Bull. Environ. Contam. Toxicol. (1989) 43:310–314
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Bulletin of Environmental Contamination and Toxicology

# Lead–Induced Biochemical Changes in Freshwater Fish *Oreochromis mossambicus*

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Lead, a non-essential and non-beneficial element has considerably added the problem of health hazard to human and experimental mammals (WHO 1977). It has also received much attention over the past few years as potentially important aquatic pollutant (Hall 1972). Fishes are of great nutritional significance and their intoxication by lead causes retardation of growth and deterioration in the nutritional value (Shaffi 1979). In fish, lead is known to cause a variety of effects like anaemia, inhibition of delta-amino levulinic acid dehydratase (ALA-D) activity (Hodson 1976), caudal fin degeneration and black tail disease (Haux et al 1981). Apart from this, very little attention has been paid to biochemical changes which develop more quickly in response to toxicants than any apparent morphological changes. Therefore, the present investigation was undertaken to evaluate the effect of lead on plasma chemistry of freshwater fish Oreochromis mossambicus. This fish was selected because of its wide availability, edibility in India and its suitability as a model fish for toxicity testing (Ruparelia et al 1986). The variables such as glucose, cholesterol and protein representing carbohydrate, lipid and protein metabolism were studied.

## MATERIALS AND METHODS

Sixty fish of both sexes (104.17±3.06 gm) used in this experiment were collected from the local pond and acclimatized to the laboratory conditions for 14 days prior to start the experiment. Fish were divided into four main groups which were divided further into three sub-groups having five fish in each. Fish were maintained in glass aquaria (92 cm x 38 cm x 37 cm; 120 L water capacity) under natural light and ambient temperature. They were fed dry fish powder daily and tubificid worms (Tubifex sp.) ad libitum on every alternate day. The medium in which fish were placed was changed every 24 h. Lead (as lead acetate) was dissolved in 100 L of aquarium water to achieve the nominal concentrations of 18.0, 24.0 and 33.0 mg $Pb^{2+}$/L. The physico-chemical characteristics of test water (pH 8.20; temperature 29°C; total alkalinity 351.20 mg/L; total hardness 210.00 mg/L and dissolved oxygen 6.10 mg/L)

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were determined as per standard methods (APHA 1985). Five fish, each from control and experimental aquaria were removed and anaesthetized with MS 222 ( 3-aminobenzoic acid ethyl ester) after 7, 14 and 21 days exposure. Blood samples were collected in heparinized vials with the help of 1 mL disposable syringe equipped with 22-guage hypodermic needle by puncturing the ventral aorta (Ruparelia et al 1986). Plasma was obtained by centrifugation and stored at -20°C until analyzed. Plasma glucose level was estimated using O-toluidine reagent (Dubowski 1962) following glucose as a standard. Total cholesterol was determined employing p-toluenesulfonic acid with single step colorimetric procedure based on Libermann-Burchard reaction (Pearson et al 1953). Total plasma protein was analyzed using Folin phenol reagent (Lowry et al 1951) and bovine serum albumin served as standard. These techniques were standardized in the laboratory on Beckman DU5 spectrophotometer and reliable reproducibility and accuracy of the results were achieved. Student's 't' test was applied for the test of significance.

## RESULTS AND DISCUSSION

The results of this study showed that lead intoxication led to significant variations in biochemical composition of plasma in fish, Oreochromis mossambicus (Table 1).

Plasma glucose level decreased from the controls in fish exposed to all the three concentrations. The decrease in glucose level was found to be significant ($p < 0.01$; $p < 0.05$) in the fish exposed to 24.0 mg/L and 33.0 mg/L of lead after 14 and 21 days. It also dropped significantly at 18.0 mg/L of lead after 21 days exposure. Thus, lead exposure caused hypoglycemia in fish. The percentage depletion in glucose level after 14 days were 6, 48 and 40% and for 21 days 27, 30 and 34%, respectively, at 18.0, 24.0 and 33.0 mg/L lead concentrations. Haux et al (1981) and Haux and Larsson (1982) in a series of experiments observed significant and persistent hypoglycemia in lead exposed fish (Salmo gairdneri). Helmy et al (1979) also noted hypoglycemia in fish (Liza macrolepis) exposed to lead nitrate. The hypoglycemic response exhibited by lead intoxicated fish might be due to the lead induced morphological and functional changes in renal tubule cells of the kidney alongwith the reduced gluconeogenesis. Such effects are common in lead exposed mammals (WHO 1977).

Plasma cholesterol level registered a fall from the controls in fish exposed to all the three concentrations. The decrease in cholesterol level was found to be significant ($p < 0.05$) after 14 days at 33.0 mg/L lead concentration. The cholesterol level also dropped significantly ($p < 0.05$, $p < 0.01$) at the concentrations of 18.0, 24.0 and 33.0 mg/L of lead after 21 days exposure. The percentage depletion in cholesterol levels at all the three concentrations after 14 days were 43, 46 and 59% and for 21 days 52, 24 and 51%.

Table 1. Effect of lead on biochemical profile of fish, O. mossambicus.

| Variable | Exposure period (days) | Control | Exposure | | |
|---|---|---|---|---|---|
| | | | 18 mg/L | 24 mg/L | 33 mg/L |
| Plasma Glucose (mg/dL) | 7 | 79.40±6.26 | 61.71±6.67 | 66.94±7.75 | 71.39±4.47 |
| | 14 | 86.63±6.92 | 80.60±13.69 | 44.20±3.60** | 51.40±4.96** |
| | 21 | 75.20±3.64 | 54.60±3.22* | 52.20±6.44* | 49.20±5.65** |
| Plasma Cholesterol (mg/dL) | 7 | 163.04±28.76 | 121.80±8.40 | 130.04±18.12 | 134.40±14.34 |
| | 14 | 149.46±30.09 | 85.25±13.50 | 79.80±11.95 | 61.20±5.37* |
| | 21 | 160.16±8.81 | 76.33±10.67** | 120.83±10.95* | 77.84±12.42** |
| Total Plasma Protein (gm/dL) | 7 | 2.17±0.23 | 3.01±0.18* | 2.52±0.15 | 2.84±0.08* |
| | 14 | 2.63±0.38 | 3.06±0.40 | 3.19±0.28 | 2.75±0.08 |
| | 21 | 2.40±0.26 | 2.46±0.26 | 3.16±0.15* | 2.96±0.03 |

Values are expressed as Mean±SE; N = 5; *$p<0.05$; ** $p<0.01$

A review of literature reveals that reports of influence of lead on circulating cholesterol level are scanty (Tewari et al 1987). The fish exposed to various concentrations of lead thus manifested hypocholesterolemia. This finding is in accordance with the findings of Tewari et al (1987) who also observed decreased cholesterol level in fish (Barbus conchonius) during lead exposure. It is presumed that reduction in circulating cholesterol level is either because of more utilization of cholesterol during corticosteroidogenesis, as it is precursor for steroid hormones, or depressed de novo synthesis. Methylmercury has also been reported to decrease the serum cholesterol level in bluegill fish, Lepomis macrochirus (Dutta and Haghighi 1986). Simultaneously an increase in protein level was also observed inthe same study. This might have resulted in high density lipoprotein in serum and was suggested to be the cause of hypocholesterolemia in mercury exposed fish. In our study too, increase in plasma protein level was observed (Table 1). Fish exposed to 18.0 and 33.0 mg/L lead showed significantly ($p > 0.05$) elevated levels of total protein after 7 days. Fish exposed to 24.0 mg/L lead for 21 days also showed significantly increased protein level. The increase in protein levels may be because of induction of protein synthesis in liver tissue by the metal. Helmy et al (1979) also observed hyperproteinemia in lead exposed fish. The precise mechanism and respective relationship involving lowering in plasma glucose and cholesterol level together with rise in protein content by lead in O. mossambicus needs indepth investigation.

Acknowledgments. The authors are grateful to Dr.S.K.Kashyap, Director, National Institute of Occupational Health, Ahmedabad, and Dr.S.K.Nigam, Deputy Director of the Institute, for their interest in the present work and providing laboratory facilities.

REFERENCES

APHA (1980) Standard methods for the examination of water and waste water. American Public Health Association, Washington,DC

Dubowski KM (1962) An O-toluidine method for body fluid glucose determination. Clin Chem 8:215-235

Dutta HM, Haghighi AZ (1986) Methylmercuric chloride and serum cholesterol level in the bluegill (Lepomis macrochirus). Bull Environ Contam Toxicol 36:181-185

Hall SK (1972) Lead pollution and poisoning. Environ Sci Tech 6: 3715-3724

Haux C, Sjobeck ML, Larsson A (1981) Some toxic effects of lead on fish. In: Pickering AD (ed) Stress and fish. Academic Press, New York, pp 340-341

Haux C, Larsson A (1982) Influence of inorganic lead in the biochemical blood composition in the rainbow trout, Salmo gairdneri. Ecotoxicol Environ Safety 6:28-34

Helmy MM, Lemke AE, Jacob PG, Alsutan YY (1979) Haematological changes in Kuwait Mullet, Liza macrolepis (Smith) induced by heavy metals. Indian J Mar Sci 8:278-281

Hodson PV (1976) Delta-aminolevulinic acid dehydratase activity fish blood as an indicator of harmful exposure to lead. J Fish Res Bd Can 33:268-271

Lowry OH, Rosenborough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biochem 193:265-275

Pearson S, Stern S, McGavack TH (1953) A rapid procedure for the determination of serum cholesterol. J Clin Endocrinol 25:8-13

Ruparelia SG, Verma Y, Kashyap SK, Chatterjee BB (1986) A new approach for the use of standard fish in toxicological study. In: Dalela RC, Madhyastha MN, Joseph MM (ed) Environmental biology: Coastal ecosystem. The Academy of Environmental Biology, Muzaffarnagar, India, pp 89-92

Shaffi SA (1979) Lead toxicity: Biochemical and physiological imbalance in nine freshwater teleosts. Toxicol Letts 4:155-161

Tewari H, Gill TS, Pant J (1987) Impact of chronic lead poisoning on the haematological and biochemical profiles of a freshwater fish, Barbus conchonius (Ham.). Bull Environ Contam Toxicol 38: 748-752

WHO (1977) Environmental health criteria. 3 Lead. World Health Organization, Geneva

Received March 18, 1988; accepted August 6, 1988.