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Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

ACUTE TOXICITY AND BIOCHEMICAL STUDIES OF LEAD NITRATE ON THE LIVER AND KIDNEY OF FRESH WATER FISH MYSTUS CAVASIUS

Jain, Shampa[1] and Deepmala Batham[2]

[1]Deptt. of Zoology
Govt. M.H. College of H. Science and science for women,
Jabalpur M.P.
[2]Deptt. of Zoology
Govt. Model Science college,
Jabalpur M.P..

Abstract

This study is aimed to examine the toxic effects of lead nitrate on freshwater fish, *Mystus cavasius* since they constitute an important link in the food chain and their contamination by heavy metal imbalances the aquatic system. The fishes were exposed to 0, 50, 55, 60, 65, 70,mg/l of lead nitrate.The lethal concentration (LC_{50}) value of lead nitrate was 55 mg/l for 96 hours exposure. *Mystus cavasius* were exposed to three sub-lethal concentrations of lead nitrate (25 mg/l, 28 mg/l and 30 mg/l) for 10 and 20 days. Heavy metals, such as environmental stressors, may alters tissue biochemical parameters in fishes. Protein content in the liver and kidney was found depleted due to proteolysis induced by lead nitrate. The liver glycogen also declined, indicating increased utilization of glucose to counteract the increased energy demand imposed by severe anaerobic stress of lead nitrate.

Key words: Mystus cavasius , biochemical, lead nitrate, protein, glycogen.

INTRODUCTION

Natural and anthropogenic sources continuously release heavy metals into aquatic ecosystem. The heavy metals after reaching to freshwaters cause serious problem due to their long persistence, bioaccumulation, biomagnification in the food chain, and toxicity to the organisms. Fish, being dominant inhibitors of aquatic environment, are considered as indicators for heavy metal pollution. [29] Some of heavy metals are essential to living organisms and they are commonly found in natural waters but high concentrations and accumulation of these may become toxic. 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. It is accumulated in soft tissues such as liver, kidneys, nervous system, and the brain. In fishes, accumulation of lead in various tissues [8,11,17,18,21] and alterations in biochemical and hematological parameters [19] have been reported. Moreover, lead-

induced changes in the histological structure of gills and kidneys have also been reported [9,12,20,22,24,25].

MATERIAL AND METHOD

Acute toxicity test on *Mystus cavasius* with selected lead nitrate heavy metal. Healthy specimens of *Mystus cavasius* were obtained from pariyat lake of Jabalpur. The collected fishes were to be maintained in the glass aquaria for seven days under laboratory condition (food). The test fishes were also examined carefully for pathological symptoms they were disinfected with o.1% solution of potassium permangnet and transferred one by one with a small hand net from the acclimatization tank to the experimental containers.

The experiment was conducted in two steps:

Experiment 1 - Acute toxicity test.

Experiment 2 - Long term exposure or sub chronic intoxication for biochemical experiments.

Acute toxicity test

Mystus cavasius juveniles with average body weight 7-8 gm were used in this study to determine the zero and 100% percentages of mortalities (Preliminary study) as well as the 96 hours LC_{50}. *Mystus cavasius* juveniles were divide in to three groups (each group contained 12 fishes) and kept in glass aquarium (59cm x 25cm x 22cm). All group of tested fish (excepts control group of fish) were exposed to different concentration of lead nitrate from 35mg/l to 65 mg/l for a period of 96 hours .

Biochemical Assay

For biochemical analysis we selected 3 sublethal concentration i.e. 25mg/l, 28mg/l, 30mg/l. for 10 and 20 days .Fishes were slaughtered & tissue sample were obtained and weighted. Liver and kidney tissues were homogenates and then centrifuged at 2000 rpm for 20 minute, and the supernatant was obtained for further biochemical assay. The estimation of Protein was done by bio analyzer star 21 plus (Erba kit). The determination of tissue glycogen was carried out by the modified Pflüger method.

Statistical analysis

Results are presented as mean ± standard errors. The data obtained in this study were statistically analyzed through tool pack 32 VBA (visual basic for application) in MS excel work sheet and also using method of ANOVA (independent sample test). Differences between control and three individual lead nitrate treated group (25mg/l, 28mg/l, and 30mg/l).

RESULTS

3.1 Acute toxicity

The results of the acute toxicity test are presented in table 1. The LC_{50} value based on probit analysis was found to be 55mg/l for 96 hrs of exposure to the lead nitrate. The results obtained showed that there was no mortality of fish in the control experiment through out 96 hrs. However there was 50% mortality at 55 mg/l, while at 65mg/l 100% mortality was observed. During this study the behavior of the control fish was normal, while the fish introduced into the different concentrations of the heavy metal showed the fish became very weak, less active and settled at the bottom.

Table 1. Rate of mortality of juvenile Mystus cavasius exposure to lead nitrate for 96 hrs

concentration	24 hrs	48 hrs	72hrs	96hrs	Mortality
Control	0	0	0	0	0
35mg/L	0	0	0	1	1
40mg/L	0	0	1	1	2
45mg/L	0	0	1	1	2
50mg/L	0	1	1	1	3
55mg/L	1	1	2	2	6
60mg/L	1	2	2	3	8
65mg/L	2	3	3	4	12

Table 2. calculate log dose and probit values

concentration	log dose	Mortality	Percentage	Probit
35mg/L	1.544068	1	8.33	3.59
40mg/L	1.60206	2	16.67	4.01
45mg/L	1.6532125	2	16.67	4.01
m50mg/L	1.69897	3	25.00	4.33
55mg/L	1.7403627	6	50.00	5
60mg/L	1.7781513	8	66.67	5.41

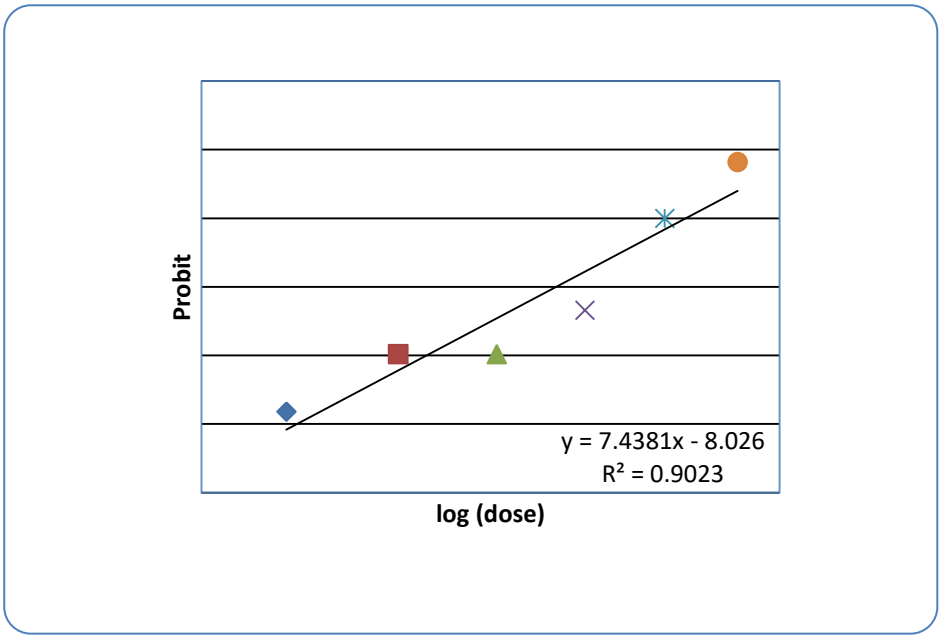

Figure : Linear relationship between probit response and log concentration of lead nitrate on juveniles of Mystus cavasius

3.2 Biochemical analysis of the liver protein and glycogen

Table3: Fish liver total protein (mg/g wet tissue) exposed to different concentration of lead nitrate at different time intervals. . The level of liver protein (Mean ± SE) in control and experimental fish.

	10 days	*20 days*
Control	250.33^{b}±7.97	250.33^{b}±7.97
25mg $Pb(no_3)_2$	140.67^{a}±6.14	153.17^{a}±9.21
28mg $Pb(no_3)_2$	148.17^{a}±4.13	143.33^{a}±5.55
30mg $Pb(no_3)_2$	137.67^{a}±4.77	131.67^{a}±3.33

Same super script did not differ significantly (P<0.05)

There is lowest significant (a)difference between control (b) 10 and 20 days interval at the dose rate of 25mg/l, 28mg/l and 30mg/l of $Pb(no_3)_2$. Depletion of liver protein was observed at all concentrations and exposure periods when compared to control.

Table 4: Fish liver total glycogen (mg/g wet tissue) exposed to different concentration of lead nitrate at different time intervals. The level of liver glycogen (Mean ± SE) in control and experimental fish.

	10 days	*20 days*
Control	13.045^{c}±0.593	13.045^{c}±0.593
25mg $Pb(no_3)_2$	1.455^{a}±0.103	3.95^{b}±0.269
28mg $Pb(no_3)_2$	2.225^{a}±0.145	5.115^{b}±0.365
30mg $Pb(no_3)_2$	3.785^{b}±0.255	4.61^{b}±0.476

Same super script did not differ significantly (P<0.05)

There is lowest significant (a) difference between control (c) interval 10 days however it is significant (b) interval 20 days at the dose rate of 25mg/l and 28mg/l where as it is significant (b) difference between control (c) both interval 10 and 20 days at the dose rate of 30 mg/l of $Pb(no_3)_2$ in glycogen. Depletion of liver glycogen was observed at all concentrations and exposure period when compared to control.

3.3 Biochemical analysis of the kidney protein and glycogen

Table 5: Fish kidney total protein (mg/g wet tissue) exposed to different concentration of lead nitrate at different time intervals. The level of kidney protein (Mean ± SE) in control and experimental fish.

	10 days	*20 days*
Control	158.83^{b}±7.66	158.83^{b}±7.66
25mg $Pb(no_3)_2$	140.83^{ab}±4.92	150.50^{b}±7.34
28mg $Pb(no_3)_2$	139.50^{ab}±5.45	148.00^{b}±7.42
30mg $Pb(no_3)_2$	135.00^{ab}±2.84	125.67^{a}±1.43

Same super script did not differ significantly (P<0.05)

There is lowest non significant (ab) difference between control(b) in interval of 10 days at all concentration but it is non significant (b) difference between control(b) at interval of 20 days at the dose rate of 25 mg/l and 28 mg/l but it is lowest significant (a)in interval of 20 days at the dose rate of 30 mg/l $Pb(no_3)_2$. Depletion of kidney

protein was observed at concentrations and exposure period where as it was lowest significant ($p<0.01$) when fish exposed to 30 mg/l $Pb(no_3)_2$ on 20 th day, when compared to control.

Table 6: Fish kidney total glycogen (mg/g wet tissue) exposed to different concentration of lead nitrate at different time intervals. The level of kidney glycogen (Mean ± SE) in control and experimental fish.

	10 days	*20 days*
Control	2.76^{b}±0.27	2.76^{b}±0.27
25mg $Pb(no_3)_2$	2.37^{b}±0.10	3.53^{c}±0.24
28mg $Pb(no_3)_2$	1.79^{a}±0.27	1.54^{a}±0.14
30mg $Pb(no_3)_2$	3.44^{c}±0.13	3.53^{c}±0.14

Same super script did not differ significantly ($P<0.05$)
There is non-significant (b) difference between control(b) in interval of 10 days at the dose rate of 25 mg/l however it is lowest significant(a) difference between control (b) in interval of 10 days and 20 days at the dose rate of 28 mg/l where as it is highest significant(c) difference between control(b) at the duration of 10 and 20 days at the dose rate of 30mg/l. kidney glycogen increased at exposure levels of $Pb(no_3)_2$ in a dose of 25 mg/l and 30 mg/l.

DISCUSSION

Wastes containing metals may arise from a variety of industrial operations such as chemical, metal processing, paint manufacture, battery manufacture etc. Metals in the water occur in forms ranging from particles of pure metal in suspension to metals ions and compounds in solution. The present study showed that the 96 hours LC_{50} value of lead nitrate was 55mg/l for *Mystus cavasius* juveniles [13]. They obtaining LC_{50} value of $Pb(no_3)_2$ for 96 hours to be 25mg/l for P.sophore. LC_{50} value for lead nitrate at 24, 48, 72 and 96 hours were 885,875,862, and 822 mg/l respectively for *Heteropneustes fossilis* [29].
In present study sub lethal doses of $Pb(no_3)_2$ produced biochemical abnormalities in liver and kidney. Lead nitrate caused drastic depletion in liver protein and slight decrease in kidney protein at all exposure levels. Protein are highly sensitive to heavy metal poisoning. Appreciable decrease in protein level of liver, muscle, intestine, gill and blood of *Heteropneustes fossilis* was noticed after the exposure of fish to nickel for 30, 60 and 90 days. [3]. Decrease in the liver and muscles protein level has been reported in *Channa punctatus* exposed to ole andrin [10]. A similar decreased liver protein level has also been found in *Cirrhina mrigala* exposed to lead acetate [4], *Cyprinus carpio* exposed to endosulfan[5] and *Heteropneustes fossilis* exposed to petroleum oil [14].

Glycogen is one of immediate fuel reserves and an important constituents which can be influenced by stress. Liver is the main carbohydrate store in fish [26] and plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose [15] in the present study, lead nitrate induced pronounced biochemical changes in *Mystus cavasius* indicating altered metabolism. In this study liver glycogen content was significantly decreased. The observation of depletion glycogen in the present study explains the increased demand of these molecules to provide energy for the cellular biochemical processs under toxic manifestations [23] similar results were observed in *Anabas testudineus* when exposed to lead nitrate [2] The slight increase in kidney glycogen were found in all concentration and duration.

This elevation may be due to inhibition of glycogenolysis or due to initiation of glycogenesis increase in glycogen level similar results were reported by [1,7].

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