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ORIGINAL ARTICLE

Effect of Lead Nitrate ($PbNO_3$) in both *in vivo* and *in vitro* systems

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ABSTRACT
Toxicology is one of the branches of Biology, serving the mankind over more than two centuries, and is still rare to implicate in research field. The present approach is to use this cosmos to check its effects and side effects (if any) which may or may not cause any change in prokaryotic and eukaryotic system. Assessment of the effects of lead nitrate was performed on two systems namely, in-vivo (in body) and in-vitro (in laboratory). In in vitro system, the study was done by considering bacteria (Gram positive, Gram negative) and fungi, their growth under normal condition and growth after $PbNO_3$treatment. Also molecular analysis was also done by applying subcutaneous injection of different concentrations of lead nitrate applied in the inseminated females and were invariably confronted with episodes like preterm delivery, gastrointestinal complications, fetotoxicity and precocious parturition of embryos. Molecular analysis was done by checking out the central dogma pathway. Whereas in the in vivo system, dose effectiveness was checked in eukaryotic body (genetically pure line albino rats) and the various physiological parameters were studied in relation to the interrelated organs.
Key Words: *Toxicology, subcutaneous injection, inseminated females,*

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INTRODUCTION

The main motive of the research work was to study the effectiveness and standardize efficacious mode of lead (nitrate salt) not only to study toxicological mechanism on Eukaryotic system by *in-vitro, in-vivo* but also molecular analysis. It was also wanted to reveal the different changes in phenotypic characters and physiological parameters. Cellular toxicological effects of lead on micro vessels and rats are quite alarming. Lead exposure can cause oxidative stress, damaging micro vessels and disrupting the blood-brain barrier. This can lead to neurotoxicity, impairing cognitive function, and even causing neurodegenerative diseases. Damage to endothelial cells: Lead exposure can alter the structure and function of micro vessels, reducing blood flow and oxygen delivery to tissues. Increased permeability: Lead can disrupt the integrity of micro vessels, allowing toxins to leak into surrounding tissues. Neurobehavioral changes: Lead exposure in rats has been linked to impaired learning, memory, and motor function. Reproductive issues: Lead can damage sperm quality and reduce fertility in male rats. Developmental delays: Prenatal lead exposure can cause birth defects and developmental delays in rat offspring. Mechanisms of Lead Toxicity & Oxidative stress: Lead generates reactive oxygen species (ROS), damaging cellular components. Calcium deregulation: Lead disrupts calcium homeostasis, altering cellular signaling pathways. DNA damage: Lead exposure can cause genetic mutations and epigenetic changes. These findings highlight the importance of reducing lead exposure, particularly in vulnerable populations like children and pregnant women [1-5].

$PbNO_3$, is an inorganic compound that is used in several common household items. The medicinal use of Lead Nitrate dates back to the middle Ages when it was used as a dye and caustic. Today lead nitrate is still used in various important works. Most important action is on the blood, producing an antiplastic effect,

rendering it more fluid and darker; the red corpuscles lose their coloring matter; the blood stagnates in the vessels and nutrition is interfered with. The nerve centers are also involved, giving rise to various symptoms of cerebral and spinal derangement. Lead nitrate is applied for various types of activities from medicine to synthetic dye [5-8].The particular usage of this chemical remedy perfectly match whenever it is used as an antiseptic along with dye or to prepare common house hold items to deal without day to day needs.

MATERIAL AND METHODS

Isolation of genomic DNA from bacterial and fungal sources [9-11]

Gram positive-*Bacillus* sp. (MTCC-2479) and Gram negative - *E. coli* (BL-121) were used as bacterial strains and were grown overnight in LB broth were transferred in a micro centrifuge tube and centrifuged. The pellet was suspended in TE buffer (Tris-EDTA, pH 8.0). 10% SDS was added and incubated. Phenol-chloroform mixture (1:1) was added and mixed. The mixture was centrifuged at 10,000 rpm. The upper aqueous phase was transferred into a new tube and re-extracted by equal volume of phenol-chloroform. The mixture was centrifuged again at 10,000 rpm. The upper aqueous was taken and mixed with 3M sodium acetate (pH 5.2) and isopropanol was added and mixed gently to precipitate the DNA. Again centrifugation was performed at 10,000 rpm to precipitate the DNA. The DNA was washed by 70% ethanol for 30 seconds and centrifuged briefly. The DNA was re-suspended in TE buffer.

The fungal broth (YEPD) was taken and 0.5M suspension buffer (Tris Cloride, pH-7.5) was mixed along with sand particles and kept in ice for 15 seconds. 1% SDS was added and incubated. The tubes were centrifuged and phenol- chloroform mixture (1:1) was added followed by centrifugation. 3M sodium acetate (pH 5.5) was added to the supernatant followed by immediate addition of absolute alcohol. Then it was placed in the freezer at -20^{o}C for 30 mins. After cooling, centrifugation was done and the pellet was washed with 70% ethanol. Finally it was suspended in TE buffer.

Agarose gel electrophoresis of DNA [12]:

Agarose gel (0.8%) was prepared by dissolving in 1X TAE buffer. The comb was inserted before pouring the gel. The gel was left for 30-40 minutes for solidification and comb was removed from the gel. Electrophoresis tank containing the gel was filled with 1X TAE electrophoresis buffer.1 volume of sample buffer was mixed with the 5 volume of DNA sample. The samples were applied to the wells formed in the gel. Ethidium bromide at a concentration of 0.5µg/ml was incorporated into the gel. The electrodes were connected and 70 volt current was applied for 1 hour. After electrophoresis, the gel was visualized under UV light.

Sodium dodecyl sulfate (SDS)-gel electrophoresis:

SDS-gel electrophoresis was done according to the methodology followed by [12]. For this, the SDS plates were assembled. Acrylamide separating gel was prepared in a conical flask and poured between the plates. Acrylamide stacking gel was prepared. The components were mixed and poured over the separating gel. Wells were prepared using combs. The samples were prepared with equal volume of sample buffer. The samples were then heated in boiling water bath and loaded. Voltage was applied depending upon the dimension of gel. The gel was allowed to run until the sample reaches the bottom of the resolving gel. The power supply was then turned off; the gel was removed from the apparatus. The gel was allowed to stain overnight. Next day, the gel was dipped in destaining solution and observed

In vivo work:

Four albino rats were taken and were treated with mili Q water for the control set, lead nitrate 200 PPB, 100 PPB and 50 PPB. After 60 days, they were sacrificed and different physiological parameters such as presence or absence of cyst, change in size of the organs and presence of any other abnormality in liver, heart, spleen and kidney were studied (table 1). Their phenotypic and hematological parameters were studied. The blood was taken from the heart on a slide and was stained with Leishmann stain, washed with water and observed under the microscope[14].

Table 1. In *in vivo* condition, four albino rats were taken; different physiological parameters and effects in different lead concentration were studied

Sl. no.	Treatment time (days)	Types of treatment	Dose	Initial weight(g)	Weight after 30 days (g)	Weight after 60 days (g)	Physiological parameter studied and changes observed after treatment.			
							Liver	Kidney	Spleen	Heart
1.	60	Water	Water 50ml/day	131.8	144.32	137.6	Normal	Normal	Normal	Normal
2.	60	$PBNo_3$: 200 PPB	10 drops in 50ml Water	190.3	189.75	193.3	Big cyst	Normal	Normal	Normal
3.	60	$PbNo_3$:100 PPB	10 drops in 50ml Water	178.3	162.2	186.25	Small cyst	Normal	Small size	Normal
4.	60	$PbNo_3$: 50 PPB	10 drops in 50 ml water	98.20	98.72	110.24	Normal	Normal	Small size	Normal

RESULTS AND DISCUSSION

Three results were observed in *in vitro* condition, i.e., bacterial growth, bacteria and homeopathy medicine, fungi and homeopathy medicine. When bacteria grow normally, there was an increase in optical density value with respect to time. When bacteria grow with lead nitrate, optical density mentioned in table 2 gets decreased as increase lead concentration inhibit the growth of bacteria so decline in the optical density value was observed in gram negative bacteria whereas gram positive bacteria showed gradual increase. Central dogma was studied and presence (+) or absence (-) of band of DNA (agarose gel electrophoresis) and protein (SDS-PAGE) is mentioned in the table 3.

Table 2. Optical density at 400 nm of cultures of bacteria and fungi

Duration of treatment	Fungi			Gram positive bacteria (*Bacillus* 2479)			Gram negative bacteria (*E. coli.* BL-121)		
	200 ppb	100 ppb	50 ppb	200 ppb	100 ppb	50 ppb	200 ppb	100 ppb	50 ppb
1st hour	0.02	0.00	0.04	0.00	0.01	0.03	0.10	0.05	0.03
2nd hour	0.03	0.03	0.01	0.19	0.20	0.20	0.07	0.08	0.06
3rd hour	0.01	0.20	0.03	0.24	0.25	0.29	0.01	0.01	0.02
4th hour	0.01	0.02	0.06	0.28	0.29	0.31	0.01	0.05	0.02
5th hour	0.01	0.03	0.02	0.29	0.29	0.29	0.00	0.04	0.02
6th hour	0.02	0.02	0.04	0.25	0.29	0.33	0.02	0.02	0.01
7th hour	0.03	0.02	0.03	0.28	0.27	0.37	0.05	0.01	0.00

Table 3. Result of agarose gel electrophoresis and SDS-PAGE

Type	Gram Positive		Gram Negative		Fungi	
	DNA	Protein	DNA	Protein	DNA	Protein
Control	+	+	+	+	+	+
$PbNO_3$ (50 PPB)	+	_+_	+	_+	+	+
$PbNO_3$ (100 PPB)	+	_	+	_	+	+
$PbNO_3$ (200 PPB)	+	--	+_	--_	+	+_

From figure 1, it can be concluded that the lead did not have any significant effect on the growth of fungi, since their growth remained static. The graph initially moved upward because of normal growth of bacteria/fungi then it decline.

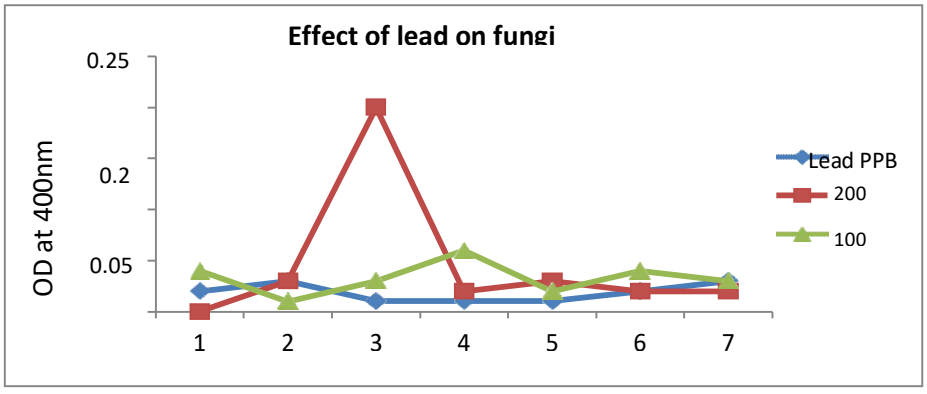

Figure 1. Effect of lead on fungi

Surprisingly the lead did not inhibit the growth of the gram positive bacteria as they show their normal growth activity with increase in optical density (figure 2). On the other hand, inhibition of growth of gram negative bacteria (figure 3).

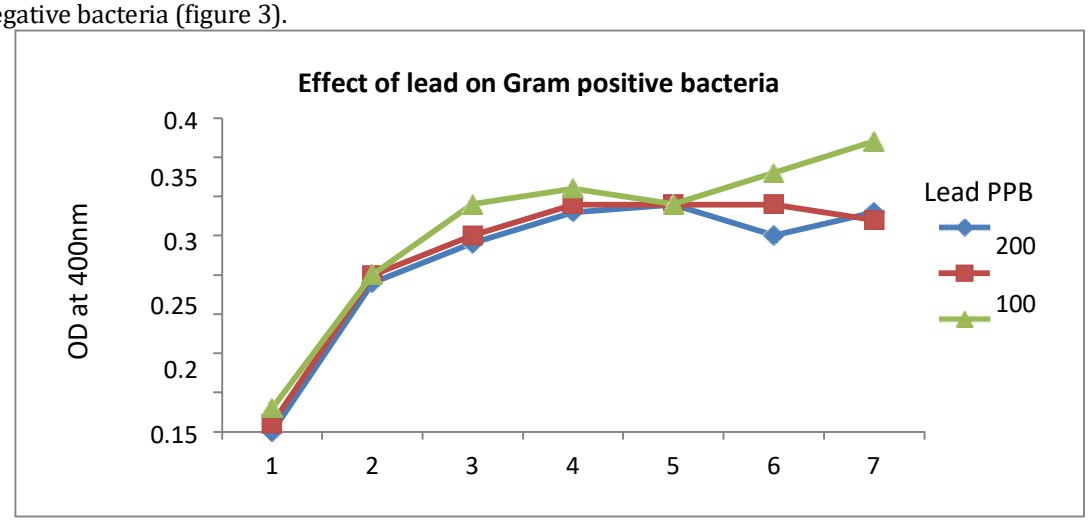

Figure 2. Effect of lead on gram positive bacteria

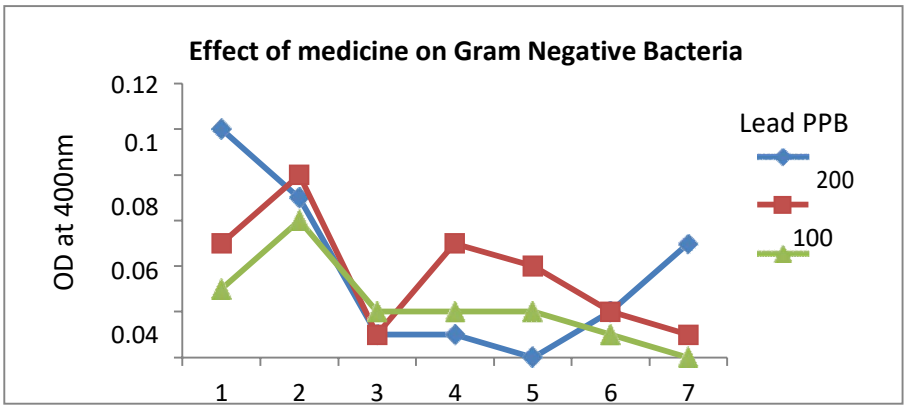

Figure 3. Effect of *Argentum nitricum* on gram negative bacteria

Microscopic view of blood film showed that the cells, those were treated with lead 200 PPB, have a shrinkage and the rest of them showed no significant change (figure 4).

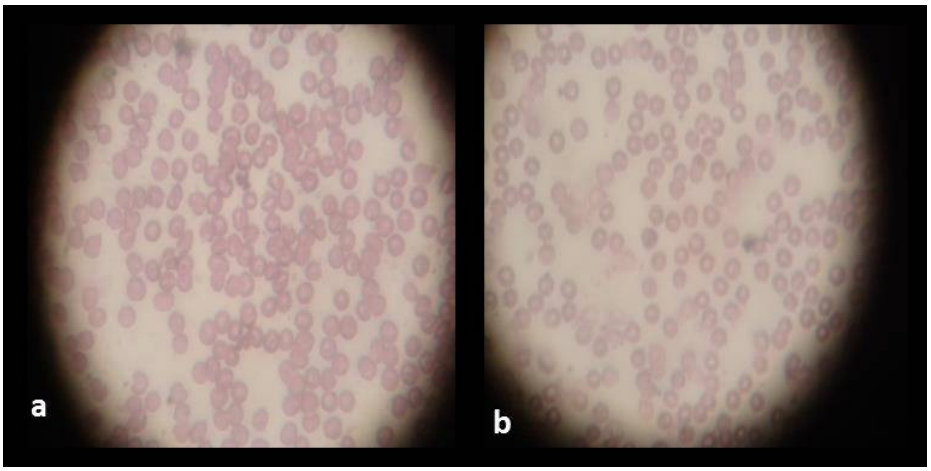

Figure 4. Microscopic slide view; (a) blood film slide for control, (b) blood film slide of $PbNO_3$-200

Treatment of mice with lead (200 PPB) led to formation of cyst in the liver (figure 5). The mice treated with higher dilution of (200 PPB) showed more number of cyst because it was more readily absorbed by the cells of its body as compared to lower dilution (50 PPB).

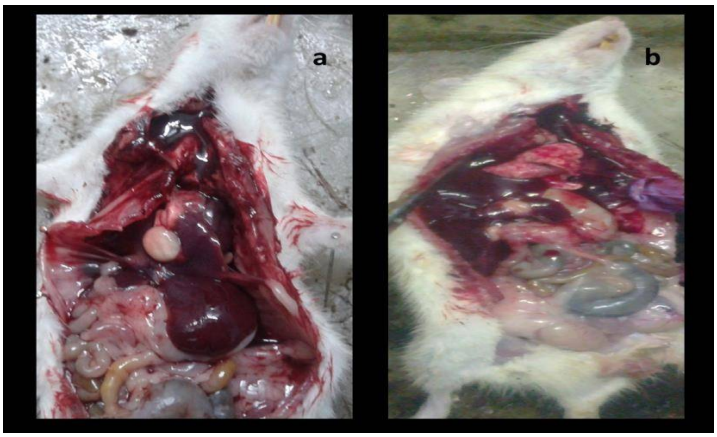

Figure 5. Mice dissection; (a) single cyst in liver of mice treated with $PbNO_3$ 100 PPB (b) double cyst in mice liver treated with $PBNO_3$ of 200 PPB.

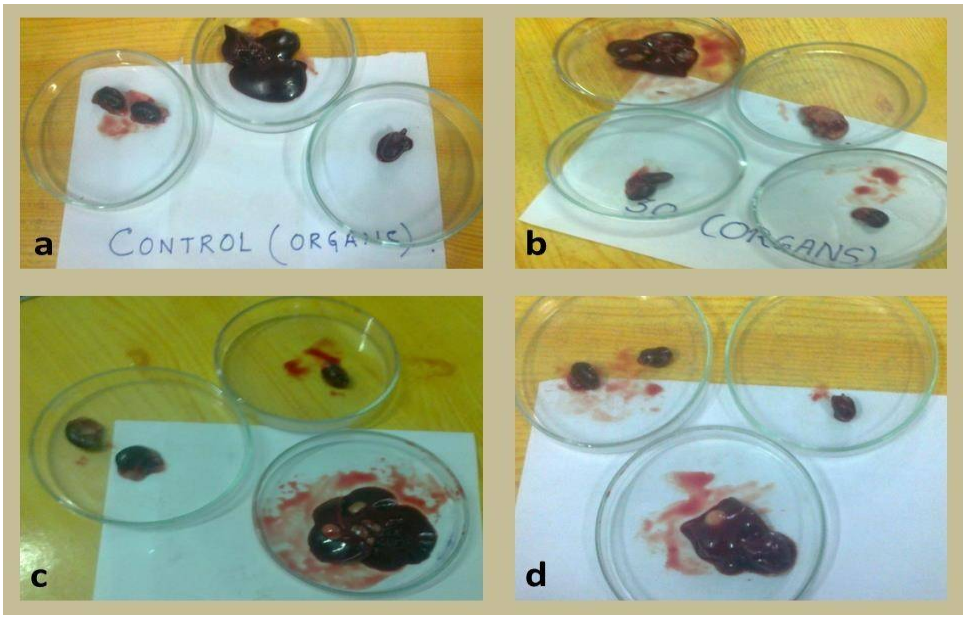

Figure 6. Histological view; (a) mice organs were used as control, (b) mice organs were treated with $AgNO_3$-30, (c) by $AgNO_3$-200 treatment, and (d) treated with crude $AgNO_3$

CONCLUSION

Aim of the experiment was to study the effect of lead nitrate on eukaryotic and prokaryotic system. Both *in vitro* and *in vivo* system. But gram-positive bacteria showed continuous increase in optical density with time due to certain unknown reasons. Also in*in vivo* condition, some physiological changes were observed such as decrease in size of spleen and formation of cyst was found in the treated albino against the untreated. Some phenotypic changes were also observed like hair fall and sudden change in weight. As we all know that Homeopathic medicine has no side effects but from this experiment, we can conclude that lead nitrate is not safe for long termuse in any form as it is highly soluble in water and a potent toxic agent.

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