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Research Paper

Effect of Lead Nitrate on serum protein profile in fish, *Clarias batrachus*

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Abstract: Lead has been recognized as a highly toxic cumulative element in man and animals. In industrialized nations during recent years, long-time exposure of animals to air-borne lead has been recognized as a health hazard. It has been found to have adverse effects on biochemical and histological conditions. Lead is included in a range of elements which have been called "heavy metals" and "trace metals" alternatively. As part of these trace metals, they enter the ocean and other waters as a result of natural processes and human activities via rivers, land run-off, dumping, the atmosphere and the sea weed. The serum proteins and albumin has been found to be decreased after lead nitrate treatment due to its toxic effect.
Keywords: *Clarias batrachus* Organophosphate, Lead nitrate, Serum protein, Albumin.

INTRODUCTION

There is worldwide concern over the impact of environmental pollutants on health and environment. These pollutants have in time progressed to take various forms such as smog toxic chemicals, mutagens and deposition products, radiation, etc. and seeped through the air, water and soil and undermined the biota, resulting in an imbalance in the normal activity of the ecosystem. Heavy metal enters the environment via a variety of routes like surface runoff from mine, waste discharge from sewage and industrial plants. Industry effluents discharged into aquatic ecosystem have created problems in water pollution. Heavy metals are among the chemicals present in the effluent that contribute to the problem. Lead is the most abundant heavy metal. Lead has been recognized as a highly toxic cumulative element in man and animals. In industrialized nations during recent years, long-time exposure of animals to air-borne lead has been recognized as a health hazard. It has been found to have adverse effects on biochemical and histological conditions. Lead is included in a range of elements which have been called "heavy metals" and "trace metals" alternatively. As part of these trace metals, they enter the ocean and other water as a result of natural processes and human activities via rivers, land run-off, dumping, the atmosphere and the sea bed. Major natural sources are

weathering, degassing, releases from terrestrial and submarine volcanoes and dissolution of marine sediments. The dominant inputs for most trace metals arc through river and land run-off but for a few elements, such as lead and mercury, the atmospheric route is also significant especially in the open ocean. Many industries release trace metals which reach the sea through a variety of routes. Generally, trace metals are discharged together with other wastes, such as sewage detergents and other organic compounds. Interaction with these wastes and various components of the sea water alters the original physico-chemical forms of these trace metals. Lead in soluble ion is both mobile and toxic.

Importance of water can be estimated from the fact that all the ancient civilization emerged on the river banks. Today water resources have been extensively exploited. Pollution of water bodies is increasing steadily due to rapid increasing population, industrialization, urbanization, increasing standards of living and various other human activities. The quality of water is the major factor concerning to human and aquatic health. Keeping this in view the present study has been undertaken and it embodies the results of the experiment conducted and facts observed on *Clarias batrachus*, as a representative live fish, subjected to various limits, acute, sub-lethal and chronic doses of the aforesaid heavy metal. The objective of the present study is to provide data on the serum protein biochemistry of *Clarias batrachus* treated with lead nitrate to work out the toxicity level of the metal under investigation.

MATERIALS AND METHODS

Rearing and maintenance of fish: The live specimen of *Clarias batrachus* commonly known as "Mangur" were brought for the present study from ponds in surrounding vicinity of Agra and fish market of Agra. The selection of Clarias batrachus as experimental fish went in for reason of its rare occurring in study area because of excess pollution and Exotic or alien fish introduction in Yamuna River and its hardy nature in terms of survival despite pollutant treatments proposed which might indicate an advantage of long stay of toxic effects in soft tissues. Above all, *Clarias batrachus* is of almost importance owing to its taste, medicinal and high market value

Experimental protocol: To assess the effect of lead nitrate the fish, *Clarias batrachus* were grouped in to five sets, four acute and one control each set consisting of six fishes.

Blood collection: The *Clarias batrachus* were taken live and cut at peduncle to collect the blood directly from caudal vein for biochemical estimations. The blood samples were collected in the centrifuge tubes.

Serum separation*:* The centrifuge tubes containing blood samples were allowed to stand in a slanting position to clot for about thirty minutes. At the clot retraction the centrifugation was done at 3000 rpm for 15 minutes to get Red blood corpuscles. The supernatant serum was carefully separated from the clot by a fine rubber bulb glass pipette transferred to air tight glass vials and stored below 0^0C in the freezer until used. However, serum was brought to room temperature before performing various biochemical tests.

Test compound (intoxicant): Lead nitrate has been selected for present study.

Determination of LC_{50}: The *Clarias batrachus* were divided into five groups (A, B, C, D and E). Each group contains ten individual. Standard solution of experimental compound, Lead nitrate was prepared by dissolving in water with the strength 300,400,500,600,700mg/L. The mortality and survival number of fish was recorded for each concentration after 96 hours. The statistical analysis of data was done by log dose/probit regression line method (Finney, 1971). Regression line was drawn on the basis of two variables, log dose and empirical probit on the

simple graph paper and used to determine the expected probit necessary for LC_{50} determination.

Biochemical estimations: Six fishes from each set (control set and experimental sets) were sacrificed for the biochemical studies after 24, 48, 72 and 96hrs treatment of Lead nitrate at sub-lethal dose ($1/5^{th}$ of LC_{50}). Serum total protein was estimated by modified Biuret and Dumas method (Span diagnostic kit code number 25931). Serum proteins reacts with copper of biuret reagent in alkaline medium to form a violet blue colored complex which appears as a result of the reaction between –CO and $–NH_2$ groups of protein. The intensity of the colored complex so developed is proportional to the total protein concentration in the sample. It is measured calorimetrically, using green filter. The serum albumin was calculated by the modified Biuret and Dumas method. Serum albumin binds up with the dye bromocresol green at pH-4.2 to form a green colored complex which is measured calorimetrically using a red filter.

Statistical calculations: In the present investigation, the statistical calculations are used after Fischer and Yates (1950).

RESULTS AND DISCUSSION

In *Clarias batrachus* the toxicity of lead nitrate results in reduced activity with enormous extrusion of mucous which practically covers the body before death. This may be due to the fact that mucous hinders the exchange of the gases resulting in the deficiency of oxygen in the blood, which in turn affects the general physiology of the body resulting into death. LC_{50} value of lead nitrate was 528.14mg/L with variance 0.001, fiducial limits (+) 2.72196 and (-) 2.71804 and regression equation Y = 5.00+6.50 (X-2.72) for the fish *Clarias batrachus*. The sub-lethal dose was $1/5^{th}$ of LC_{50} i.e. 105.62mg/L.

Serum total protein: The serum total protein ranged from 288-315 with an average of 300.50 mg/dl. The serum total protein after intoxication of lead nitrate at 24hrs ranged from 250-265 with an average of 258.00 mg/dl, while after 48hrs ranged 234-249 with an average of 240.50 mg/dl, while after 72hrs ranged from 200-215 with an average of 211.00 mg/dl, and after 96hrs ranged from 165-179 with an average of 175.00 mg/dl. The decrease in serum total protein with increase of exposure to lead nitrate is significant after 24hrs, 48hrs; highly significant after, 72hrs and very highly significant after 96hrs (Table-1, Fig. 1). The serum total protein showed decreasing trend on exposure to sub-lethal concentration of lead nitrate at different time intervals (24, 48, 72 and 96 hours) in *Clarias batrachus*. The decrease in serum total protein with increase of exposure to lead nitrate is significant after 24hrs, 48hrs; highly significant after, 72hrs and very highly significant after 96hrs. The decrease in total protein may be due to the inhibition of RNA synthesis disturbing the protein metabolism. This is also supported by Medda *et al.* (1992), who observed that when lead nitrate exposure increased further decrease in total protein level may be due to inhibition of metabolizing enzymes in presence of toxicant. Similar trend of protein decrement was also observed by Arasta *et al.* (1996) in Indian catfish *Mystus vittatus* and Mastan *et al. (*2008) in *clarias batrachus* after lead nitrate exposure.

In the present investigation, serum total protein may be depleted probably because of excessive renal excretion or due to the liver disorder after the pesticide exposure. This is corroborated with the finding of Garg, *et al*. (1989). Saxena *et al.* (2005) evaluated the toxicity of Roger to *Clarias batrachus* by studying its morphological and patho-physiological impact and found that it causes depletion in the serum total protein. Garg *et al*. (1989) observed that fall in serum protein may be due to the impaired function of kidney or due to reduced protein synthesis owing to liver cirrhosis. Dalela *et al*. (1981) also reported that decrease in serum protein levels of

pesticide treated *Mystus vittatus* may be due to the excretion of proteins by kidney due to kidney disorder or impaired protein synthesis as a result of liver disorder. In the present investigation reduction in the serum total protein may also be attributed to intensive proteolysis which contributes to the increase in the free amino acids to be fed into TCA cycle as ketoacids.

Serum albumin: The serum albumin ranged from 155-162 with an average of 158.00 mg/dl. The serum albumin after intoxication of lead nitrate at 24hrs ranged from 129-134 with an average of 132.50 mg/dl, while after 48hrs ranged 110-116 with an average of 115.90 mg/dl, while after 72hrs ranged from 98-102 with an average of 100.00 mg/dl, and after 96hrs ranged from 90-93 with an average of 92.56 mg/dl. The decrease in serum albumin with increase of exposure to lead nitrate was significant after 24hrs, 48hrs; highly significant after 72hrs and very highly significant after 96hrs (Table-2, Fig. 2). The serum albumin showed decreasing trend on exposure to sub-lethal concentration of lead nitrate at different time intervals (24, 48, 72 and 96 hours) in *Clarias batrachus*. The decrease in serum albumin with increase of exposure to lead nitrate was significant after 24 hrs, 48 hrs; highly significant after 72 hrs and very highly significant after 96hrs. Similar to the present findings, Ansari and Kumar (1988) have reported the decline in protein (both albumin and globulin) and nucleoprotein (DNA and RNA) in zebrafish after diazinon treatment. Further, Singh and Bhati (1994) have reported changes in haematobiochemical parameters after treatment of zinc chloride in *Channa striatus*.

Table-1. Effect of lead nitrate on total protein (mg/dl) in *Clarias batrachus* (Linn.)

Serum total protein (mg/dl)	Control	Exposure hours			
		24	48	72	96
Range	288-315	250-265	234-249	200-215	165-179
Mean	300.50	258.00	240.50	211.00	175.00
±S.Em.	±1.50	±1.10*	±0.90*	±0.67**	±0.50**

Table-2. Effect of lead nitrate on serum albumin (mg/dl) in *Clarias batrachus* (Linn.)

Serum albumin (mg/dl)	Control	Exposure hours			
		24	48	72	96
Range	155-162	129-134	110-116	98-102	90-93
Mean	158.00	132.50	115.90	100.00	92.56
±S.Em.	±1.54	±0.67*	±0.65*	±0.50**	±0.22**

S.Em.- Standard error of mean
NS- Non-significant
*- Significant
**- Highly significant
***- Very highly significant

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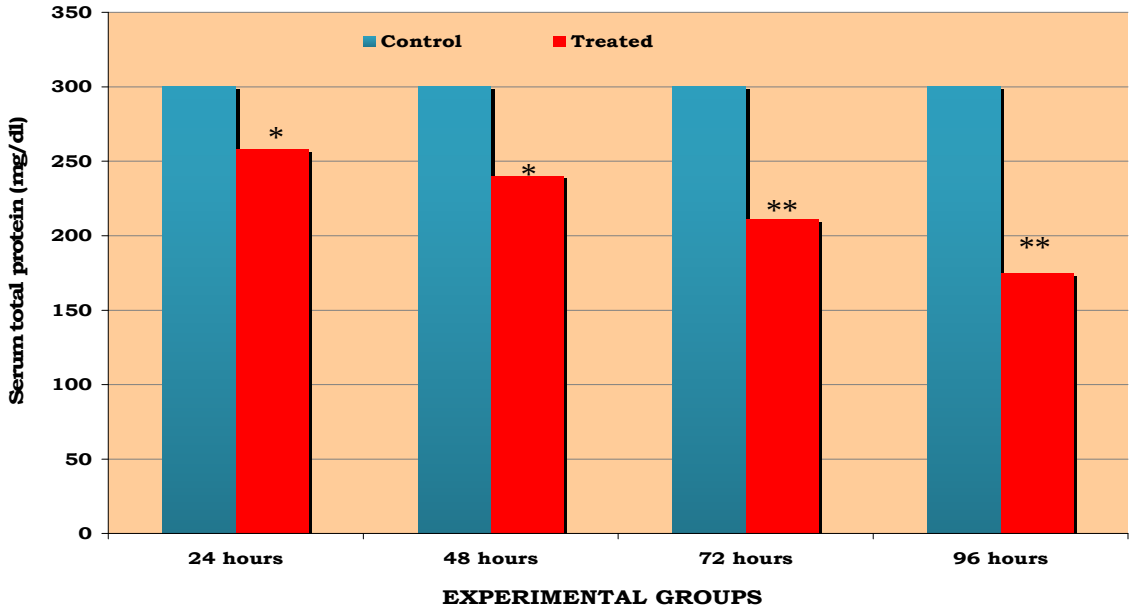

Fig. 1 : Effect of lead nitrate on serum total protein (mg/dl) in *Clarias batrachus* (Linn.)

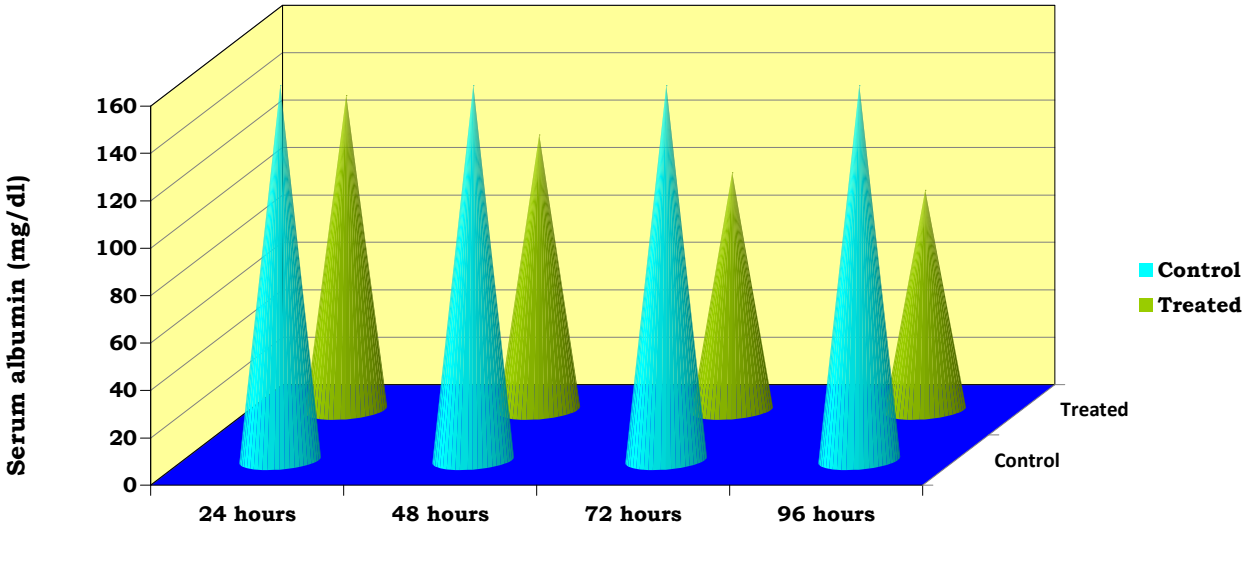

Fig. 2: Effect of lead nitrate on serum albumin (mg/dl) in *Clarias batrachus* (Linn.)

*** Non-significant; ** Significant; *** Highly significant; **** Very highly significant**

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