Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Lead induced toxicity in *Labeo rohita*: cortisol, antioxidant enzymes and liver biomarker levels

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Article info

Received 26.05.2025
Received in revised form 22.06.2025
Accepted 16.07.2025

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Quratulain, Ismat, N., Kousar, T., Ullah, M., Lubna, Ali, K., Khan, T. M., Ali, S., Farooq, U., & Azmat, H. (2025). Lead induced toxicity in Labeo rohita: cortisol, antioxidant enzymes and liver biomarker levels. Regulatory Mechanisms in Biosystems, 16(3), e25125. doi:10.15421/0225125

The long-term persistence of heavy metals in water bodies makes their contamination a serious concern. Among heavy metals, lead (Pb^{2+}) is most abundant in the aquatic environment, where its negative effects on the fauna are severe. This work aims to investigate the median-lethal concentration (LC_{50}) of lead against *Labeo rohita*, and lead-induced oxidative stress by analyzing antioxidant enzymes (SOD, CAT), cortisol profile, lipid peroxidation and aminotransferases activity (ALT, AST and ALP). The final value of LC_{50} determined by probit analysis was 71.58 ± 0.61 mg/L. The results indicated that fish mortality is a function of Pb^{2+} concentration and duration of exposure. After determination of LC_{50}, experimental fish were treated with 1/3rd, 1/5th and 1/10th of LC_{50}, with one control group for 28 days. The cortisol level was significantly increased from week 1 to 4. The maximum cortisol level observed was 124.95 ± 0.88 µg/mL. SOD and CAT activity significantly increased with increase in the concentration and duration. The Pb-exposed groups showed a significant rise in lipid peroxidation (LPO) and liver enzyme activity. This study identified lead as a stress-inducing factor in fish. So, the use of lead should be reduced to protect valuable biodiversity. The study also reinforces the urgent need to monitor and regulate lead discharge into aquatic environments to safeguard aquatic biodiversity and protect public health through the food chain.

Keywords: bioaccumulation; hepatic dysfunction; oxidative damage; stress biomarkers; toxicology; heavy metal exposure.

Introduction

Around the world, aquatic pollution has been a major concern. Human activities are the main cause of pollution by loading chemicals in aquatic ecosystems such as heavy metals and pesticides, which can have detrimental effects on the environment (Al-Saeed et al., 2023). Among other pollutants, heavy metal poisoning of water sources has escalated to a potentially catastrophic level. Heavy metal contamination in water bodies is largely due to industrial effluents and landfill leachate discharge (Ali et al., 2022). Heavy metals pose a serious threat to both terrestrial and aquatic ecosystems, ultimately to terrestrial animals, humans and aquatic life because of their toxic, accumulative, and non-biodegradable properties (Tchounwou et al., 2012). These heavy metals can enter the body of aquatic organisms through food, inhalation, and skin absorption and cause structural deformation of proteins, enzymes, DNA, and a number of other biomolecules by binding with them (Javed et al., 2016).

A highly toxic metals is lead (Pb^{2+}), which is present only in very low concentration naturally. But lead is being produced in large quantities by several industrial processes, e.g. smelting, mining and manufacturing of paint, water pipes and fuel. It is a cumulative toxin designated as one of the 129 priority pollutants by the Environmental Protection Agency (Kumar et al., 2007). Lead can pose a serious risk to aquatic life and is the major cause of the decline in ecological health. Lead has negative impacts on aquatic organisms, causing mortality at lethal doses and impotency, as well as behaviour, and growth performance alterations at sub-lethal concentrations. Pb can cause hepatic and renal damage as well as growth retardation, and it generates stress, resulting in cortisol and metabolic enzyme impairment (Kiran et al., 2021).

Fish is the main source of protein for humans and is regarded as one of the most important dietary sources of omega-3 fatty acids worldwide. It is also rich in unsaturated and polyunsaturated fats (Vali et al., 2022). Being top consumers in the aquatic food chain, fish absorb heavy metals through their gill surface and gastrointestinal tract wall, which allows them to accumulate the metals in their body organs at higher levels, specifically in the liver and kidneys. Fish intake may risk human health if fish accumulate heavy metals beyond the permitted concentration (Ali et al., 2018). Hence, fish can be used as an experimental biological model to estimate toxicological biomarkers. The current study used the indigenous fish *Labeo rohita* as a water quality indicator. *Labeo rohita* is a large carp native to South Asia (Javed et al., 2016) and has emerged as a key product in the aquaculture industry, ranking as the world's tenth most produced fish. *Labeo rohita*, is highly valuable commercially because it is present extensively in the freshwater culture system of Asia and has a mouth-watering flavour (Barange, 2018).

Even at low concentrations, lead exposure can be lethal for fish due to bioaccumulation. Increased Pb accumulation in the fish body indicates the possibility of harmful health impacts on consumers (Kiran et al., 2021). Lead (Pb^{2+}) can cause behavioural changes, oxidative stress, and antioxidant imbalance. Stress response starts at the cellular and molecular levels. If these responses are not controlled, they could upset the homeostatic balance, change the physiology and metabolism of an organism and possibly harm its ability to function. Endocrinological alterations, such as elevated amounts of catecholamines and corticosteroids, are examples of primary stress responses (Tejchman et al., 2021). Cortisol is considered one of the most common stress indicators towards pollutants in fish. Pb^{2+} disturbs cortisol secretion at higher concentrations through an immediate reaction on adrenocortical cells and increased cortisol production (Souza-Talarico et al., 2017).

Oxidative stress is described as a condition in which reactive oxygen species (ROS) concentration is elevated for a short or long period, disrupting cellular metabolism and control, and causing damage to cellular components (Wei et al., 2020). Lead is a redox inactive metal that causes oxidative stress in aquatic animals by disturbing the process of antioxidant enzymes, which results in increased generation of ROS. By the Fenton reaction, hydrogen peroxide converts into the hydroxyl radical, which creates oxidative stress and damages proteins, lipids, and DNA/RNA (Kim & Kang, 2015). Catalase (CAT) and superoxide dismutase (SOD) are two primary biochemical mediators that combat ROS. As a result, it has been proposed that these antioxidant enzymes can be used to investigate the effects of lead toxicity in a variety of animals, including fish (Kim & Kang, 2017).

Lipids undergo lipid peroxidation, a chemical process that has the power to interfere with membrane structure and function. This damage to cell membranes by heavy metals results in an imbalance between the production and breakdown of enzyme proteins (Padi & Chopra, 2002). Biological membranes are impacted by lead, might result in lipid peroxidation (LPO). Oxidative stress by Pb causes the enzyme-aminolevulinic acid dehydratase (ALAD) to produce lipid peroxidation and aminolevulinic acid in biological membranes. Because the products are produced by peroxidation of membrane lipids, TBARS with malondialdehyde (MDA) is a sensitive biomarker for detecting lipid peroxides (Antonio-Garcia & Masso-Gonzalez, 2008).

Lead poisoning causes hepatic damage, implying cellular leakage of hepatic marker enzymes into the bloodstream as well as a breakdown in the hepatic membrane architecture functional integrity (Offor et al., 2017). Assessment of alterations in liver enzyme activity is a good diagnostic tool since they directly represent metabolic abnormalities and cellular damage in specific organs (Vinodhini & Narayanan, 2009). AST, ALT, LDH, and ALP are commonly employed in the diagnosis of pollution damage in fish tissues such as the liver, kidneys, muscles, and gills. There is little information available about disruption caused by lead in enzymatic antioxidant activities as well as the cortisol profile of *L. rohita*. So, this work investigates the acute toxicity of lead and its oxidative stress in *L. rohita*, by examining different enzymatic and non-enzymatic biochemical biomarkers.

Materials and methods

Experimental organism and handling. The trials were carried out in the Fisheries Lab of the University of Veterinary and Animal Sciences, which is located in Lahore, Pakistan. *Labeo rohita* of average weight 30 ± 5 g was taken from the Manga Mandi fish hatchery, Punjab, Pakistan. Before the experiment, the fish were placed in two large rectangular cemented tanks (294 cm x 90 cm x 87.3 cm) with fresh water flowing through them for two weeks, for acclimatization (Hunn et al., 1968) to laboratory conditions.

Metal acute toxicity assay. For acute (96-h) toxicity testing, 100 individuals of *L. rohita* were selected randomly and placed in 10 glass aquaria having a water capacity of 80 litres for each replicate. A stock solution of lead nitrate $Pb(NO_3)_2$ was prepared. A total of 10 concentrations in geometric series were set simultaneously to determine 96-hr LC_{50} of lead against *L. rohita* The optimum value of pH, temperature and total hardness of water was monitored at 7.5, 30 °C and 200 mg/L, respectively. The test was conducted following OECD (2019) standard guidelines and fish mortality was noted after each 24 hours. Based on fish mortality, the value of LC_{50} of lead nitrate was calculated by Probit analysis (Finney, 1971).

Metal chronic toxicity assay. For chronic (28 days) toxicity tests, 160 fish were divided into 4 groups with 1 replicate each. These groups were treated with different concentrations of lead such as T1 with 1/3rd, T2 with 1/5th, T3 with 1/10th of LC_{50} and T4 as control without any concentration of lead. Stock solution of lead nitrate $Pb(NO_3)_2$ was used. During the exposure period, the water was replaced on alternate days and the lead concentration was maintained the same in respective glass aquaria. Fish was fed with commercial feed of 30% crude protein. Mortality of fish was observed regularly during the whole experiment.

Physico-chemical parameters. The fish were kept in a laboratory setting with consistent water temperature, pH, and hardness of 30 ± 0.5 °C, 8.0 ± 0.5, and 180 ± 10 mg/L, respectively. The physicochemical parameters, viz. pH, dissolved oxygen (mg/L) were measured by a multimeter (HANNA (HI-98194), Romania), water temperature (°C), electrical conductivity (µS/cm) were measured by using Digital Electrical conductivity meter (AD-3000, Adwa, Romania), while total hardness (mg/L), total ammonia (mg/L), and carbon dioxide were measured by following methods explained in APHA (2012).

Sampling. Fish were collected after each 14 days of the experimental trial. Blood samples were taken from the caudal vein with hypodermic micro syringes (Jiangsu Zhiyu Medical Instrument Co. Ltd, Taixing City, China). Then, samples were centrifuged for 15 minutes at 3200 revolutions per minute per gram of tissue (Sumera et al., 2018). The acquired serum was stored at –20 °C temperature for the period between centrifugation and its usage for the estimation of plasma cortisol and liver biogeneration biomarkers. After collecting the blood sample, fish were dissected and the liver was separated for enzyme assay.

Estimation of plasma cortisol. The active Cortisol ELISA kit, Catalog No. CO368S was used to measure cortisol levels in blood plasma (Madison et al., 2015).

Liver degeneration biomarker. Bioactive diagnostic (Bad Homburg, Germany) kits were used to measure liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) spectrophotometrically in triplicate at 340 nm. Bioactive diagnostic (Bad Homburg, Germany) kits were used to measure alkaline phosphatase (ALP) in triplicate at 405 nm.

Catalase. CAT activity was assayed by the method of Chance & Maehly (1955). The perfused liver was homogenised in 50 mM phosphate buffer (pH 7.0) in a 1:9 ratio and centrifuged for 15 minutes at 16,000g. The supernatant was separated as an enzyme source. 2 mL phosphate buffer, pH 7.0, 0.45 mL H_2O_2, and 0.025 mL enzyme source made up the reaction mixture. At a wavelength of 250 nm, the enzyme activity was measured in micromoles of H_2O_2 metabolized/milligram protein/minute by using a UV spectrophotometer (Analytik Jena, Germany).

Super oxide dismutase. The inhibition of photo reduction of nitroblue tetrazolium (NBT) by SOD was determined for measuring its activity using the technique of Beauchamp & Fridovich (1971). The perfused liver was homogenised in a 10% w/v potassium phosphate buffer (pH 7.5) and centrifuged for 15 minutes at 16000 g. The supernatant was separated as an enzyme source. 100 mM phosphate buffer, 10 mM EDTA, 130 mM methionine, 750 mM NBT, 60 mM riboflavin, and the enzyme source made up the whole reaction mixture. The reaction was started by adding riboflavin, the samples were exposed to fluorescence for 30 minutes, and the colour was measured at 560 nm against a reagent blank held in the dark. The activity was calculated in units per milligramme of protein.

Lipid peroxidation. The lipid peroxidation was calculated using the method described by Niehaus & Samuelsson (1968). Liver tissue was homogenized in a 50 mL falcon tube containing 50 µL BHT (butylated hydroxytoluene) and 15 mL distilled water. The mixture was centrifuged for 15 min at 16,000 g. Then, 1 mL of supernatant was transferred to a 15 mL plastic tube, 2 mL TBA/TCA (thiobarbituric acid/trichloroacetic acid) was added, and the tube solution was homogenized by vortexing. Then, the solution was heated for 15 minutes in a water bath at 90 °C. After that, the tubes were vortexed for a few minutes before being centrifuged at 4 °C for 15 minutes. The supernatant was collected in cuvettes, and the absorbance was measured in a spectrophotometer at 531 nm wavelength. The resulting absorbance was compared to a tetramethoxypropane standard.

Statistical analysis. The data obtained for 96-h LC_{50} were subjected to Probit analysis (Finney, 1971). The results of the plasma cortisol, antioxidant enzyme assays, and physicochemical parameters were subjected to one-way ANOVA. The significance level among parameters was compared using Duncan's Multiple Range Test at $P \leq 0.05$ (Steel et al., 1996).

Results

Median lethal concentration. Labeo rohita of average weight 30.0 ± 5.0 g were used to perform acute toxicity tests. The results of the 96h acute toxicity test are presented in Table 1. It was discovered that, as expected, when the concentration of lead in the fish increased, the percentage of mortality increased. The value of LC_{50} for lead nitrate calculated by probit analysis was found to be 71.58 ± 0.61 mg/L.

Table 1
Labeo rohita mortality (%) observed during 96 hour exposure to lead

Pb^{2+} concentration, mg/L	Fish mortality, % during 96 hour Pb^{2+} exposure			
	replicate 1	replicate 2	replicate 3	mean
0.0	–	–	–	–
5.0	–	–	–	–
10.0	–	–	–	–
15.0	–	–	–	–
20.0	–	–	–	–
30.0	10	10	–	6.67
40.0	10	20	20	16.67
50.0	30	30	30	30.00
60.0	50	40	40	43.33
70.0	50	50	60	53.33
80.0	60	60	70	63.33
90.0	70	70	80	73.33
100.0	90	80	90	86.67
110.0	100	90	100	96.67
120.0	100	100	100	100.00

Note: the value of LC_{50} for lead nitrate calculated by probit analysis.

The experimental trial was conducted based on preliminary data to study the effect of 1/3rd, 1/5th, and 1/10th of LC_{50}. The physico-chemical parameters were measured during the whole experiment mentioned are mentioned in Table 2.

Plasma cortisol level. The cortisol level (µg/mL) was observed to increase with increase in the concentration and duration of exposure to lead. The value in the control group was significantly ($P \leq 0.05$) less than in the treated groups throughout the experiment. Among the treated groups, the maximum value (124.95 ± 0.88 µg/mL) was observed in T1 on the 28th day of exposure, and the minimum value (60.61 ± 0.84 µg/mL) was observed in T3 on the 14th day of exposure. The plasma cortisol level of *L. rohita* is presented in the Table 3.

Antioxidant enzymes activity. The SOD activity significantly increased from first to fourth week. The value in the control group was significantly lower than in the treated groups. Among treatment groups, SOD activity was significantly ($P < 0.09$) high in T1 (49.72 ± 2.24 IU/mg protein) exposed to 1/3rd of LC_{50}, and a significantly low value was observed in T3 (17.31 ± 2.21 IU/mg protein) with 1/10th of LC_{50} (Table 4). The catalase activity of *L. rohita* is shown in Table 5. Catalase activity was significantly increased in the treated groups as compared to the control. The maximum value (70.45 ± 1.03 IU/mg protein) was observed in T1 (1/3rd of LC_{50}), and the minimum (32.22 ± 0.93 IU/mg protein) in T3 (1/10th of LC_{50}) on the 14th day. In the control group, the catalase value was significantly ($P \leq 0.05$) lower as compared to the treated groups.

Table 2
Physico-chemical parameters of water recorded on daily basis during experimental period (x ± SD, duration of experiment – 28 days)

Parameters	T_1 (1/3rd LC_{50})	T_2 (1/5th LC_{50})	T_3 (1/10th LC_{50})	T_4 (control)
Water temperature, °C	29.73 ± 0.24^a	29.97 ± 0.14^a	30.50 ± 0.34^a	29.90 ± 0.41^a
Dissolved oxygen, ppm	7.67 ± 0.54^a	7.87 ± 0.26^a	7.74 ± 0.21^a	7.50 ± 0.01^a
pH	8.11 ± 0.22^a	8.25 ± 0.06^a	8.19 ± 0.41^a	8.21 ± 0.54^a
EC, µS/cm	1963.99 ± 0.34^a	1956.96 ± 0.23^a	1975.99 ± 0.26^a	1968.09 ± 0.20^a
TDS, ppm	1656.68 ± 0.64^a	1686.77 ± 0.54^a	1652.16 ± 0.46^a	1636.95 ± 0.20^a
Total ammonia, mg/L	0.67 ± 0.20^a	0.51 ± 0.31^a	0.67 ± 0.30^a	0.53 ± 0.10^a
Total hardness, mg/L	185.49 ± 0.50^a	181.36 ± 0.20^a	185.9 ± 0.50^a	190.70 ± 0.20^a
Carbon dioxide, mg/L	0.78 ± 0.20^a	0.72 ± 0.10^a	0.76 ± 0.30^a	0.76 ± 0.40^a

Table 3
Cortisol level (µg/mL) of *Labeo rohita* recorded
in the experiment (x ± SD, n = 5, duration of experiment – 28 days)

Samplings	T_1 (1/3rd LC_{50})	T_2 (1/5th LC_{50})	T_3 (1/10th LC_{50})	T_4 (control)
14th day	83.90 ± 0.92^d	71.72 ± 1.13^c	60.61 ± 0.84^b	35.60 ± 0.45^a
28th day	124.95 ± 0.88^d	100.35 ± 1.04^c	86.32 ± 1.10^b	39.42 ± 1.06^a

Note: the data was subjected to one-way ANOVA and the significance level among parameters was compared using Duncan's Multiple Range Test at $P \leq 0.05$; means with different superscripts in a row show statistically significant ($P \leq 0.05$) difference.

Table 4
Superoxide dismutase (IU/mg) protein activity of *Labeo rohita*
(x ± SD, n = 5, duration of experiment – 28 days)

Samplings	T_1 (1/3rd LC_{50})	T_2 (1/5th LC_{50})	T_3 (1/10th LC_{50})	T_4 (control)
14th day	27.85 ± 1.80^c	25.27 ± 1.60^c	17.31 ± 2.21^b	12.95 ± 1.11^a
28th day	49.72 ± 2.24^d	37.81 ± 2.26^c	22.07 ± 1.77^b	11.95 ± 0.79^a

Note: see Table 3.

Table 5
Catalase (IU/mg) proteins activity of *Labeo rohita*
(x ± SD, n = 5, duration of experiment – 28 days)

Samplings	T_1 (1/3rd LC_{50})	T_2 (1/5th LC_{50})	T_3 (1/10th LC_{50})	T_4 (control)
14th day	47.25 ± 0.64^d	40.02 ± 1.26^c	32.22 ± 0.93^b	27.02 ± 0.93^a
28th day	70.45 ± 1.03^d	57.95 ± 0.98^c	42.05 ± 0.96^b	25.95 ± 0.85^a

Note: see Table 3.

Aminotransferases estimation. Biochemical parameters showed a significant ($P \leq 0.05$) difference between the control and treated groups. The enzymatic activity of ALT, AST and ALP in serum was found to be increased by increasing the concentration and duration of lead exposure. The lowest value was found in the control group as compared to the treated groups. Within the treated groups, the maximum value of ALT, AST and ALP was found to be 65.13 ± 2.10, 93.96 ± 2.05 and 553.33 ± 2.51 U/L, respectively, in T1 (1/3rd of LC_{50}) on the 28th day of exposure. On the other hand, minimum values 31.83 ± 1.59, 31.99 ± 2.02 and 372.04 ± 5.29 U/L were found to be in T3 (1/10th of LC_{50}) on the 14th day. The observed level of ALT, AST and ALP is presented in Tables 6, 7 and 8, respectively.

Table 6
Alanine aminotransferase (ALT, U/L) activity in the serum
of *Labeo rohita* (x ± SD, n = 5, duration of experiment – 28 days)

Samplings	T_1 (1/3rd LC_{50})	T_2 (1/5th LC_{50})	T_3 (1/10th LC_{50})	T_4 (control)
14th day	53.46 ± 2.05^a	38.26 ± 0.70^b	31.83 ± 1.59^c	22.04 ± 2.64^d
28th day	65.13 ± 2.10^a	45.30 ± 1.01^b	38.36 ± 0.77^c	21.04 ± 1.73^d

Note: see Table 3.

Table 7
Aspartate aminotransferase (AST, U/L) activity in the serum
of *Labeo rohita* (x ± SD, n = 5, duration of experiment – 28 days)

Samplings	T_1 (1/3rd LC_{50})	T_2 (1/5th LC_{50})	T_3 (1/10th LC_{50})	T_4 (control)
14th day	81.33 ± 1.52^a	55.66 ± 2.51^b	31.99 ± 2.02^c	18.04 ± 1.04^d
28th day	93.96 ± 2.05^a	69.33 ± 2.51^b	37.98 ± 2.04^c	21.33 ± 2.08^d

Note: see Table 3.

Table 8
Alkaline phosphatase (ALP, U/L) activity in serum
of *Labeo rohita* (x ± SD, n = 5, duration of experiment – 28 days)

Samplings	T_1 (1/3rd LC_{50})	T_2 (1/5th LC_{50})	T_3 (1/10th LC_{50})	T_4 (control)
14th day	514.04 ± 3.60^a	456.04 ± 2.01^b	372.04 ± 5.29^c	308.30 ± 2.51^d
28th day	553.33 ± 2.51^a	490.30 ± 4.04^b	403.01 ± 6.24^c	306.60 ± 9.45^d

Note: T1: (1/3rd) LC_{50}, T2: (1/5th) LC_{50}, T3: (1/10th) LC_{50}, T4: (control) means with different superscripts in a row show a statistically significant ($P \leq 0.05$) difference.

Lipid peroxidation analysis. By exposing *L. rohita* to sublethal concentrations of lead, the level of lipid peroxidation increased up to 24.87 ± 0.50 nmoles/g on the 28th day in T1 with maximum lead concentration, and the minimum value of 15.43 ± 0.45 nmoles/g was found in T3 with the minimum lead concentration on the 14th day of exposure. The control group showed minimum values when compared with treated groups (Table 9).

Table 9
Lipids peroxidation (nmoles/g) of tissue in serum
of *Labeo rohita* (x ± SD, n = 5, duration of experiment – 28 days)

Samplings	T_1 (1/3rd LC_{50})	T_2 (1/5th LC_{50})	T_3 (1/10th LC_{50})	T_4 (control)
14th day	19.46 ± 0.42^a	17.70 ± 1.02^b	15.43 ± 0.45^c	9.06 ± 0.40^d
28th day	24.87 ± 0.50^a	19.50 ± 0.70^b	17.07 ± 0.99^c	10.04± 0.50^d

Note: see Table 3.

Discussion

Fish were exposed to lead nitrate for 96 hours. The LC_{50} value obtained by the current study is 71.58 mg/L. The findings of this present research study indicated that the mortality in the experimental fish *L. rohita* was increased by increasing the concentration of lead. Many researchers found the same results when they exposed different species to $Pb(NO_3)_2$ toxicant (Mary et al., 2014; Choubey et al., 2015; Jain & Batham, 2016). Hence, our recent findings show that the fish *L. rohita* is susceptible to $Pb(NO_3)_2$ and that increasing the exposure period and concentration, as expected, reduces their survival ability. The same results were found by Pandit & Rajkumar (2015), however their LC_{50} value was different from the current study, which may be due to differences in fish age and size.

The present study evaluated the tolerance limit of *L. rohita* against different sub-lethal doses of lead by investigating its effect on different biochemical biomarkers. The plasma cortisol level was observed through blood plasma after lead exposure. Changes in blood parameters are early indicators of pathophysiological abnormalities brought on by various toxicants since blood is a sensitive tissue that is influenced by environmental influences. They are therefore a great way to monitor the health of fish (Parveena et al., 2013). Pb increases the production of CRH (cortisol-releasing hormone), which in turn increases the cortisol level by stimulating neurons in the hypothalamus (Nair & Ajit, 2008). The release of the polypeptide corticotropin releasing hormone (CRH) into the bloodstream by the hypothalamus initiates the mechanism of action of cortisol. The anterior pituitary gland secretes adrenocorticotrophic hormone (ACTH) in response to additional stimulation from CRH.

ACTH then triggers the production and release of cortisol and other glucocorticoid hormones by the interrenal tissue by activating the melanocortin2 receptor (MC2-R) in the adrenal cortex (Spiga et al., 2011). The fact that Pb^{2+} operates as a stressor was validated in this present study. There was a significant difference found between all treatments as compared to the control. The treated groups showed a drastic increase in cortisol levels from 1st to 4th week. Similar results were found by Sumera et al., (2018) who reported an increased level of plasma cortisol in *L. rohita* after five weeks of exposure to lead. Thang et al. (2017) also reported a significant increase in the levels of plasma cortisol in *Oreochromis* sp. when exposed to lead and arsenic. Ramesh et al. (2009) reported a significant increase in plasma cortisol levels in *Cyprinus carpio* when exposed to environmental lead.

Fish liver tissues are considered an indicator of water contamination more frequently than any other organ. The liver is a primary metabolic organ, and the level of pollution in the aquatic environment is closely correlated with the level of contaminants in fish liver (Tapia et al., 2012). Toxicants disrupt the fish's physiological condition and cause cell organelle distortions, which may increase the function of certain enzymes (Vinodhini & Narayanan, 2009). Heavy metal poisoning causes oxidative stress, which can cause activation of antioxidant enzymes. The amount of reactive oxygen species (ROS) is strictly regulated by the cellular antioxidant system in order to maintain redox homeostasis. Numerous physiological processes are regulated by ROS, which are byproducts of cellular signalling pathways. The primary hub for ROS synthesis among the several locations in the cell is the mitochondria. Uncoupled electrons that are released by oxygen reduction result in production of superoxide radicals ($O^{2–}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (–OH) (Stevens et al., 2019). The first line of defence against superoxide radical toxicity is the SOD family of metalloenzymes, which play a significant antioxidant role in aerobic species. SOD is essential for tissue protection against free radicals (Kohen & Nyska, 2002). Superoxide anion dismutation is catalysed by SOD to produce H_2O_2 and H_2O and protect cells from superoxide anion damage. In our present study, superoxide dismutase activity was observed to be higher than normal and its activity increased by increasing the concentration and exposure duration of lead. By comparing all treatment groups to the control, a substantial difference was observed. The outcomes indicate the increasing trend (T1 > T2 > T3 > T4) of SOD among all treatments. Similar results were found by Dai et al. (2018). With increasing lead exposure and concentrations in the liver, the SOD value showed a parabolic trend. Latif et al. (2017) reported a significant increase in SOD activity by exposing two species of fish (*Catla catla* and *Cirrhina mrigala*) to lead chloride for 28 days. Similarly, Basha & Rani (2003) reported an elevation in SOD activity in *O. niloticus* liver and kidney tissues when exposed to three sub-lethal concentrations of cadmium.

CAT accepts H_2O_2 generated by SOD as substrate and decomposes it into H_2O and O_2, so free radicals are eliminated. Adipogenesis, inflammation, insulin signaling, metabolic balance, and cellular energy-sensing networks are all adversely affected by the buildup of reactive oxygen species (Rius-Pérez et al., 2020). After being exposed to heavy metals, the fish body eliminates ROS by increasing production of antioxidants in response to oxidative stress. The catalase value was observed to be increased by increasing the concentration and exposure duration of lead. The comparison of all treatments to the control revealed a substantial difference. It was observed that during the exposure period, the amount of catalase in treated fish significantly increased. Similar to our research, Dai et al. (2018) indicated a higher level of CAT activity in liver after 8 weeks of lead treatment and in the kidneys after 4 weeks of treatment. Similarly, Vinodhini & Narayanan (2009) explained that catalase activity in the renal tissues of common carp steadily went up for 16 days of exposure before decreasing on the 32nd day. Similar results were found by Basha & Rani (2003) for cadmium-induced toxicity in *O. mossambicus*. They reported an elevated activity of catalase for 15 days of exposure and a decrease after 30 days.

When the production of free radicals increases, they disrupt cellular activities by damaging the structure of biochemicals (fats, proteins and nucleic acids). Free radicals interact with polyunsaturated fatty acids (PUFAs) which are the main constituent of membrane lipids in cells, resulting in lipid peroxidation (LPO). LPO involves the direct reaction of lipids and oxygen to create semi-stable peroxides and free intermediate radicals, and this process of pathological free radicals results in phospholipid degradation. Lipid oxidation in the cell membrane leads to a loss of integrity by changing the fluidity of the membrane, among other pathological states (Wang et al., 2015). Our study findings showed a significant ($P \leq 0.05$) increase in lipid peroxidation in liver tissues. It was indicated that the LPO value was increased by increasing concentration and duration of the treatment groups. The results of the current study correspond to Bangeppagari et al. (2014), where the results also showed an increase in LPO in *L. rohita* when treated with lead. Similarly, Aruljothi & Samipillai (2010) found an elevation in LPO due to arsenic exposure in *L. rohita*. Farombi et al. (2007) likewise discovered an increase in LPO after heavy metal exposure in *Clarias gariepinus*.

Serum aminotransferases such as ALT, AST, and ALP are frequently used as indicators of liver damage. In the present research study, we examined the activity of aminotransferases in *Labeo rohita* after induced lead toxicity. As lead quantity and duration increased, the enzymatic activity increased significantly ($P \leq 0.05$). On the 28th day, the highest value of liver enzymes was recorded in group 1 with the maximum lead concentration (1/3rd of LC_{50}). It has been demonstrated that an increase in the plasma level of enzyme activity directly leads to significant pathological changes in the permeability of cell membranes or hepatocellular injury, which may be related to cellular degeneration, most likely in the liver, heart, or muscle. Similar to our results, Mahmoud et al. (2013) and Olojo et al. (2012) found an increment in ALT, and AST levels in *C. gariepinus* when exposed to lead. Tabrez et al. (2021) found that the level of aminotransferases increases in *Mystus* species by bioaccumulation of different heavy metals. Heavy metal exposure caused a rise in serum ALT, ALP, and AST in *Tilapia zillii*, *Mugil cephalus*, *Oncorhynchus mykiss*, and *Barbus luteus*, according to previous research (Mahamood et al., 2021).

Conclusion

This study conclusively demonstrates that lead (Pb^{2+}) exposure exerts pronounced toxic effects on *L. rohita*, a commercially and ecologically significant freshwater species. The calculated LC_{50} value of 71.58 ± 0.61 mg/L confirms *L. rohita*'s sensitivity to lead nitrate under acute exposure. Chronic exposure at sub-lethal concentrations (1/3rd, 1/5th, and 1/10th LC_{50} over 28 days elicited a clear dose- and duration-dependent increase in physiological stress, as evidenced by elevated plasma cortisol levels. Furthermore, the activity of key antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) significantly increased in response to oxidative stress induced by lead, indicating a compensatory defense mechanism. Concurrently, a marked rise in lipid peroxidation (LPO) and aminotransferases (ALT, AST, and ALP) was observed, suggesting oxidative damage and hepatic dysfunction. Collectively, these findings underscore the hepatotoxic and stress-inducing potential of lead, even at sub-lethal levels, and affirm the use of *L. rohita* as a sensitive bioindicator species for aquatic heavy metal pollution. The study also reinforces the urgent need to monitor and regulate lead discharge into aquatic environments to safeguard aquatic biodiversity and protect public health through the food chain.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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