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The efficacy of Ascorbic acid against Lead Nitrate Impact on the Histology of Liver of Common Carp (*Cyprinus carpio)*

Musrat Majeed[1]*, Rajendra Chauhan[2], Farooq Ahmad Mir[2] and Shaista Masarat[2]
[1]*Department of Zoology, Government MVM College Bhopal (Madhya Pradesh), India.*
[2]*Barkatullah University, Bhopal (Madhya Pradesh), India.*

(Corresponding author: Musrat Majeed)*
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ABSTRACT: Lead is reported as heavy metal that induces physiological dysfunction and blood disorders. They are often firmly attached to polypeptides and proteins and are water soluble but non-degradable. Diverse activities continue to have an impact on the aquatic environment, altering the climate and causing health hazards to fish, despite past study issuing cautionary notes. This study assessed the toxicity of lead nitrate to the freshwater fishes *Cyprinus carpio*, to determine the hematological toxicity, histopathology and mortality and survival rate. Fishes were exposed to sub lethal lead nitrate concentrations 5ppm for 15 and 30 days of exposure and (percentage)% mortality was noted after 24, 48, 72, and 96 hours. The determination of LC50 of the Lead nitrate during the present experimental period. 10 mg/l to 60 mg/l Lead nitrate was used to assess the rate of mortality at each concentration. 60 mg/l was seen to be LC100 for Lead nitrate. 50% mortality was recorded in experimental groups exposed to 30-40 mg/l of Lead nitrate therefore, the acute 96h LC50 value for the present experimental fish, *Common carp* was calculated to be 35 mg/l (ppm).In the present research, the significant hematological (WBCs, RBCs, Hb, neutrophils, Basophils MCHC and Lymphocytes) and histopathological alteration in liver tissue of common carp was recorded upon the exposure to different concentration of lead. The results concluded that lead has a strong influence on hematological and histopathological parameters of common carp during chronic toxicity.

Keywords: Lead nitrate, *C.carpio,* mortality, probit, LC50, dysfunction.

INTRODUCTION

Common carp is one of the freshwater fish that has significant economic value so that the community widely cultivates carp. Besides being kept in ponds, common carp are also often kept in the fields together with rice plants Aquatic pollution by heavy metals is a major threat to human health and to aquatic life (Afaghi 2020). At present, the impact of heavy metals on aquatic fauna is attracting widespread attention, especially in studies linked to industrial contamination. These heavy metals in the aquatic systems are due to different anthropogenic and natural sources, including industrial or domestic waste water, application of pesticides and fertilizers, leaching from landfills, shipping and harbor activities and atmospheric deposits and geological weathering of the earth crust .Lead is a substance that exists in the environment in a wide range of physical and chemical forms. When present in amounts over the usual range, lead has a negative impact on fish behaviour. Most of the lead that is present in the environment is inorganic and exists in various oxidized forms. Pb is the ionic species that persists the longest in the environment and is thought to be the form in which aquatic creatures biochemically collect the most Pb. Fishes live close to their surroundings which contains different heavy metals, bacteria, remnant pharmaceuticals, lead nitrate, microplastics, pesticides, etc. Therefore, cumulative impacts of these toxicants may cause harmful effects on aquatic flora, fauna, and other living beings of the food web. However, a variety of parameters, such as fish age, pH, and water hardness, affect how hazardous is lead nitrate. The study of ecotoxicology takes into account an immune system, which is almost ubiquitous in all multicellular organisms and serves as a direct conduit between an organism and its surroundings. Lead is widely used in paint and is mostly released into the environment through the exhaust pipes of various automobiles. Its degeneration will lead to a drop in fish quality and an increase in disease susceptibility. Abiotic factors and the metal concentration of water both decrease the immune system's cellular and humoral components. The most common metals that can be dangerous to humans at low concentrations include lead, cadmium, nickel, arsenic, chromium, and mercury (Al-fatlawi *et al.*, 2015).

MATERIALS AND METHODS

Lead nitrate (Ranbaxy India), to be used for the preparation of various toxic concentrations, (stock solution) by adopting the dilution techniques. Fish Used in tests and their Collection With the aid of fishermen, a specific group of *C. carp* (weight 80-120gm, length 12-

18 cm) n=10) fishes that were healthy and free of disease was procured from nearby fish markets.

A. Acclimatization, food and Feeding

To treat any cutaneous issues, the fish were initially immersed in a 0.02% $KMnO_4$ solution for two minutes. The fish were placed in aquariums, with an average weight 80-120 gm. Fishes were given no food to help the fish adjust to their new surroundings. They underwent a 15-dayacclimatization period in a laboratory glass container filled with non-chlorinated tap water while being fed a pelleted diet. To prevent a hypoxic environment, aerated tubed motorized pumps were used for aquarium maintenance. Monofilament netting was used to cover aquariums to prevent fish from leaping out during testing.

B. Introductory tests

By adhering to the safety precautions and guidelines provided by the (APHA, 2017). The physicochemical characteristics of the tap water were evaluated. The tap water used to keep fish in aquariums had a pH range of 6.9 to 7.2, a temperature of 26 to 27 °C, dissolved oxygen levels of 5 to 6 ppm, and a total of 224.

Chemical analysis of water used for experiments

Sr. No.	Parameters	Unit	Values
1.	Temperature °C	°C	27±2.5
2.	pH		7.5
3.	Dissolved Oxygen	ppm	5-6
4.	Carbon Dioxide	ppm	3-4
5.	Total Dissolved Solids	ppm	12
6.	Alkalinity I Phenolpthalene II Methyl Orange (as $CaCO_3$)	ppm	 Nil 456
7.	Total hardness	ppm	224
8.	Calcium hardness	ppm	48
9.	Chloride (Cl^-)	ppm	38

Acute Toxicology. Using various toxicant concentrations, the experiment was carried out using the renewal bioassay method according to standards throughout the course of 96 hours. The acclimated fish were divided into five experimental groups, each with ten fish, for the experiment, which was carried out in a static system in glass aquariums. Before being combined with aquarium water, the sub-lethal dosage of test compounds was freshly synthesized in distilled water. Each test medium's concentration was gradually increased, and the amount of metal concentration was kept constant at the mortality rate from 0 % to 100.

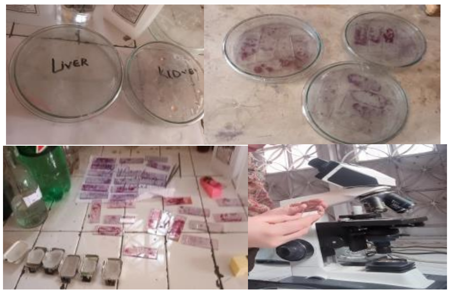
Table 1.

Lead Nitrate Conc. (mg/l)	No. of Fish	No. of Dead Fish	A	B	
0.00	10	0	0	0	0
10.00	10	1	10	0.5	5
20.00	10	3	10	1.5	15
30.00	10	5	10	3	**30**
40.00	10	6	10	5	**50**
50.00	10	8	10	6.5	65
60.00	10	10	10	8.5	85
			∑axb = 250		
	70		**LC50 = 35 mg/l**		

The Lead Nitrate concentrations, groups and calculations of the acute 96 h LC50 value of Lead Nitrate in *Cyprinus carpio.*

Mortality Response. The experiment's mortality rates were recorded at intervals of 24, 48, 72, and 96 hours, and dead fish were immediately removed from the test liquid to stop any organic deterioration and oxygen loss. %. A small number of pilot tests were carried out to determine the deadly concentration, and several concentration ranges were chosen to assess the death rate. To obtain consistent results, each experiment was conducted three times. The division of experimental groups based on the LC50 value and the greatest degree of Pb contamination of natural freshwater bodies found in the literature survey.

A lot of mortality were seen prior and after the experimental set due to change in environment from natural to labortaory condition, toxicant entered their body their mouth remained open with the eventual death of the fishes. The results showed adverse effects of Pb on fishes.



RESULTS

Behavioral Reactions. According to Rand fish behavioral reactions during the toxicity test included convulsions, equilibrium state, fin movement, hyperactivity, and swimming rate in both the exposed and control groups.

During the current study, it was noted that the *Cyprinus carpio* exposed to different concentrations of $Pb(NO_3)$ induces behavioral changes. Specifically, at a high concentration of toxicant after 2-3 days, fishes showed darting movement and swimming disturbances. As the concentration of toxicant increases, fishes appeared to induce mucus secretion over the gills causing rapid movement of opercula to make great efforts for movement. However, the control group of fish maintained in normal water were found to be active throughout the experiment. After 3-4 days of toxicant exposure opercula beat counts were found lower than that of the control group. Thus, toxicant entered their body, their mouth remained open with the eventual death of the fishes. The results showed adverse effects of Pb on fishes.

Table 2: Behavioral changes in Lead Nitrate exposed *Cyprinus carpio*

Sr. No.	Parameter/Event	5 ppm $Pb(NO_3)$	10.0 ppm $Pb(NO_3)$	15 ppm $Pb(NO_3)$	20 ppm $Pb(NO_3)$
1.	Behavioural change	--	--	+	++
2.	Restlessness	--	--	+	++
3.	Erratic swimming	--	+	+	+
4.	Jerky body movement	--	+	--	+
5.	Rolling the body	--	--	+	+
6.	Convulsion	--	--	--	+
7.	Mucous secretion	--	--	--	+
8.	Loss of equilibrium	--	--	+	+
9.	Rapid-opercular movement	--	+	++	++
10.	Difficulty in breathing	+	+	++	++
11.	Lethargic	--	+	+	+

+++ Intensely; ++ moderately; + lightly; -- No

Assessing (LC50) Concentration. The median lethal concentration (LC50) or median tolerance limit is the toxicant concentration at which 50% of the test species perish during a specific period or the concentration deadly to 50% of the test population. The following techniques were used to estimate the LC50 values of toxicants:

(i) Direct interpolation method: For 96 hours, a toxicity curve's transformation was drawn between % mortality vs. concentration

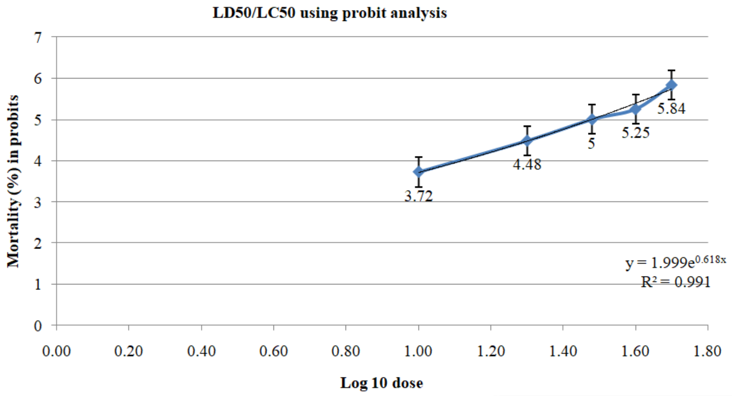
(ii) Finney's Probit technique. Examining the dose-response data using this manner is routine practice. Finney's data was used to calculate the Probit values of % mortality (Fig. 1). A line corresponding to the 50% mortality at 96 hours was drawn on the curve between the log concentrations and Probit kill values.

(iii) Using SPSS Probit, analyses in Table 3. Using Probit analysis and SPSS 21 statistical analysis software, the actual LC50 data was examined. Probit analysis is based on linear regression technique after transformation of toxicity data in the parametric procedure and extensive history of statistical applications. Through best-fit line, it was also possible to determine the LC50 value (with 95% confidence intervals), the correlation between mortality (Probit) and the log10 concentrations, and the regression line equation. And the study revealed mortality of the fishes was directly proportional to the concentration. No mortality was recorded among the control groups of all the experiment. However, it was also proved that an increase in concentration of test chemical was required as a lethal dose for these freshwater fishes.

Total mortality of fish was recorded after exposure of 96 hours.

Table 3: Showing the mortality of *Cyprinus carpio* at 96h after treatment of different lead nitrate concentration.

$PbNO_3$ Conc. (ppm)	Mortality	No. of fish exposed
00	00	10
5	01	10
10	04	10
15	06	10
20	8	10



Movement frequencies: Fish under stress exhibit a significant variation in movement frequencies. Any heavy metal, toxin, or poison has the potential to cause stress. Opercular Beat Frequency (OBF) and Tail Beat Frequency (TBF) measurements were made to confirm the impact of lead nitrate's lethality on movement frequencies. Several authors have claimed that the first increases in OBF and TBF may be connected to the impulsive reaction to shock.

Behavioral Reactions

Tail Beat Frequency: In table the findings of the tail beat frequency (TBF) for various Lead nitrate concentrations exposed singly are shown as mean SE.

Table 4: Summary of TBF values of *Cyprinus carpio* exposed to various concentrations of Lead Nitrate.

Lead Nitrate Conc.	Exposure Duration			
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
5.0	1.1±0.3	2.4±1.5	3.3±1.0	7.0±0.5
10.0	1.9±0.1	3.9±0.6	5.6±2.0	8.3±0.6
15.0	2.8±0.5	4.9±0.9	7.2±1.3	10.2±1.2
20.0	3.3±0.2	6.0±0.9	9.2±0.3	12.3±1.2

The fishes (n=10, for each treatment) were exposed to six concentrations of percentile of LC50 of Lead Nitrate. The values were enumerated by simple physical examination of the individual fish.

The results are expressed as mean±SE of five replicas for each treatment

Histopathology: The general histology analysis revealed instances of injury in Common carp tissues following exposure to concentrations of Lead nitrate 5ppm for 15 days and 5ppm of lead nitrate along with 450 gms of ascorbic acid while other group were treated with same concentration 5ppm lead nitrate alone and 5ppm along with 450 gms of ascorbic acid for 30 days. Both the control and treated groups' liver samples had their histopathological alterations examined and the protective role of Vitamin C has also been demonstrated. The liver is essential for xenobiotic metabolism and excretion, as well as digestion and storage. As a result, structural changes are to be predicted under certain hazardous situations.

LIVER

An outer peripheral connective tissue and an inner lining of endothelial cells makes up each sinusoid. In the control group, the liver exhibited a normal architecture with hepatocytes presenting a homogenous cytoplasm and a large central or sub central spherical nucleus. Lobular organization of hepatic tissue consists of cord-like structures with hepatic plates eminently visible along blood sinusoids. The sinusoids and their nuclei are lined by the flattened and elongated endothelial cells.

The blood sinusoids drains into a central vein. Hepatocytes of *Cyprinus carpio* appear to be polygonal in shape and is comprised of a single spherical nucleus with centrally located nuclei. Exocrine pancreatic tissue (hepatopancreas) is a pronounced feature in the liver of

Cyprinus carpio and is clearly visible as darkly stained tissue around the hepatic portal veins. Macrophage centers are found in the normal liver sections of *Cyprinus carpio*, mainly in the vicinity of hepatopancreatic tissue (Fig. 1 Control).

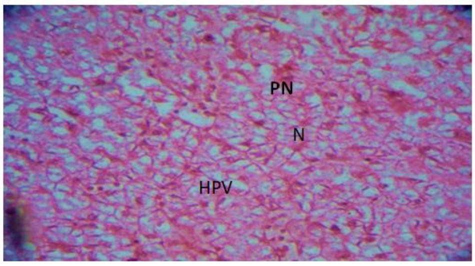


Fig. 1.

Treatment with 5 ppm of lead nitrate for the period of 15 days. The histopathological changes were severe in the liver for the period of 15th day. Liver sections were exhibited the dilation of sinusoids, cytoplasmic vacuolation, nuclei in place of their central position have shifted to the cell periphery and de-shaped hepatocytes were observed under microscope (Fig. 2).

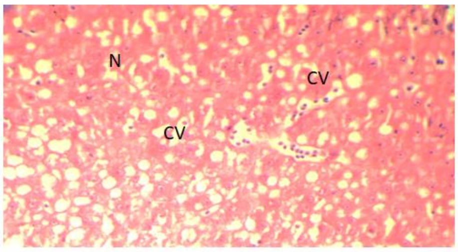


Fig. 2.

Treatment with 5 ppm of lead nitrate for the period of 15 days along with Vitamin C. The hepatic parenchyma was exhibiting the presence of minor cytoplasmic vacuolation, hepatocytes with centrally located nucleus. Disorganization of hepatic cords was seen due to rupture of cell membranes of hepatocytes. Infiltration of leucocytes within dilated blood sinusoids was reported (Fig. 3).

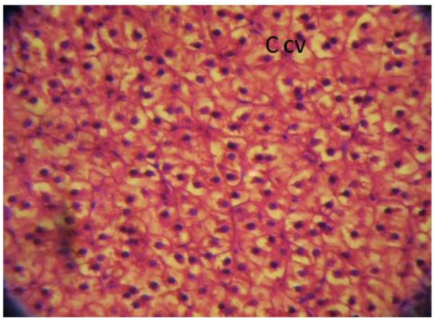


Fig. 3.

Treatment with 5 ppm lead nitrate for the period of 30 days. Semi thin sections of the Liver of the common carp under microscopic examination after exposure to 5 ppm lead nitrate for the period of 30 days showed dilatation and wall thickening of some blood vessels, resulting in congestion with blood cells and considerable necrosis of the hepatocytes was observed in this group (Fig. 4).

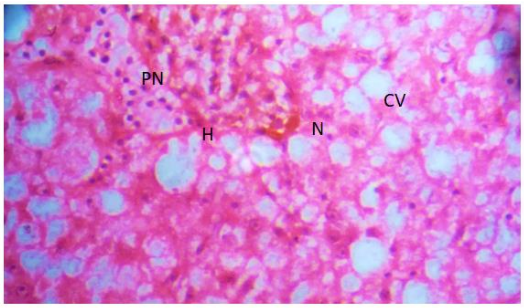


Fig. 4.

Treatment with 5 ppm lead nitrate for the period of 30 days along with Vitamin C. The liver tissue of common carp for the period of 30 days with Ascorbic acid as a protective measure exhibiting the presence of very extensive cytoplasmic vacuolization, sinusoidal spaces were dilated and filled with blood, Vacuoles in cytoplasm of hepatocytes with the pycnotic nuclei and nuclear degeneration was observed (Fig. 5).

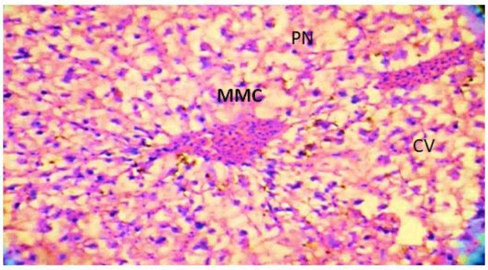


Fig. 5.

DISCUSSION

In environmental toxicology, fish are an invaluable test model for determining the fatal and sub-lethal effects of aquatic contaminants. Measuring histopathological and haematological alterations in fish is a sensitive and accurate method of assessing xenobiotic effects. It was examined the importance of the histopathology as a useful biomarker for the assessment of environment quality to investigate the toxicity of heavy metal as well as other pollutants that can compromise the quality of aquatic ecosystem. Variety of histotological changes were observed in gills, kidney, and liver of *Cyprinus carpio* (Mustafa *et al*., 2017).

The assessment of LC50 also showed that the lead concentration in freshwater may be fatal to all aquatic life. Lead exposure has an effect on human health, causing 1.06 million deaths and almost 24.4 million healthy life years to be lost. It has been established that pb and other heavy metals of anthropogenic sources are significant pollutants in aquatic ecosystems. Strong regulations for the discharge of heavy metals in aquatic ecosystems should be implemented in order to save the valuable biodiversity. Since the bioassay is an essential tool for determining the impact and outcome of toxicants in the aquatic environment. Research reveals Heavy metal exposure to fish in polluted areas caused toxins to enter the biological system and caused metabolic abnormalities, which led to the discovery that lead was highly toxic even at low concentrations. Reports reveal the amount of Pb present in freshwater bodies varies from 18 to 1,559 µg/L which cause alterations in behavior, stress which is very sensitive indicator of potential toxic impacts, Fish exposed to different doses of toxicants exhibited marked behavioral changes (ILA 2015; Church *et al*., 2017; Sarma *et al.*, 2103; Sharma, 2019).

Fishes appeared to induce mucus secretion over the gills causing rapid movement of opercula to make great efforts for movement. Similar symptoms were also observed by Senthamilselvan *et al.* (2015) in Lates calcarifer exposed to chromium and mercury metal. (Kumar *et al.,* 2015) observed the initial increase of mucus secretion, loss of buoyancy, and balance with change in body coloration in *Clarias batrachus* after exposure to copper sulfate. The mortality Probit of LC50 values was determined for the lethal concentration of toxicants $Pb(NO_3)_2$ during acute exposure periods. Results of the present study indicate that the LC50 values of *C. punctatus* and *H. fossilis* were found 158.171 mg/l and 280.074 mg/l respectively. From the Probit analysis, the points at which the toxicants began to cause lethal effects on the fish varied significantly (Selvanathan, 2011). The lethal concentration required to cause 10% mortality in fish exposed to toxicants was also determined, and it was demonstrated that as the level of bioaccumulation increased, the organisms could become lethal. The results show that the concentration of Pb metal has a direct effect on the LC50 values (Mahnaz, 1822). The assessment of the acute toxicity of Pb on *Clarias gariepinus* and found lethal values ranging from LC10 to LC95. Different researchers have observed similar results on LC50 in different fish species in response to various toxicants. Hence, our current findings are in agreement with many workers (Pandit *et al*., 2017). Exposure of Pb at all the concentrations showed a reduced growth rate and hence, inversely proportional to growth which is in accordance with the present results and the mortality was increased as the concentrations of $Pb(NO_3)_2$ increased in the freshwater fish *C. punctatus* (Dube *et al.,* 2017). These findings were supported with the observation by many researchers in different species exposed to $Pb(NO_3)_2$ toxicant. Hence, our current findings demonstrated that common carp (*Cyprinus carpio*) are sensitive to $Pb(NO_3)_2$ decreasing their survival capacity when increasing the exposure time explained in his study that there are differences in the value of LC50 found in the same fish species for the same heavy metal (Reddy, 2016; Ullah, 2016). And it is important note that lead is an extremely poisonous metal, LC50 results demonstrated that the level of lead contained in freshwater is potentially fatal to all aquatic animals (Paul *et al*., 2019; Ali *et al.*, 2016). Thus requirement for ascorbic acid varies based on the type of fish in addition to interspecies differences such as fish strain, size, and age. The amount of ascorbic acid that must be supplied for proper function depends on the type of vitamin that is introduced to the diet (Lim and Webster, 2001). Ascorbic acid is essential for the majority of vertebrates, including fish, as a key water-soluble antioxidant and co-factor in several hydroxylation reactions in the tissues (Lim *et al.,* 2000).

Ascorbic acid (vitamin C), a necessary component for supporting the physiological processes of numerous animals, including fish, must be included in aqua feeds. Due to a lack of the ascorbic acid-producing enzyme L-gulonolactone oxidase, the majority of fish are incapable of producing vitamin C through biosynthesis. Fish, however, require an external supply of vitamin C. In their natural habitat, vitamin C would be found in their natural foods, but with extensive fish farming, it becomes necessary to supplement.

The result of the current study showed that the metal lead nitrate induced chronic toxic effects in the form of behavioral alterations fish hepatocytes had varied numbers of vacuoles and mild to moderate granulation in the cytoplasm, Therefore, this study will assist in formulating the use of Pb metal and should be rigorously controlled and regulated by creating suitable legislation to impede its bioaccumulation in the water ecosystem that mitigates the negative impact on aquatic fauna. While as the data also suggest that Vitamin C can act synergistically in preventing the histological damage caused by lead nitrate. It may be recommended that Vitamin C can be used effectively to minimize the lethal impact of lead nitrate if used in low concentrations. It is important to acknowledge that further research is necessary to determine the optimal concentration of Vitamin C and the duration of treatment required to fully prevent the toxic effects of lead nitrate.

CONCLUSIONS

The impacts of heavy metals on fish are more challenging to visualize because of the aquatic ecosystem's dynamic nature. Similar research was performed to determine the amount of lead present in water and it affects natural populations of freshwater fish by altering their physiology, abilities, and skills. The results have significant implications for ecological risk assessments and could make it difficult for fish to survive in their natural habitat. Since consumption of fish food containing heavy metals can have an impact on human health by spreading through the food chain, there is a constant need to identify heavy metal toxicity. Long-term exposure to the genotoxicity that occurred in common carp might have a major negative impact on the health of fish.

RECOMMENDATION

Overall, the findings imply that Vitamin C can function synergistically in reducing the histological damage produced by lead nitrate. Vitamin C can be used successfully to decrease the negative effects of lead nitrate if given in small quantities. It is crucial to realise that further study is required to discover the ideal Vitamin C dosage and the length of therapy needed to completely fend off the adverse effects of lead nitrate.

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Conflict of Interest. None.

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