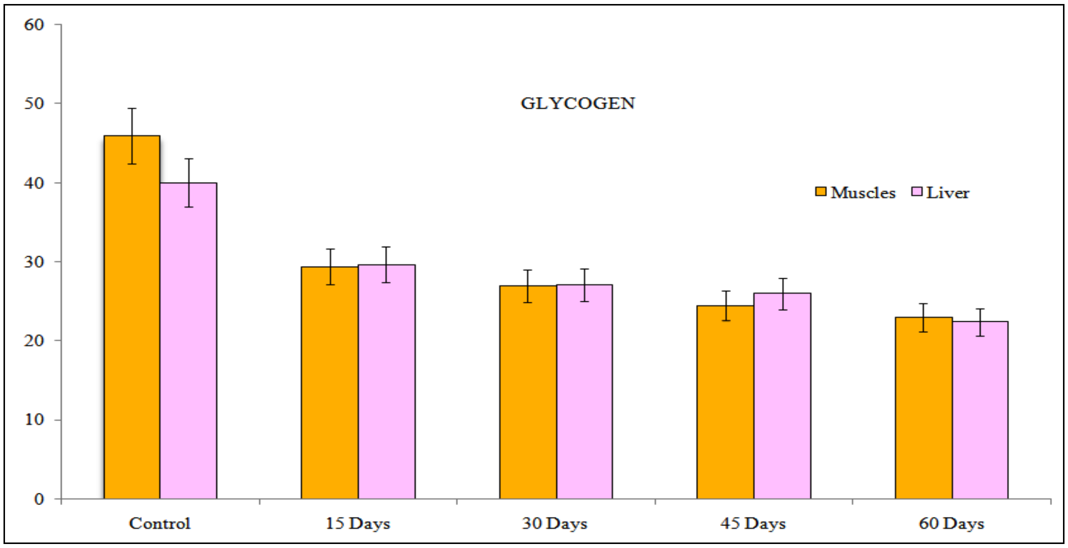

Fig 2: Variations in the level of glycogen in liver and muscle tissues of fresh water fish *Labeo rohita* fingerlings after 15, 30, 45 and 60 days exposure to lead nitrate.

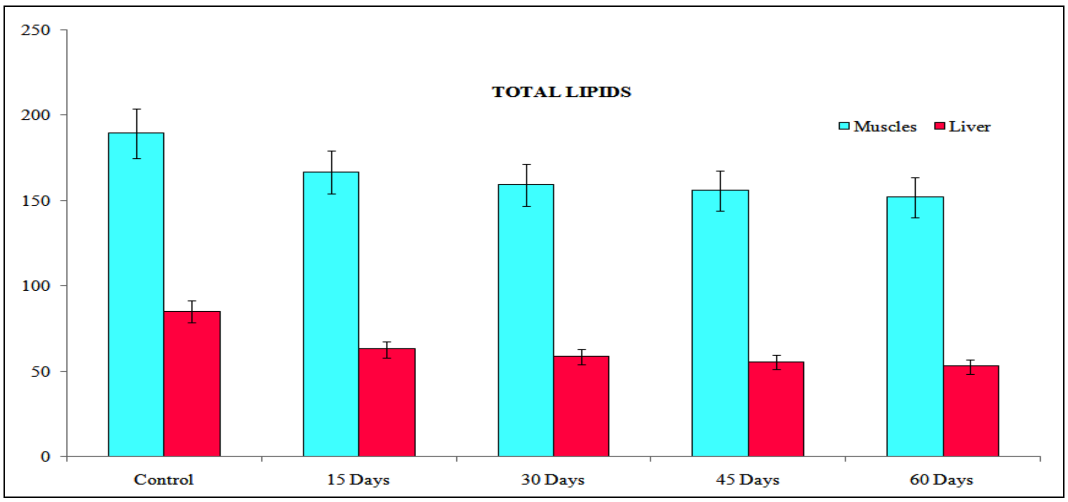

Fig 3: Variations in the level of total lipids in liver and muscle tissues of fresh water fish *Labeo rohita* fingerlings after 15, 30, 45 and 60 days exposure to lead nitrate.

Heavy metals occur in the environment both as a result of natural processes and as pollutants from human activity (Jordao *et al.*, 2002) [13]. According to World Health Organization (1991), metal occur in less than 1% of the earth's crust, with trace amounts generally found in the environment and when these concentrations exceed a stipulated limit, they may become toxic to the surrounding environment.

Fish, a common source of protein contains a greater quantity of protein than any other living organism (Karthikeyan, 2012) [15]. Proteins are the primary structural and functional polymers in living systems. They have a broad range of activities, including catalysis of metabolic reactions and transport of vitamins, minerals, oxygen and fuels, and for the maintenance of osmotic and ionic regulation. A dynamic equilibrium exits between proteolysis and synthesis which is mainly responsible for protein turnover and homeostasis in any tissues (Tulasi and Jayantha Rao, 2013) [48]. Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms (Singh and Sharma, 1998) [36]. Muscle is the chief component on which nutritive value of fish may be assessed, sand glycogen, protein and lipid are the main constituents of this tissue. The liver plays an important role in the synthesis of proteins.

In the present analysis, the total protein level was found to be decreased significantly ($P<0.01$) up to 15 days of exposure in muscle and liver tissues. The decrease of protein may be due to extreme proteolysis to overcome the metabolic stress, A significant decrease ($P<0.05$) was also observed during 30, 45 and 60 days of exposure period. The fall in protein level of exposure may be due to increased catabolism and decreased anabolism of proteins. The changes and decrease in protein level might also be due to inhibition of metabolizing enzymes by administration of toxicants.

In consistent with our results, several other investigations also revealed a decrease in protein profiles with toxicants. The protein content decreased in the liver, brain and kidney tissues of *Channa punctatus* during lihocin treatment (Abdul *et al.*,

2010) [1]. Jrueger *et al.*, (1968) [14] reported that the fish can get the energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus, the depletion of protein fraction in liver, brain and kidney tissues may have been due to their degradation and possible utilization for metabolic purposes. Kannan *et al.* (2010) [17] reported the decreased protein content on gill, brain and muscle of *Mystus vittatus* when exposed to mercuric chloride. Decrease in the liver and muscles protein level has been reported in *Channa punctatus* exposed to oleandrin (Tiwari and Singh, 2004) [46]. A similar decreased liver protein level has also been found in *Cirrhina mrigala* exposed to lead acetate (Ramalingam *et al.*, 2000) [31], They have also stated that, alterations in the protein levels might be due to the adaptation of the animals to metal stress. The present study supported by previous reports of Vutukuru (2003) [51]. He has observed changes in the biochemical parameters of the same fish species when exposure to chromium toxicity. Decreased protein level may be attributed to stress mediated immobilization of these compounds to fulfill an increased element for energy by the fish to cope with environmental condition exposed by the toxicant (Jankins and Smith, 2003). Present findings are in good agreement with the above findings by different workers. The present study clearly supports the earlier workers that tissue proteins are used to meet the increased energy demand posed by stress.

Glycogen is one of the immediate fuel reserves and an important constituent which can be influenced by stress. Glycogen is an essential component of living cells and source of energy for animals which include simple sugar with micro and macro molecular substances. Glycogen, in the form of carbohydrate is stored in organisms especially in the liver and muscles. Evidences reveal that exposure of lead (poisoning) brings the changes in the biochemical profiles of the fish. The observations indicate that alteration in the normal behaviour and biochemical parameters serve as an index of the toxic effects on different tissues in fishes. In the present study, glycogen content of liver and muscle tissues showed a decreasing trend as duration of exposure increased. Depletion of glycogen in the tissues is an indication of typical stress response in fish challenged with lead nitrate toxicity.

In the present study, the fish showed stress condition during exposure period as fast swimming, fast opercular movements, dashing with the walls of aquarium and reduced feeding. Thus, during such type of stress conditions, the glycogen reserves are depleted to meet out the energy demand as studied by Tiwari and Singh (2009) [47]. According to Chezhian *et al.* (2010), the decreased level of glycogen may be due to the induced activation of adrenal pituitary glucocorticoid hormones, which stimulate the hepatic glucose production thereby elevating blood glucose level to meet the critical need of energy under effluent stress in the Estuarine fish, *Lates calcarifer.* In carbohydrate metabolism, glycogen plays an important role. The disturbance in glycogen profile was considered as one of the most outstanding lesions to the biological systems due to the action of heavy metals (Ramalingam, 1988; Veeraiah *et al.,* 2013) [30, 49]. Dezwann and Zandee (1973) [6] stated that "decrease in glycogen and its consequent depletion in tissues may be attributed to hypoxia since it increases carbohydrate consumption" and under hypoxic conditions, the animals derive its energy from anaerobic breakdown of glucose, which is available to the cell by the increased glycogenolysis (Mary Chandravathy and Reddy, 1995; Rajamannar and Manohar, 1998; Rajamanicam, 1992) [25, 32, 33]. A similar trend of decrease in glycogen content of fish exposed to metallic stress was observed by many investigators (Radhakrishnah *et al*., 1992, Jagadeesan, 1994; Mary chandravathy and Reddy, 1995; James *et al*., 1995; Arasta *et al*., 1996; Tilak *et al*., 2001, 2003; Martin *et al*., 2001; Vutukuru, 2003; Thangam and Sivakumar, 2004; Sonawane *et al*., 2004; Kulkurani *et al*., 2005; Arockia Rita and Jhon Milton, 2006 Venkataramana *et al*., 2006; Prabhakara Rao and Radhakrishnaiah, 2006; Veeraiah *et al*., 2013; Karanjkar and Deshpande, 2016) [25, 49, 10, 3, 44, 46, 23, 24, 51, 42, 37, 2, 50, 29, 49, 18].

Thus, in the present experimental analysis it was observed that exposure to chronic toxicity of heavy metal lead nitrate in the fingerlings of the fish *Labeo rohita* caused changes in the total glycogen level which may be attributed to toxic stress, resulting in the disruption of enzyme associate with carbohydrate metabolism.

Lipid is an important normal body constituent used in the structure of cell membranes, synthesis of bile acid and steroid hormones. The results presented in graphs shows a significant decrease in lipid content in the studied tissues of the test fish, generally, the lipid contents in muscle and liver tissues were found to be decreased significantly ($P<0.01$ and $P< 0.01$) with the periods of exposure.

A number of workers have reported decline in lipids level of various organs and tissues under toxic stress of various chemical. Leela *et al.* (2000) [21] have observed a significant decrease in the total lipid content of liver, muscle and gill of *Tilapia mossambica* under the stress of phosalone. The alterations of lipid content may be due to its utilization in cortical steroidogenesis and also impairment in the synthesis of lipid. Decrease in lipid content in tissues suggested that the lipid have been channelized to meet the metabolic demand for extra energy need to mitigate the toxic stress. The biochemical constituents in liver and kidney of fish *Channa gachua* after sub-lethal exposure to chlorpyrifos have falls down considerably. Katti and Sathyanesan (1983) [16] reported the decreased lipid level in fresh water fish *Clarius batrachus* under the stress of lead nitrate. In grass carp the levels of total protein, cholesterol and glucose indices were reduced significantly by the exposure of atrazin (Thirumurugan *et al.*, 2011) [43]. The low level of lipid recorded in the exposed fish, of the present study might have been used for energy production for other metabolic functions in which these products play a vital role during stress conditions (Pathan *et al*., 2009) [28]. As an explanation of the loss of lipid noticed in this study, it may be suggested that, pollutants inhibited lipid synthesis and started mobilizing the stored lipids either through β-oxidation or through a gradual unsaturation of lipid molecules (Jha and Jha, 1995).

Conclusion

In the present study, it is concluded that lead nitrate has a profound influence on the biochemical analysis of major carp fingerlings *L.rohita* and lead nitrate is toxic to aquatic organisms. The results simply a better understanding on the toxicological end point of this specific heavy metals and provides significant information on safe levels in the aquatic environment.

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