

Research Article

## Toxic effect of lead nitrate on testicular functions in male Swiss albino mice

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### Abstract

Lead, heavy metal, has been associated with various health disorders including respiratory, digestive, nervous and reproductive systems. Male reproductive disorders are rising worldwide in parallel to increased exposure to harmful chemicals through anthropogenic sources. The aim of this study was to find the effect of lead nitrate on sperm quality, serum testosterone level and testicular histopathology in male Swiss albino mice. Male Swiss albino mice were divided as Control and three groups of lead nitrate treated mice (LN-15, LN-30 and LN-45) with four animals in each group. An oral method was applied to administer 52 mg/kg body weight of lead nitrate to each mice of LN-15, LN-30 and LN-45 for 15, 30 and 45 days, respectively. Distilled water was administered to the control group for 45 days. Sperm quality and serum testosterone level was studied for each group on completion of exposure to each group. There was a significant decline in sperm count ( $10.81 \pm 0.97$  to  $5.85 \pm 1.22$ ) in LN-45; sperm motility ( $74.0 \pm 5.95$  to  $47.75 \pm 5.97$ ) in LN-30 and ( $74.0 \pm 5.95$  to  $40.75 \pm 7.81$ ) in LN-45; normal sperm morphology ( $77 \pm 3.11$  to  $42.0 \pm 8.04$ ) in LN-30 and ( $77.50 \pm 3.11$  to  $35.5 \pm 5.19$ ). Serum testosterone levels decreased significantly ( $5.29 \pm 0.84$  to  $2.61 \pm 0.51$ ) in LN-45 group. Lead nitrate adversely affected testicular functions and increased adversity with a longer exposure duration in Swiss albino mice. It can reduce male fertility by hampering the formation of normal mature sperms.

**Keywords:** Heavy metals, Lead nitrate, Male reproductive functions, Sperm, Testosterone

## INTRODUCTION

Humans are exposed to heavy metals (HM) because of their immense use in industries, agriculture, household, and technological applications. Heavy metals are found naturally but anthropogenic activities are the main reasons for the rise of their occurrence in the earth's atmosphere (Al Osman *et al.*, 2019; Wirth and Mijal, 2010). Heavy metals on entering the body can accumulate in the liver, kidney, brain, and other organs and tissues, exerting their toxicity as evidenced by disturbing biological functions. Metals such as lead, arsenic, cadmium, and mercury are categorized as toxic heavy metals. Lead among heavy metals is a non-essential metal and has been associated with various diseases (Al Osman *et al.*, 2019; Wirth and Mijal 2010; Rai *et al.*, 2019). World Health Organization (WHO) has raised concern among its member states about safeguarding children, workers, and women of childbearing age from lead toxicity. WHO has categorized lead

among the ten chemicals having major effects on the health of humans (WHO, 2021). Lead has a long history of usage, dating over 5000 years back by mankind for various purposes (Jarup, 2003). However, even after its addition to gasoline is banned in most countries, its usage has not been restricted in some sectors (Assiet *al.*, 2016)). This has facilitated its entry into human, as evidenced by recent studies, which have observed it in a detectable amount in human blood (Charkiewicz and Backstrand, 2020). It is used in battery plates, sulphuric acid production, soldering tools, atomic react shields, paints, ceramics, chemical factories, aviation gasoline, printing, bearings, and construction industries. Old construction demolition and renovations, including the removal of old paints, are also prominent sources of lead exposure (Wirth and Mijal, 2010; Rai *et al.*, 2019; Charkiewicz and Backstrand, 2020).

Lead present in the soil, food, and dust enters the body either through the nose or mouth (Wirth and Mijal, 2010; Charkiewicz and Backstrand, 2020). Utensils

used in the kitchen made of metals, glasses or painted ceramics containing lead can leach out in the cooked or stored food items. Consumption of food products, which are partially or completely processed, including materials used during packaging, may contain lead. Vegetables and fruits grown near industrial plants can easily accumulate lead through the roots and subsequently become the medium of its entry within the body (Charkiewicz and Backstrand, 2020). Pipes and materials used in the plumbing system of the households which are made using lead as a component, may cause the leaching of lead in the consumed water (Wirth and Mijal, 2010 ; WHO, 2021). Lead is found to have negative effects on the body organs of both human and experimental animals. On absorption within the body, it gets stored in the blood and bones and disturbs the normal function of the brain, liver, and kidney (Andjelkovic *et al.*, 2019; Asrar *et al.*, 2021; Stewart *et al.*, 2006 ;Kasperczyk *et al.*, 2013). Lead has a negative impact on the reproductive health of human and has been associated with the low semen quality (Taha *et al.*, 2013), diminished fertilization outcome (Benoff *et al.*, 2003), and unsuccessful pregnancy (Lee *et al.*, 2020). Its exposure to the laboratory animal has shown deleterious effects on the reproductive system. (Bas and Kalender, 2016; Dumitrescu *et al.*, 2015). Observing the scenario of the present time, it was considered useful to study the effect of various concentrations of lead on the male reproductive system in animal models. Selection of route, dose, and time of exposure to toxic chemicals is crucial in inducing their testicular toxicity or other reproductive disorders in males. Though past works on animals show the effect of lead on male fertility potential as depicted through the testicular morphology and relevant sperm quality, yet this effect seems to be variable as per the species of experimental animals, type of compound used, methods of dose administration i.e., quantity, route, and exposure duration. Also, the studies reporting the comparative effect of lead on sperm quality and testosterone level among rats and mice depending on the exposure time are very few, and the results are inconsistent (Bas and Kalender, 2016; Acharya *et al.*, 2003; A. Thoreux *et al.*, 1995; Wadi and Ahmad, 1999; Eman Hassan, 2019; Richard, 2016). The present study was made to find the effect of lead nitrate on sperm quality and serum testosterone level in male Swiss albino mice.

## MATERIALS AND METHODS

### Methodology

#### Chemicals

Thermo Fischer Scientific manufactured lead nitrate used in the experiment and it was purchased from a scientific store in Patna, Bihar.

### Experimental animals and diets

The experimental work was conducted at Mahavir Cancer Sansthan and Research Centre Phulwarisharif, Patna, Bihar, with the Committee for the purpose of control and supervision of Experiments on Animals (CPCSEA, Registration number 1129/PO/ReBi/S/ 07/ CPCSEA). The work was approved by the Institutional ethical committee. The animal handling and experiments were done as per the guidelines provided by CPCSEA, New Delhi, India. About eight weeks to ten weeks old male Swiss albino mice with body weights between 26 grams to 30 grams were selected and kept in the animal laboratory of the institute for acclimatization. They were kept in room maintained at 24°C to 26°C temperature with alternate light dark periods of 12 hours each. Two animals were kept in each propylene cage of small size. The recommended laboratory animal dietary food and water were provided as the daily feed. Fresh drinking water was provided each day in bottles fixed in cages.

### Experimental design

The animals were left for acclimatization for one week before the onset of the experiment. The animals were randomly categorized into four groups of "Control" and lead nitrate treated groups named "LN-15", "LN-30" and "LN-45" with four animals in each group. An oral gavage procedure was applied to administer distilled water to the control and lead nitrate to the other three groups. Lead nitrate at a dose of 52 mg/kg body weight was administered to each mice in treated groups during the experimental period that is 15 days (LN-15), 30 days (LN-30), and 45 days (LN-45). The dose of lead nitrate was decided referring to the LD-50 found in our laboratory and various reported works which studied the effect of lead nitrate on mice and rat. (Sharma *et al.*, 2010; Bas and Kalender, 2016) The dose of lead administered in this experiment was approximately 1/40 of LD50 found in our laboratory.

### Weight of whole body and testes

The weight of each animal's body was observed timely during the experimental duration. Body weight of all the animals was noted at the onset and on the completion of dose duration, and weight of the testes was taken after the animals were sacrificed.

### Preparation of sperm suspension and blood collection

After a complete duration of dosing, all animals were euthanized, and testes with epididymis were dissected and taken out. Animals were sacrificed, and blood obtained from each was centrifuged at 3000g for 15 minutes to obtain the serum, which was stored at -20°C for testosterone estimation. Sperms were obtained from

the epididymis, and their suspension was made in 0.9% normal saline to study sperm quality.

### Sperm count, sperm motility and sperm morphology

Sperm count was evaluated according to previously mentioned procedures (Vega *et al.*, 1988; WYROBEK and BRUCE, 1975; SHETTY and BAIRY, 2015). Sperm observation was done under the light microscope of Olympus. Sperm count was done in the chamber and calculated per ml of caudal sperm reserve. Sperm motility was evaluated by placing sperm suspension on the glass slides and calculated as the percentage of total sperm counted. Sperm morphology was evaluated by staining sperms with only Eosin Y dissolved in normal saline (WHO, 2010) and calculated as a percentage of total sperms observed. In all these parameters, two observations were made for the same slide, of which one was blind, which implies that one observer for microscopic study was not knowing the identity of the sample. The average of these was recorded as the final result. Two hundred sperms were calculated to find the percentage of motility and normal morphology. The average of the two observations was recorded.

### Estimation of serum testosterone

Testosterone (T) level in the serum was determined through an Enzyme-linked immunosorbent assay (ELISA). The assay was conducted through a kit and observed under ELISA Reader (AM 2100) of Thermo Fisher, U.S.A.

### Histopathological study

The testes of all groups were dipped in normal physiological solution, and fat tissue was removed and then fixed in 10% formalin for 48 hours. The standard protocol was applied for tissue dehydration in a series of graded ethanol and embedded in paraffin wax. With the help of microtome (Micron HM 340E, Thermo scientific, USA), five-micron thick sections of the blocks with tissue were cut and kept on slides. These sections were stained with Hematoxylin and Eosin (H&E), and slides were observed under a light microscope.

### Statistical analysis

Results were depicted as mean  $\pm$  standard deviation (SD) for four mice in each group. One-way analysis of

variance (ANOVA) was applied to analyse the total variation in each data set. Tukey's multiple comparison test was applied. This statistical analysis was performed using GraphPad Prism Program (GraphPad Software, Inc. San Diego, U.S.A).

## RESULTS

The body and testes weight of the lead-treated mice decreased gradually with increased duration of lead exposure (Table 1). Sperm count of LN-15 group was reduced non-significantly compared to the control group. Sperm count was reduced in LN-30 and in LN-45 groups significantly ( $p < 0.05$ ) (Table 2). Total motile sperm was reduced non-significantly in LN-15 group and significantly in LN-30 ( $p < 0.05$ ) and LN-45 ( $p < 0.05$ ). (Table 2). A significant increase in sperms with abnormal morphology in LN-15, LN-30, and LN-45 groups was observed ( $p > 0.05$ ) (Table 2). Hooked tail end, coiled tail, complete loop of tail, and bent mid piece were the most common abnormalities in the lead treated groups. Also, sperms with double heads were found more in number among them, which was comparatively negligible in the control group (Fig. 1). Serum testosterone levels of LN-15 and LN-30 differed non-significantly from the control, but the level of testosterone in LN-45 decreased significantly ( $p < 0.05$ ) (Table 2).

The histopathological study of testes of the lead-treated groups (LN-30 and LN-45) showed a marked increase in necrosis and clumping of cells within seminiferous tubules. A lack of normal spermatid formation and damaged germinal epithelium layer was observed in LN-45 group. The Leydig cells appeared normal in LN-15 group and LN-30 groups (Fig. 2).

## DISCUSSION

For the last four decades, some studies have reported decrease in the semen quality and increased cases of male reproductive disorders (Hagai *et al.*, 2017; Sengupta *et al.*, 2016; Carlsen *et al.*, 1992). About 48 million couples worldwide have fertility issues, among them, 50% of cases involve male factors. The initial steps undertaken to diagnose male infertility were to evaluate sperm quality and hormone level, including

**Table 1.** Average weight of the body of mice in gram (gm) at the onset and at the end of the experiment and testicular weight in milligram (mg) (n=4).

Groups of animals (n=4)	Initial body weight (gm)	Final body weight (gm)	Gain in body weight (gm)	Testicular weight (mg)
Control	30.25	34.50	4.25	222 $\pm$ 4.9
LN-15	30.50	31.0	0.5	211 $\pm$ 8.4
LN-30	31.0	31	0	203 $\pm$ 9.4**
LN-45	30.50	32.5	2.0	193 $\pm$ 3.3***

**Table 2.** Sperm parameters and serum testosterone (in nanogram(ng/ml)) in different experimental groups. p-value is depicted as \* for p<0.01, \*\* for p<0 .001 and \*\*\* for p<0.0001

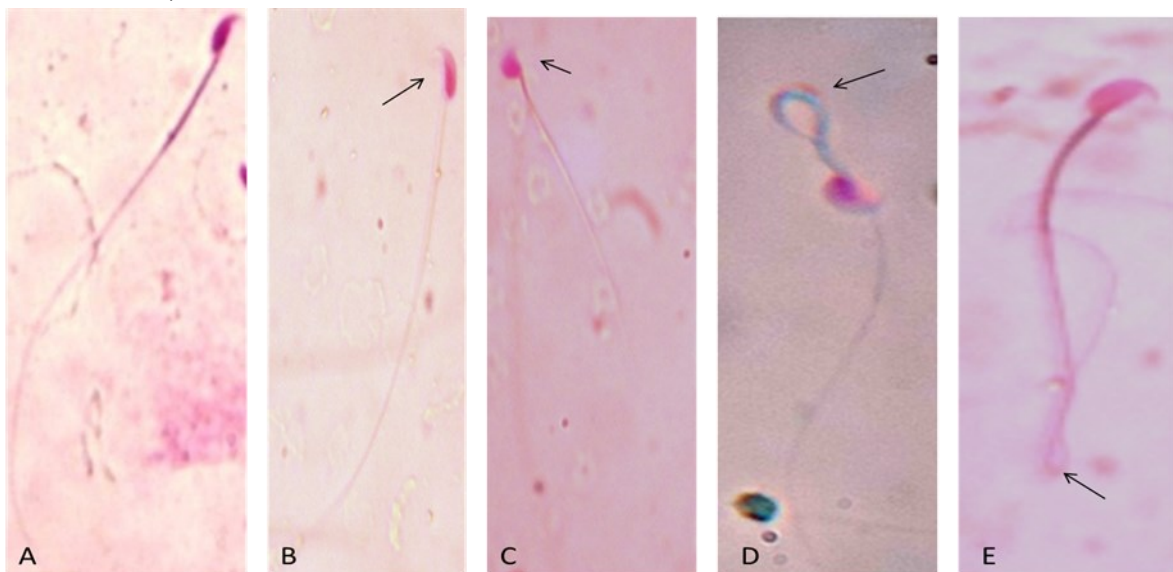
Groups of animals (n=4)	Sperm count (10 <sup>6</sup> /ml)	Sperm motility (%)	Sperm morphology (%)	Serum Testosterone (ng /ml)
Control	10.81± 0.97	74.0± 5.95	77.50 ± 3.11	5.29 ±0.84
LN-15	9.43± 1.44	64.25± 8.96	63.50±11.27	4.04 ±1.12
LN-30	7.94± 1.26*	47.75±5.97**	42.0 ± 8.042***	4.6 ± 0.67
LN-45	5.85± 1.22***	40.75±7.81***	35.5 ± 5.19***	2.61 ±0.51**

testosterone (WHO; Agarwal *et al.*, 2021; Vigeh, 2011). The present study showed that lead nitrate could adversely impact male fertility during longer exposure duration, as evidenced by a marked decrease in testicular weight and sperm parameters after 45 days of lead exposure (p<0.05) Lead hindered the process of spermatogenesis, resulting in the condition of oligozoospermia, teratozoospermia, and asthenozoospermia. The decline in sperm motility and normal sperm morphology in all three lead groups compared to the control strongly supports the deleterious effect of lead on sperm maturation process resulting in defective tail formation (Table 2, Fig.1)

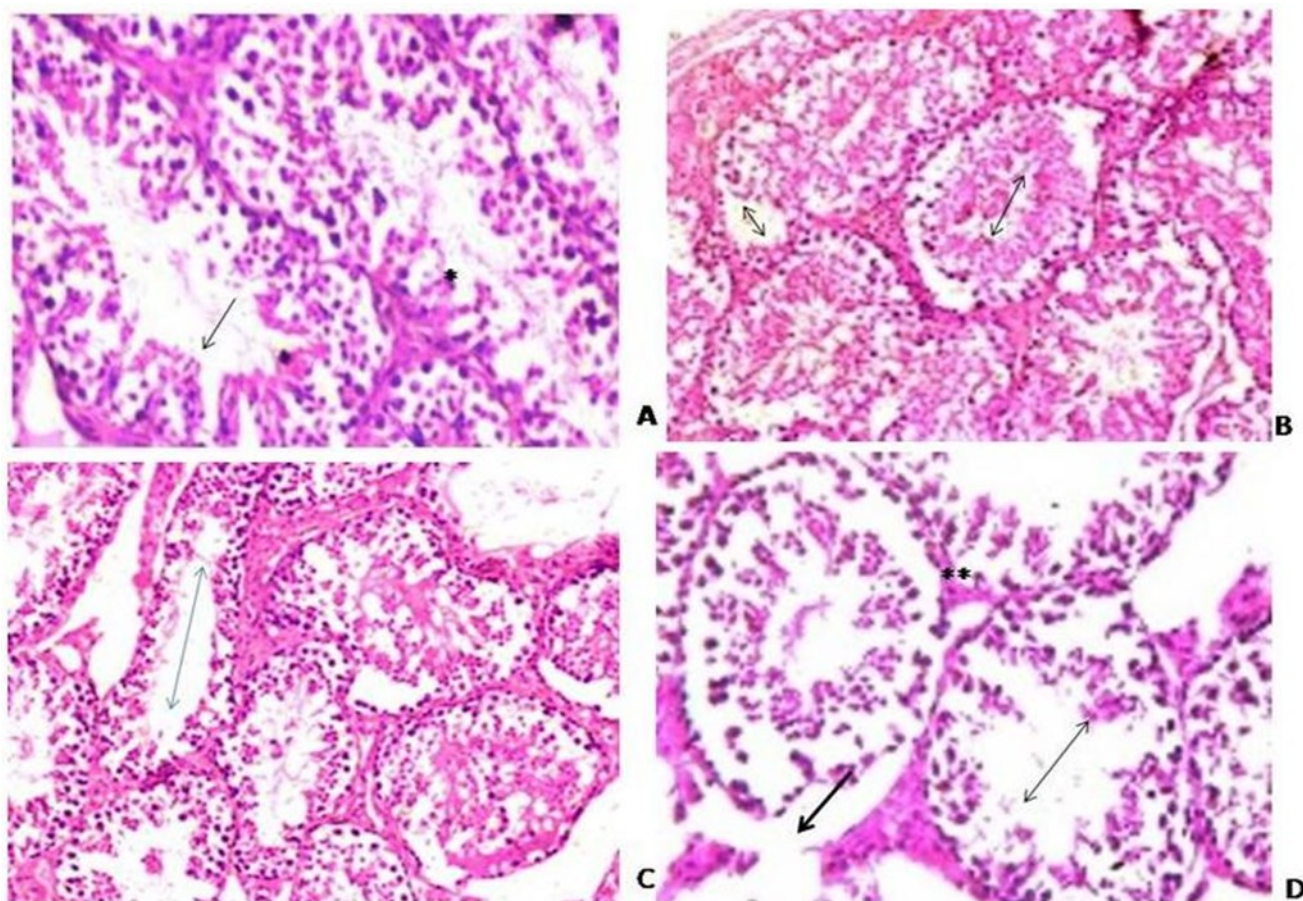
Lead is known to displace zinc in metallothionein (Wirth and Mijal, 2010) Zinc is needed for spermatogenesis and is required during the conversion of histone to protamine in the sperm maturation process (Wang *et al.*, 2019; Gatewood *et al.*,1990). Zinc also plays a role in the lipid stabilization of cell membranes. Replacement of zinc by lead may hinder histone to protamine conversion, which may increase the production of sperms with abnormal morphology and damaged chromatin structure(Wang *et al.*, 2019; Fallah 2018). Also, studies on humans have shown the association of increased blood lead levels with an increment in sperm chromatin damage (Hsu *et al.*, 2019).

Lead is also suggested to affect calcium-mediated processes as it can replace calcium in zona adherens junctions damaging the blood testes barrier. This may allow the lead to enter the testicular tissue and deregulate its functions (Wirth and Mijal, 2010).The effect of lead on testosterone levels in serum and plasma has been studied in mice and rats previously. However, they provided contradictory results; either there was a decline in testosterone level (Eman Hassan, 2019 and A. Thoreux Manlay *et al.*,1995) or it remained unaffected (Wadi and Ahmad,1999).

In the present study, lead affected the serum testosterone level for longer exposure duration than in the LN -45 group (p<0.05). The significant decline of testosterone after 45 days of treatment (p>0.001) may be due to the effect of lead on Hypothalamus - Pituitary - Gonadal axis (HPG-axis) in long-term exposure. The normal range of testosterone level on 30 days of treatment, along with a significant decline in motility and normal morphology of the sperm, suggests a more intense and direct effect of lead on the maturation process of sperm compared to HPG axis. However, this could not be confirmed in the present study as there is a limitation that other hormones like luteinizing and follicular stimulating hormones were not studied.



**Fig. 1.** Sperm morphology observed after staining with Eosin Y. (A) Normal sperm with intact acrosome and proper tail; (B) Sperm with narrow head; (C) Sperm head lacking normal acrosome; (D) and (E) Sperm with coiled tail



**Fig. 2.** Photomicrograph of testicular tissue stained with hematoxylin and eosin observed under light microscope. Control (A): Normal spermatogenesis was observed in the control group. In the Lead treated group, LN-15; (B) necrosis and clumping of spermatids were observed in LN-30; (C), necrosis increased, and there was empty space within tubules. In LN-45 (D), damaged germinal epithelium layer with prominent increase in necrosis and decrease in sperm formation was observed

The histopathological study of testes after lead treatment in this study shows similar effect as reported earlier (Elgawish and Abdelrazek, 2014; Bas and Kalener, 2016). However, here it is evident that the same dose of lead can affect the testicular tissue differently depending on the duration of exposure. Clumping of spermatocytes and spermatids occurred in the lumen of seminiferous tubules of LN-15 and LN-30 groups, which may be caused by congestion in the blood testes barrier. Diminished formation of elongated spermatids was observed in some tubules of LN-30 group. However, in the LN-45 group, lead caused a significant decrease in the formation of spermatocytes and spermatids and damaged the germinal epithelium layer.

### Conclusion

Overall, lead nitrate was seen to have a deleterious effect on male testicular functions in Swiss albino mice. The selection of dose and duration of exposure selected in this experiment may prove beneficial for future

investigation on lead-induced male reproductive toxicity and in search of its chelating agent. The lead had a deleterious effect on sperm motility and sperm morphology after 30 days and 45 days of lead exposure in Swiss albino mice. Level of serum testosterone level decreased significantly after 45 days of treatment with lead nitrate. Subchronic exposure to lead nitrate can adversely affect sperm quality and serum testosterone levels in albino mice.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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