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In Vivo Assessment of Toxic Effects of Lead Nitrate on *Daniorerio* (Zebrafish)

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ABSTRACT

In the presentscenario, aquatic pollution is a huge concern. It is mainly caused by industrial, agricultural, and various domestic activities. In the animal body, lead has no biological function. The objective of the present study is to determine the toxic effects of lead nitrate on Daniorerio. Fish were procured and grouped into six groups one unexposed group and five exposed groups. Fish were exposed to different concentrations of lead nitrate 10, 20, 30, 40, and 50 mg/L for 96 hours to determine LC50.Based on the mortality of fish for 96 hours, the LC50 of lead nitrate for Daniorerio is 40 mg/L. There is a positive correlation between concentration and mortalities. Behavioralchanges of zebrafish at different concentrations of lead nitrate were observed. Zebrafish exposed to lead nitrate exhibit anomalousbehavior. Physiological responses such as slow locomotion, gulping of air, swimming with jerky movements, schooling disturbed, rapid opercular activity, and erratic swimming were observed during the early hours of lead nitrate exposure, after which it became habitual. In the control group, fish exhibit natural behavior. Therefore, during the disposal of heavy metals, restrictive regulations should be imposed to conserve valuable biodiversity.

Keywords: *Lead nitrate, Toxicity, LC50, Zebrafish, Behaviour*

Introduction

In the present scenario, the effect of heavy metals on aquatic fauna is gaining global concern, particularly in relation to industrial pollution [1]. The unregulated discharge of agricultural pesticides into aquatic bodies led to environmental issues for organisms in the aquatic habitat [2, 3].Heavy metal pollution of the aquaticbodies results from direct geological weathering , atmospheric deposition or through the evacuation of agricultural, municipal,manufacturaland residential waste products including agriculture wastewater treatment [4, 5, 6].Fish can absorb heavy metals either via skin , gills or ingestion of contaminated food [7, 8].After ingestion of metal by fish, they are transferred to the organs and tissues, via blood circulation where they form precipitation [9, 10].Lead is a detrimental heavy metal and non-essential that can be found as cerussite($PbCO_3$), galena (PbS), and anglesite ($PbSO_4$) in rocks, the earth's crust, water, and soil [11].Lead is an industrially significant heavy metal used in manufacturing paints, pottery, batteries, explosives,and other various essential products of daily life [12].The main sources of irrigation contamination are agricultural, household, and industrialized waste, which are released into ordinary water bodies [13]. Lead is a known toxic heavy metal contributed primarily by natural availability and human activities [14].Heavy metals such as lead have no nutritional value, and they cause aquatic pollution in theenvironment [15]. Lead is considered a highly toxic metal, as it is proclaimed to be responsible for decease or sublethal changes in the growth, behavior and reproduction of fish [16].Due to anthropogenic activities, fish belong to the vertebrate group that reacts first when the aquatic environment is polluted with contaminants [17].In polluted areas, fish exposed to heavy metals result in the transmission of chemical substances into the biological systems and cause biochemical disturbances [18, 19, 20]. Higher LC because higher concentrations need to cause 50% of mortality in organisms [21].Acute toxicity studies is used to determine the concentration of test substances or the quantity of toxicants that causes adverse effects on a group of test organisms in a short-term exposure under a controlled environment [22]. Toxicity refers to an individual's reaction to a chemical substance at a particular concentration or dosage for a specific period [23].Evaluation of median lethal dose or concentration (LC50 or LD50) is crucial as it can be used as an indicator of the resistance level of population response to metals [24].In the comparison of the zebrafish genome with the human genome, it was concluded that 70% of human genes had analogues of the zebrafish gene with the same function, and 84% of genes related to human disease have zebrafish gene analogs[25].These fish are cheaper and easier to maintain compared to lab mice. They required small spaces to maintain large numbers of fish. They reproduce at a fast rate. 200-300 eggs are produced daily; thus, abundant offspring are produced in a short period. They take 90 days to mature [26]. Taking this into account, the present work was initiated to evaluate the percentage of mortalities to find the LC50 of lead nitrate and behavioral changes.

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Materials and Methods

Procurement and maintenance of Zebrafish

In our experiment, an animal model (*Daniorerio*) was procured from a local seller shop and transported to the lab. This study was carried out in the Department of Zoology at Veeranari ChakaliIlamma Women's University during June and July 2023. Zebrafish with an average weight (350–500 g) and length

(4–4.5 cm) were used in the experiment. In the experiment, water was exposed to air to make it chlorine-free. The Aquarium was filled with 6 L of dechlorinated water, and the fish were acclimatized prior to the experiment for 14 days. All exposure takes place at 14:10 h (light/dark) at 26-29 °C. Fish were acclimatized before the experiment for 14 days. A total of 65 fish were procured and maintained. In both control and experimental aquariums, an air pump was provided for continuous air supply. Lead nitrate stock solutions were prepared and renewed every 24 hours. For one day, fish were starved prior to the experiment. Fish were fed with commercial food pellets daily during the experimental period. Water was renewed daily.

Test chemical

The test chemical used in the experiment was lead nitrate.

Experimental design

The toxicity of the lead nitrate experiment was conducted in the lab for 4 days. Fish were procured and organized into 6 groups 1 control and 5 experimental groups. Each group contains 10 fishes. Lead nitrate was made at 10, 20, 30, 40, and 50 mg/L. Double distilled water was used to prepare the heavy metal stock solution. Eventually, after 24-96 hours, mortality of fish was evaluated to calculate LC50 using the Finney probit analysis method. A different concentration of lead nitrate was freshly prepared in distilled water before being mixed with aquarium water. In each medium, the concentration of the toxicant was increased gradually, and the quantity of metal (lead nitrate) concentration was maintained to determine the mortality rate from 0 to 100%. After introducing lead nitrate, mortality of fish was recorded at 0, 24, 48,72, and 96 hours.

Every day, dead fish were removed and counted from the aquarium. The data obtained from the experiment were used to evaluate the LC50 value of lead nitrate.

Evaluation of median lethal concentrations:

The concentration at which half of the population of test species dies during a particular period is known as median lethal concentration (LC50) or median tolerance limit.

The LC50 values of toxic substances were evaluated by using the following methods:

Direct interpolation method:

It involves converting the toxicity curve plotted between percentage of mortality and concentration for 96 hours.

Finney probit method:

It is a standard method to evaluate the dose-response data. Based on the percentage of mortality,probit values were obtained from Finney's table given below. Probit kill values and log concentration curve was plotted by pointing a line representing 50% of death at 96 hours.

Behavioral observation

Alterations of fish behavior was monitored before and after the introduction of toxicant (lead nitrate).

Statistical analysis

Calculate LC50 using the Finney probit analysis method[27] and correlation to determine the relation between concentration and mortalities.

Results

Median lethal concentration

Table-1 Rate of mortality of zebrafish exposed to lead nitrate for 96 hours

	24 hrs		48 hrs		72 hrs		96 hrs		Total	
Concentration mg/L	Dead fishes/total fishes	Mortality (%)	Dead fishes/total fishes	Mortality (%)	Dead fishes/total fishes	Mortality (%)	Dead fishes/total fishes	Mortality (%)	Dead fishes/total fishes	Total mortality (%)
Control	0/10	0	0	0	0	0	0	0	0	0
10	0/10	0	0	0	0	0	1	10	1	10
20	0/10	0	0	0	1	10	1	10	2	20
30	0/10	0	2	20	1	10	1	10	4	40
40	2/10	20	1	10	1	10	1	10	5	50
50	2/10	20	2	20	3	30	2	20	9	90
									LC50=43.518	

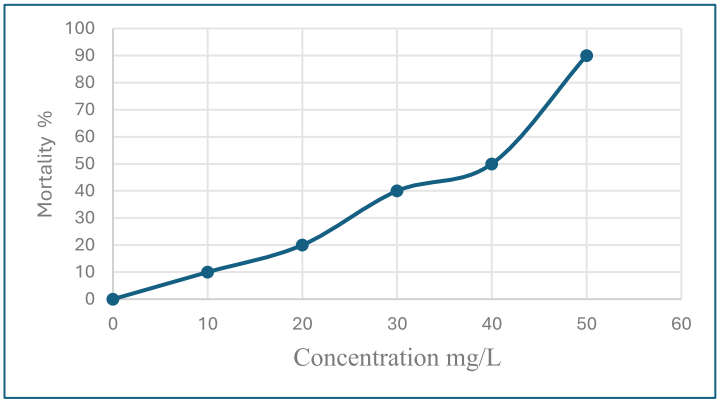

Fig-1 Different concentration of lead nitrate and mortality

Table-2 Calculated log dose and probit value

Concentration mg/L	Log dose	Mortality	Percentage %	Probit of kill
10	1	1	10	3.72
20	1.30103	2	20	4.16
30	1.47712	4	40	4.75
40	1.60206	5	50	5
50	1.69897	9	90	6.28

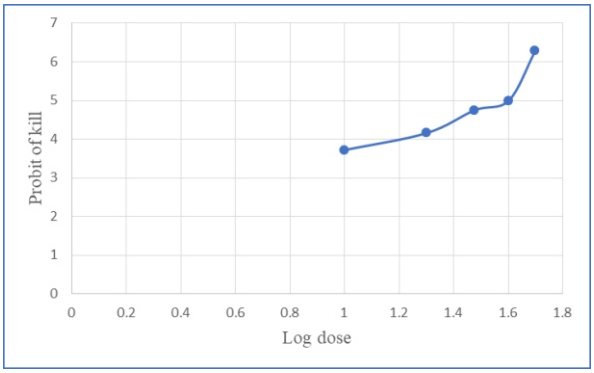

Fig-2 Plot of log conc. of lead nitrate versus probit kill of zebrafish showing LC50 at 96 hours

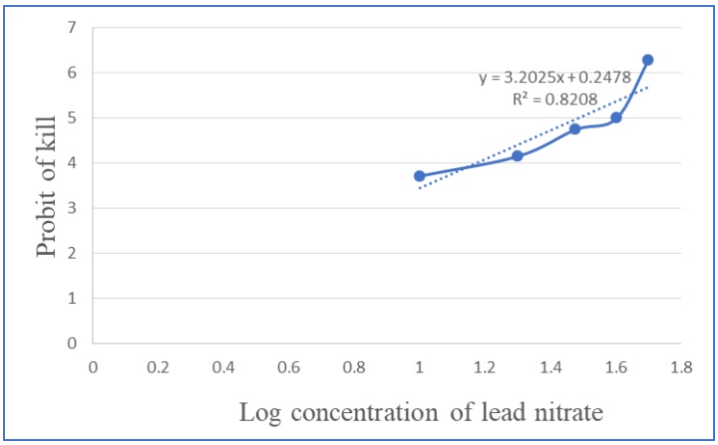

Fig-3 Linear relationship between probitresponse and log concentration of lead nitrate

The concentration of lead nitrate ranges from 10 mg to 50 mg/L to test forlethal toxicity for 96 hours. After exposure of 96 hours, mortality of fish was recorded. No mortality was observed in the control group. LC50 lead nitrate is 40 mg/L for zebrafish. Different concentrations of lead nitrate and mortality of zebrafish values are shown in Tab-1, and the concentration and percentage of mortalities are shown in Fig-1 with calculated LC50 values. The relation between lead nitrate toxicity and mortality in zebrafish is shown in Tab-3. Table 2 shows log dose versus probit kill values of zebrafish representing LC50 at 96 hours. Fig-2 shows the log concentration of lead nitrate and probit kill it exhibit a linear relationship. 10 mg/L of lead nitrate causes the death of 10% of zebrafish. Subsequently increased in the concentration of toxicant, i.e., lead nitrate, gradually at 40 mg/L; it causes the death of half of the population of zebrafish; hence for zebrafish, 40 mg/L is toxic. In addition, 50 mg/L of lead nitrate causes 90% mortality in zebrafish for 96 hours.The calculated probit and log dose are displayed in Fig-3.

Concentration mg/L	Mortality (%)
0	0
10	10
20	20
30	40
40	50
50	90

	Column 1	Column 2
Column 1	1	
Column 2	0.964109	1

Tab-3 displays the correlation between concentration and mortalities. There is a strong positive correlation between the concentration of lead nitrate and mortalities of zebrafish. In Tab-4, there was a strong positive correlation of 0.96179 between the concentration of lead nitrate and mortalities.

Lead nitrate toxicity induces behavioural response in *Daniorerio*

Experimental group

Zebrafish exposed to lead nitrate exhibit anomalous behaviour. Therefore, lead nitrate enters the body of fish and keeps the mouth open, which ultimately results in the death of fish. The results represent that lead nitrate causes adverse effects on fish. During the toxicity test, the zebrafish were constantly observed, and they showed symptoms of slow locomotion, disturbed schooling, rapid opercular activity, and erratic swimming. With an increase in the concentration of toxicant, the activity increases with a few hours; it becomes occasional, and fishes settle down in an aquarium and become lethargic. Fishes exhibit increased behavior changes on addition of lead nitrate stock solution; later it became occasional.

Control group

The control group of fish exhibits grouped schooling behaviour. The rate, duration, and type of behavioural changes increased with increased lead nitrate concentration. In the control group of fishes there were mortalities or behavioural alterations.

Discussion

Fish live in an environment thatcontains diverse heavy metals,remnants, pesticides, pharmaceuticals ,coliforms, etc. The cumulative effects of these toxicants cause detrimental effects on aquatic flora ,fauna, and other living organismsof the food web [28, 29]. Heavy metal such as lead is characterized as the most toxic substance[30].In the present study it was observed based on the mortality of fish for 96 hours, the LC50 of lead nitrate for Daniorerio is 40 mg/L. Hence, for zebrafish, 40 mg/L is toxic. Heavy metal toxicity to the organisms is assessd based on the absorbed dose, route of exposure, and exposure duration [31, 32, 33,34].Small concentrations of lead, cadmium and other heavy metals are lethal and highly dangerous in long-term exposureIn air-breathing fish, the LC50 of lead nitrate for *C. batrachus*and*C. punctatus* were 346.6 and 177.8 mg/L respectively.[35]. The toxicity of Pb on *Cyprinuscarpio* (Common carp) wasreported [37], and the 96-hour LC50 was 328 mg/L. [38] illustrates that there are different LC50 values for the same species exposed to the same heavy metal. It was found that few fishes are highly sensitive to the toxic effects of one metal, and less sensitive towards another toxic heavy metal equally at the same concentration. Metals enter intoliving organisms by inhalation, ingestion, or through the skin, and their existence may cause severe toxicity in humans and animals [39]. According to one study [40]the status of heavy metals contamination in aquatic fauna and flora is bioaccumulative and hazardous. Heavy metals are primarily considered toxic substances for aquatic systems because they are highly toxic, and living organisms tend to accumulate them [41] .A study reported the toxicity of lead to *Oreochromisniloticus.*The 96-hr LC50 value of $Pb(NO_3)_2$ was 44 mg/L[42]. This value was greater than our results, indicating that zebrafish is highly sensitive than *Oreochromisniloticus.* The 96-hour LC50 of lead nitrate to *C. punctatus* (158.171) [43]and *H. fossilis*(280.070). LC50 of lead 21.63 mg/L and cobalt 69.83 mg/L.[44] In both groups, *C. punctatus* and *H. fossilis* mortality increases with an increase in the concentration of toxicant, i.e., lead nitrate. This study indicates LC50 of lead 21.63 mg/L and cobalt 69.83 mg/L.[45] It was concluded that lead is highly toxic to zebrafish in comparison to cobalt. 96-hour exposure of *Mystuscavasius* to lead nitrate LC50 value was 55 mg/L [46]. There exists a direct relation between concentration of toxicant and mortality [47] .The values of toxicity tolerance vary in different toxicants against different test species and sizes.[48]. There is a strong correlation between physiological alteration and behavioral disturbance; hence, it provides ecological relevancy to the physiological measurement of toxicity [49]. Physiological responseslike behavioural changes shown by the animal, are sensitive indicators of chemically produced stress in aquarium organisms [50]. During the toxicity test, the zebrafish were constantly observed, and they showed symptoms of slow locomotion, disturbed schooling, rapid opercular activity, and

erratic swimming. Inhibition of cholinesterase, alterations in the levels of neurotransmitters, decreased thyroid and gonadal hormone levels, and sensory deprivation are generally reported and related to behavioral disturbance (51).Thetarget organ is the central nervous system which commonly participates in systemic poisoning [52], which results in loss of movement and coordination, tremors, and unbalanced behavior that leads to hyperexcitability and convulsions [53]. Similar finding in *Labeorohita*exposed to lead nitrate fish become lethargic, activity decreases while increasing in the first few hours [54]. After acute exposure to water lead concentration, zebrafish larvae exhibit altered swimming patterns accompanied by elevated oxidative stress [55]. Similar results are observed by different researchers [56, 57, 58,59,60]. Hence, our present results agree with many researchers. The target organ constantly participating in systemic toxicity is the central nervous system. Behavio ral observations are used to monitor the quality of aquatic environments and pollution severity for physiological systems reflected in fish behaviour (i) faulty exchange of gases in gills, (ii) imbalance in stress-mediated hormones, (iii) impaired osmoregulation, and (iv) impaired metabolism [61].

Conclusion

In conclusion, the current findings indicate that lead nitrate is highly lethal to zebrafish and causes behavioural anomalies at different concentrations. Determination of LC50 helps us to figure out tolerated levels of heavy metal in an aquatic environment. An acute toxicity experiment tells about the health conditions of a specific aquatic environment. The present finding concluded that 40 mg/L is lethal for zebrafish, which causes the death of half of the population, and an increase in the concentration of lead nitrate mortalities of fish increases, and fish exhibit various behavioural changes. It is determined that lead nitrate has an intense impact on behavioural changes in zebrafish. Lead nitrate is lethal to aquatic fauna. Heavy metals such as lead nitrate endpoint provide notable information about the safe level of heavy metal in aquatic habitats. Acute toxicity tests estimate the effect of pollution due to heavy metals and the development of water quality measures to conserve aquatic environments.

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