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Effect Chronic Lead Toxicity on Biomarkers in Rohu (Labeo Rohita) Fingerlings

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Abstract: *Heavy metal contamination in aquatic ecosystems poses a significant threat to fish health and aquaculture sustainability. This study evaluated the effects of chronic lead acetate exposure on biochemical biomarkers in Labeo rohita fingerlings. The 96-hour LC□□ value was determined to be approximately 30 mg/L using probit analysis, and a sublethal concentration (3.15 mg/L) was selected for a 28-day exposure. Water quality parameters were maintained within recommended limits. Results revealed significant biochemical alterations in lead-exposed fish, with increased activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), indicating hepatic dysfunction and physiological stress. In contrast, superoxide dismutase (SOD) activity decreased, suggesting oxidative stress and compromised antioxidant defense. These findings demonstrate that sublethal lead exposure disrupts enzymatic and antioxidant systems in L. rohita. The study highlights the utility of biochemical biomarkers in assessing heavy metal toxicity and emphasizes the need for effective monitoring and regulation of aquatic pollution.*

Keywords: *Lead toxicity, Labeo rohita, oxygen consumption, sublethal toxicity, bio-markers. chronic exposure.*

I. INTRODUCTION

Heavy metal contamination in aquatic ecosystems has emerged as a critical environmental issue due to its detrimental effects on aquatic organisms (Mahmuda et al., 2020; Sarkar et al., 2016). These metals accumulate in water bodies through both natural processes and anthropogenic activities, including agricultural runoff, landfill leachates, port operations, and the discharge of industrial and domestic effluents (Ezemonye et al., 2019). Increasing population pressure, intensified agricultural practices, and rapid industrialization have further contributed to the influx of pollutants, adversely affecting the physiological, biochemical, and structural integrity of aquatic fauna (Sarkar et al., 2022; Shahjahan et al., 2019, 2021).Among environmental pollutants, heavy metals are particularly concerning due to their persistence, non-biodegradable nature, and long-term ecological impacts (Tamizhazhagan et al., 2016). Fish are widely recognized as reliable bioindicators for monitoring metal contamination in freshwater ecosystems (Azmat, 2012). Lead (Pb), a highly toxic heavy metal, commonly exists in inorganic oxidized forms and enters aquatic organisms through contaminated water and food sources. It tends to accumulate in metabolically active organs such as the liver, gills, and kidneys (Jackson, 2005).Exposure to lead has been shown to disrupt multiple physiological processes in fish, including enzyme activity, endocrine function, hematological parameters, and tissue architecture (Shahjahan et al., 2022). Even at sublethal concentrations, lead can impair feeding behavior, growth performance, and biochemical composition, particularly affecting proteins and carbohydrates, which serve as primary energy reserves (Amin et al., 2017). The extent of toxicity is influenced by factors such as fish age, water pH, and hardness (Nussey, 2000). Additionally, oxygen consumption is a sensitive indicator of metabolic status and is frequently used to assess stress responses in aquatic organisms (Schmidt-Nielsen, 2007).

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In this context, the present study was undertaken to evaluate the chronic effects of lead acetate exposure on biochemical biomarkers (ALT,AST, ALT and SOD) in *Labeo rohita* fingerlings, with the aim of understanding lead-induced metabolic alterations.

II. MATERIALS AND METHODS

Experimental Fish Acclimatization

Healthy fingerlings of *Labeo rohita* (mean total length: 3.93 ± 0.226 cm; mean body weight: 2.21 ± 0.05 g) were obtained from a fish hatchery located in Paithan, District Chhatrapati Sambhaji Nagar (Aurangabad). The fish were transported to the laboratory in aerated polyethylene bags and acclimatized under laboratory conditions for 15 days in a 1000-liter cement tank maintained at 34 ± 2°C. During acclimatization, fish were fed a commercial fish diet twice daily, and the water was renewed every 24 hours.Post-acclimatization, the fish were transferred to 25-liter capacity aquaria (plastic troughs) for experimentation. Water quality parameters, including temperature, dissolved oxygen, pH, and alkalinity, were maintained in accordance with the guidelines recommended by the United States Environmental Protection Agency (USEPA, 1976).

Acute Toxicity and LC□□ Determination

Acute toxicity of lead acetate was assessed following standard protocols by (USEPA 1995) and (OECD, 2000). Fish were divided into ten groups (n=10 per group) and exposed to varying concentrations of lead acetate ranging from 10 to 65 ppm for a period of 96 hours using a static bioassay method. Mortality was recorded at 24-hour intervals. The 96-hour median lethal concentration (LC□□) was determined using Probit analysis (Finney, 1952; Finney DJ, 1970).Experiments followed institutional ethical guidelines for fish handling.

Sublethal Exposure

Based on the LC□□ value obtained, a sublethal concentration (1/10th of LC□□; 3.15 ppm) was selected for chronic exposure studies. Fish were randomly divided into two groups: a control group and an experimental group (n=10 per group). The treatment group was exposed to 3.15 ppm of lead acetate for a period of 28 days. During the exposure period, water was renewed every 24 hours, and fish were monitored for behavioral and physiological changes.

Analysis of Biomarkers

The liver tissue samples were homogenized in cold phosphate buffer saline (0.1 M pH 7.4) using a Waring blender.Then, this homogenate was filtered and centrifuged at 400 x g at 4^{o}C and supernatant was stored at 4^{o}C and used for biochemical estimation. Liver homogenate was prepared and the clear supernatant was used for the estimation of the enzymes ALT, AST and SOD.Total Alanine transaminase (ALT), Aspartate transaminase (AST) Superoxide dismutase (SOD) in the tissues were analysed.

Estimation of Alkaline Phosphatase:

0.5 ml of borate buffer, 0.5 ml of substrate and 0.1 ml supernatant will be mixing, and incubated at room temperature for 1 hour. After incubation the enzyme reaction will arrested using 5.9 ml of 0.05 N NaOH and mixed well. The colour intensity was measured at 650 nm. The mixture contain above all the sample will used as a blank. The Tyrosine will used as the standard.

Assay of serum aspartate aminotransferase (AST)

Serum AST was assayed by using the diagnostic kit based on the method ofRetiman and Frankel, (1957).The amount ofoxaloacetate was measured by converting it into pyruvate by treating with anilinecitrate and then reacting the pyruvate with 2,4-dinitrophehylhdrazine (DNPH) tofrom 2,4-dinitrophenylhydrazone derivative which is brown colored in alkalinemedium.

Assay of serum alanine aminotransferase (ALT)

Serum ALT was assayed by using the diagnostic kit based on the method ofRetiman and Frankel, (1957).

Superoxide dismutase (SOD)

Enzyme was assayed by taking 0.5 ml of 1:10 dilution of tissue homogenate followed by addition of 0.3 ml of sodium pyrophosphate buffer, 0.025 ml of PMS (200 µM) and 0.075 ml of NBT. The reaction was started by the addition of

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0.075 ml of NADH. After incubation at 30 °C for 90 seconds, the reaction was stopped by the addition of 0.25 ml glacial acetic acid.Then the reaction mixture was stirred vigorously and shaken with 2.0 ml of n-butanol.The mixture was allowed to stand for 10 minutes and centrifuged, n-butanol alone was served as blank. The color intensity of the chromogen was read at 560 nm. One unit of enzyme activity was defined as the amount of SOD capable of inhibiting 50 % of nitrite formation under assay condition.

III. RESULTS

Physico-chemical Analysis of Water:

Water parameters (Temperature: 23.2 ± 0.2°C, pH: 7.7 ± 0.3, alkalinity: 284 ± 0 mg/L, dissolved oxygen: 6.5 ± 0.4 mg/L) were maintained within recommended ranges (Table 1) throughout the experiment.

Table 1: Physico-chemical analysis of water parameters.

Parameters	Mean ± SDResults	Recommended range
Temperature	23.2 ± 0.2	18- 35°C
pH	7.7 ± 0.3	6.5- 8.7
Alkalinity	284 ± 0	50- 400 mg/L
Dissolved Oxygen	6.5 ± 0.4	5–8 mg/L.

The probit regression analysis of lead acetate toxicity demonstrated a clear dose-dependent increase in mortality of *Labeo rohita* over 96 h. Mortality increased from 10% at 10 mg L^{-1} to 100% at 60 mg L^{-1}, indicating high sensitivity of fish to lead exposure. The probit value of 5 (corresponding to 50% mortality) intersected the regression line at approximately 30 mg L^{-1}, establishing the 96-h LC□□ of lead acetate. The steep slope of the regression curve reflects a narrow margin between sub-lethal and lethal concentrations, highlighting the acute toxic potential of lead in freshwater fish.

Table 2: Study of lethal concentration of Lead acetate (96 h LC_{50}) by probit analysis on rohu fingerlings

Lead acetate: LC_{50} value for 96 Hrs

Sr. No	Conc (mg/L)	log 10 conc	Total No of Rohu fishes	No of Rohu fish alive	Percentage (%) Mortality	Probit value
1	10	1	10	9.5	5	3.36
2	15	1.17	10	9	10	3.72
3	20	1.3	10	8	20	4.16
4	25	1.39	10	6	40	4.75
5	30	1.47	10	5	50	5
6	35	1.54	10	5	50	5
7	40	1.6	10	4	60	5.25
8	45	1.65	10	2	80	5.84
9	50	1.69	10	1	90	6.28
10	60	1.77	10	0	100	6.64

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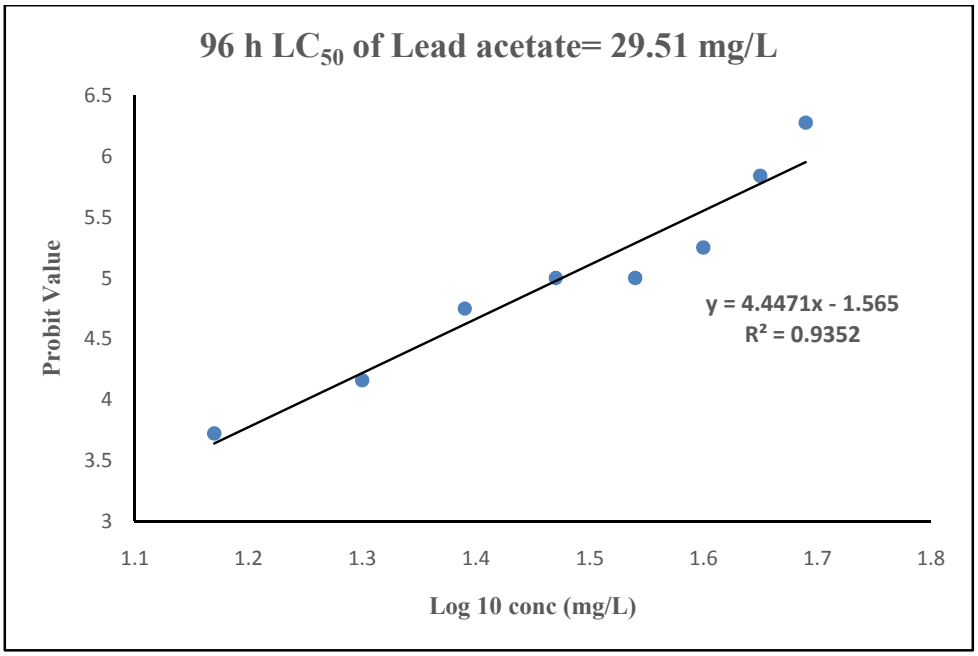

Figure 1: Probit Analysis of Lead acetate LC_{50} estimation

Table 3: Biochemical Analysis (Biomarker)

Enzymes activity(U/L)	Control	Pb
AST	299±0.69	361±0.06
ALT	134±0.45	174±0.08
Alkaline Phosphatase	8.72±0.34	10.3±0.68
SOD	6.91±0.82	4.63±0.48

Chronic exposure to 3.15 ppm lead acetate ($1/10^{th}$ LC_{50}) over 28 days significantly altered various biomarkers (ALP, AST, ALT, SOD) (Table 3).

AST (Aspartate Aminotransferase):AST activity showed a significant increase in the Pb-exposed group (361±0.06 U/L) compared to the control (299±0.69 U/L). This elevation indicates hepatocellular damage and leakage of intracellular enzymes into circulation, suggesting toxic effects of lead on liver tissue.

ALT (Alanine Aminotransferase):ALT levels were markedly elevated in Pb-treated fish (174±0.08 U/L) relative to the control group (134±0.45 U/L). Since ALT is a liver-specific enzyme, its increase reflects hepatic injury and impaired metabolic function due to lead toxicity.

Alkaline Phosphatase (ALP):ALP activity increased from 8.72±0.34 U/L in control to 10.3±0.68 U/L in the Pb group. This rise suggests possible biliary dysfunction or altered membrane transport, indicating physiological stress and liver impairment under lead exposure.

SOD (Superoxide Dismutase):SOD activity significantly decreased in Pb-exposed fish (4.63±0.48 U/L) compared to control (6.91±0.82 U/L). The reduction indicates oxidative stress and weakened antioxidant defense, as lead interferes with enzymatic free radical scavenging mechanisms.

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IV. DISCUSSION

The present study clearly demonstrates that chronic exposure to sublethal concentrations of lead acetate induces significant biochemical alterations in *Labeo rohita* fingerlings, reflecting physiological stress and metabolic dysfunction. The observed increase in mortality with increasing lead concentration during acute toxicity testing confirms the high sensitivity of *L. rohita* to lead contamination, which is consistent with earlier findings that heavy metals exert dose-dependent toxic effects on aquatic organisms (Ezemonye et al., 2019; Taslima et al., 2022). The narrow margin between lethal and sublethal concentrations further indicates the potential ecological risk posed by lead even at low concentrations.

The significant elevation in hepatic enzymes such as AST and ALT in the Pb-exposed group suggests hepatocellular damage and impaired liver function. These enzymes are normally localized within hepatocytes, and their increased activity in tissue homogenates reflects cellular membrane damage and enzyme leakage due to toxic stress. Similar elevations in transaminase activity have been reported in fish exposed to heavy metals, indicating liver dysfunction and altered protein metabolism (Vaseem and Banerjee, 2014; Zulqarnain et al., 2024). The increase in ALT, being more liver-specific, further confirms that lead toxicity primarily targets hepatic tissues and disrupts normal metabolic processes.

Alkaline phosphatase (ALP) activity also showed a noticeable increase in the treated group, which may be attributed to altered membrane transport and possible biliary dysfunction. Elevated ALP levels are often associated with stress conditions and tissue damage, particularly in the liver and associated organs. This finding aligns with previous studies where heavy metal exposure resulted in increased ALP activity due to enhanced lysosomal activity and membrane permeability changes (Tewari et al., 1987; Sivabalan, 2022).

In contrast, the antioxidant enzyme superoxide dismutase (SOD) exhibited a significant decline in activity in lead-exposed fish. SOD plays a crucial role in scavenging reactive oxygen species (ROS), and its reduction indicates oxidative stress and weakened antioxidant defense mechanisms. Lead is known to generate oxidative stress either by enhancing ROS production or by inhibiting antioxidant enzymes, thereby disrupting cellular redox balance (Shahjahan et al., 2019; Ahsan Raza et al., 2024). The decreased SOD activity observed in this study suggests that prolonged lead exposure overwhelms the antioxidant defense system, leading to oxidative damage in fish tissues.

Overall, the biochemical responses observed in this study indicate that lead exposure disrupts enzymatic activity, induces oxidative stress, and impairs liver function in *Labeo rohita*. These alterations can adversely affect growth, metabolism, and survival of fish, ultimately impacting aquaculture productivity and ecosystem health. The findings are in agreement with earlier reports highlighting the detrimental effects of heavy metals on fish physiology and their usefulness as biomarkers for environmental monitoring (Azmat, 2012; Shahjahan et al., 2022).

V. CONCLUSION

The present study demonstrates that chronic exposure to sublethal concentrations of lead acetate exerts significant toxic effects on *Labeo rohita* fingerlings, as evidenced by alterations in key biochemical biomarkers. The elevated levels of AST, ALT, and ALP indicate hepatic dysfunction and cellular damage, while the decline in SOD activity reflects impaired antioxidant defense and increased oxidative stress. These findings confirm that even low concentrations of lead can disrupt metabolic and physiological processes in fish, highlighting the sensitivity of *L. rohita* as a bioindicator species. Overall, the study underscores the ecological risks of lead contamination in aquatic environments and emphasizes the need for continuous monitoring and effective regulation to protect fish health and ensure sustainable aquaculture practices.

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