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# Age and Dose-Specific Testicular Histomorphometry and Hormonal Profile in Lead-Intoxicated Rats

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## ABSTRACT

In the present study, we report the relationship between doses of oral lead exposure, duration of exposure, and the hormonal and testicular histomorphometric changes in male Wistar rats at specific stages of postnatal development. Male juvenile Wistar rats (average weight of 34 g) were administered lead acetate at concentrations of 0.5% (5000 ppm), 1.0% (10,000), and 1.5% (15,000 ppm) via drinking water. Rats were killed at 3 weeks, 6 weeks, 9 weeks, and 12 weeks of postnatal development, representing the early juvenile, late juvenile, peripubescent and early post-pubescent stages of life. Sera were analyzed for gonadotropins (Gn) and testosterone (T). Testicular histomorphometry was studied by the H&E and toluidine blue techniques; while caudal epididymal sperm cells were analyzed for density and motility. Serum T and Gn levels were significantly reduced ($P<0.05$) in the lead-exposed rats at the peripubescent and early post-pubescent periods. Sperm density showed significant decreases in the lead-treated rats only at the early post-pubescent age; while sperm motility was adversely impacted not only at postnatal week 12, but also at week 9 and week 6. In addition, testicular histology showed marked loss of germ and interstitial cells that was most severe at postnatal week 12, while germinal epithelium height and seminiferous tubule diameter were significantly attenuated in the lead groups at each of the age categories studied. These findings indicate that the adverse effects of juvenile oral lead on male reproductive profile are most pronounced in the peripubescent and early post-pubescent postnatal periods.

**Keywords:** Lead, testicular, histomorphometry, sex hormones, Age

## INTRODUCTION

Lead (Pb) is a ubiquitous divalent metal that is known to be toxic to biological systems when ingested beyond the acceptable blood concentrations of 10µg/dl and below [1]. Environmental sources of Pb include lead pipes (for domestic water supply), lead smelting activity, paint factories, and leaded gasoline, etc[2]. Moreover, lead may contaminate sources of drinking water that are close to mining and ore processing sites [1].

Being a divalent metal, lead readily makes use of the calcium ion channels to gain access to cells, where it impairs such calcium-dependent cellular processes as neural signalling and cell motility, etc [3]. Indeed, children exposed to high concentrations of Pb from contaminated water have been reported to present with low intelligence quotient [1]. Similarly, Pb is capable of impairing sperm motility and is a known testicular toxin [4,5].

Because the relative subpopulations of germ and somatic cells in the testicular parenchyma and interstitium vary depending on the age of the organism (from childhood to senescence), the deleterious effects of lead on the testis may depend not only on its blood concentrations, but also on the age of the organism (duration of cumulative exposure) [6]. Supraphysiological blood concentrations of lead may therefore severely impact the reproductive parameters of one particular age group than the other [6]. This may also perturb the serum levels of gonadotropins and androgens, with significant impact on the morphology and function of the testicles [7].

The aim of the present work was to study the relationship between doses and duration of lead exposure and testicular histomorphometric and hormonal profiles in rats at different postnatal stages of development, from the juvenile stage to the early post-pubescent period.

## MATERIALS AND METHODS

### Chemicals

Lead acetate is a product of Kiran Light Laboratories (India), and was purchased from Yomi- Esthony Company, Ilorin. Other reagents were of analytical grades and were procured locally.

***Animals***

Weaned male Wistar rats (average weight of 34 g) were obtained from John Alfred Animal Facility, Ilorin. The animals were acclimatized for 14 days prior to the commencement of oral Pb acetate treatment. All experimental rats were maintained on standard rodent chow and water was served *ad libitum. Care and handling of animals was in accordance with the National Academy of Sciences' Guide for the Care and Use of Laboratory Animals*[8]*; as well as the University of Ilorin's Ethical Review Committee's guideline on the use of rodents for research.*

***Oral lead acetate treatment and euthanasia of rats***

Lead acetate was administered orally to groups of male Wistar rats (n=5 each) via the drinking water at concentrations of 0.5%, 1.0% and 1.5% [9], which delivered daily doses of 5000 ppm, 10000 ppm and 15000 ppm, respectively, for either 3 weeks (early juvenile period), 6 weeks (late juvenile period), 9 weeks (peri-pubescent period) or 12 weeks (post-pubescent period). At the end of each treatment period, rats were placed under light anaesthesia with diethyl ether and the cauda epididymides were harvested for sperm analysis. For each rat, blood was collected via cardiac puncture into plain sample bottles and allowed to stand briefly for 30 minutes, after which it was centrifuged at 15,000 rpm for 10 minutes to obtain serum for testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) assays. Furthermore, all animals were subjected to whole body perfusion, first with normal saline, and then with the fixative (4% paraformaldehyde in phosphate buffered saline), and the perfusion-fixed testicles were transferred into 4% paraformaldehyde solution for histological processing.

**Sperm Analysis for the Pb-treated and control rats**

Sperm from the caudal parts of the epididymides was analysed for density (count) and motility as was previously described by Oyewopo, Togun [10]. Briefly, Sperm count was estimated with the aid of the Neubauer improved cell-counting chamber. In addition, the ratio of the motile to non-motile sperm cells (sperm motility) was estimated for the Pb-treated and control groups.

**Bioassay for serum gonadotropins (FSH and LH) and testosterone**

Sera from Pb-treated and control rats were assayed for testosterone concentrations by the enzyme immunoassay (EIA) technique as described by Gower [11] using the testosterone EIA kit from Cayman Chemical (MI, USA). In addition, serum follicle stimulating hormone (FSH) and luteinizing hormone were assayed by enzyme-linked immunosorbent assay (ELISA) using respective assay kits from Monobind Inc. (Lake Forest, USA).

**Testicular Histology and Histomorphometry**

Paraformaldehyde-fixed testicles were sectioned at 5 µm with the aid of the rotary microtome and then processed for light microscopy by the haematoxylin and eosin (H&E) and Toluidine blue methods as described in Bancroft, Stevens [12]. With the aid of ImageJ (NIH), haematoxylin and eosin sections of the testes were analysed for morphometric parameters. In the control and Pb-treated rats, the diameter (external diameter) of the seminiferous tubules, and the height of the seminiferous (germinal) epithelium were estimated to the nearest µm.

**Statistical Analysis**

Data were analysed using two-way analysis of variance (ANOVA), with the aid of GraphPad Prism software (GraphPad Inc., USA). Results are presented as mean ± standard error of the mean (mean±SEM). The means of the variables measured among the Pb-exposed and non-exposed age-matched control groups were compared using Bonferroni *post hoc* test. P value less than 0.05 ($p<0.05$) was taken as statistically significant. All graphs were drawn using the GraphPad Prism (GraphPad Inc., USA).

## RESULTS

**Lead-induced Loss of Testicular Germinal and Steroidogenic Cells is Age- and Dose-Dependent**

Exposure of rats to high concentrations of Pb via their drinking water resulted in age- and dose-dependent loss of germ and steroidogenic interstitial cells (Fig. 2A and Fig. 3). Histologic evidence of germ cell loss was obvious in rats' testes at every dose of oral Pb administered (0.5%, 1%, and 1.5%). Such Pb-induced histological changes showed massive depletion of the germinal epithelium, which was most pronounced at postnatal week 12 in the Pb-treated rats (Fig. 2). Furthermore, histological evidence showing loss of testicular interstitium and the associated steroidogenic cells (interstitial cells of Leydig) was obvious in Toluidine blue sections of the testes of the Pb-exposed rats by week 12 of postnatal life. However, in rats receiving the highest dose of Pb in their drinking water (1.5%), histological evidence of interstitial tissue depletion occurred as early as week 6 of postnatal life, while the interstitium appeared intact at week 3(Plates D1, D2 and D3 in Fig. 3).

**Oral Lead Attenuates Seminiferous Tubule Diameter and Germinal Epithelium Height**

At each dose and duration of Pb acetate treatment, the diameters of the seminiferous tubules were grossly attenuated (diminished), while the height (thickness) of the germinal epithelium had also reduced significantly compared with the controls ($P<0.05$) (Fig. 4A and 4B). Meanwhile, the relative testicular weights (ratios of testicular weights to total body weights) did not differ significantly between the Pb-treated groups of rats and the controls (Fig. 5).

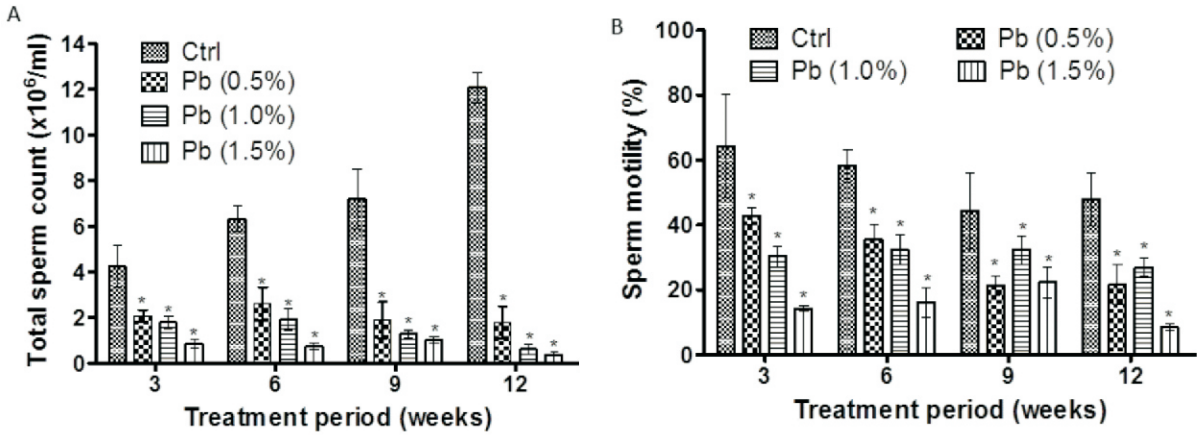


**Figure1:** Caudal epididymal sperm count (A) and sperm motility (B). At each dose and duration of oral Pb acetate treatment, sperm count and sperm motility decreased significantly compared with the controls. **P*<0.05 vs. controls.

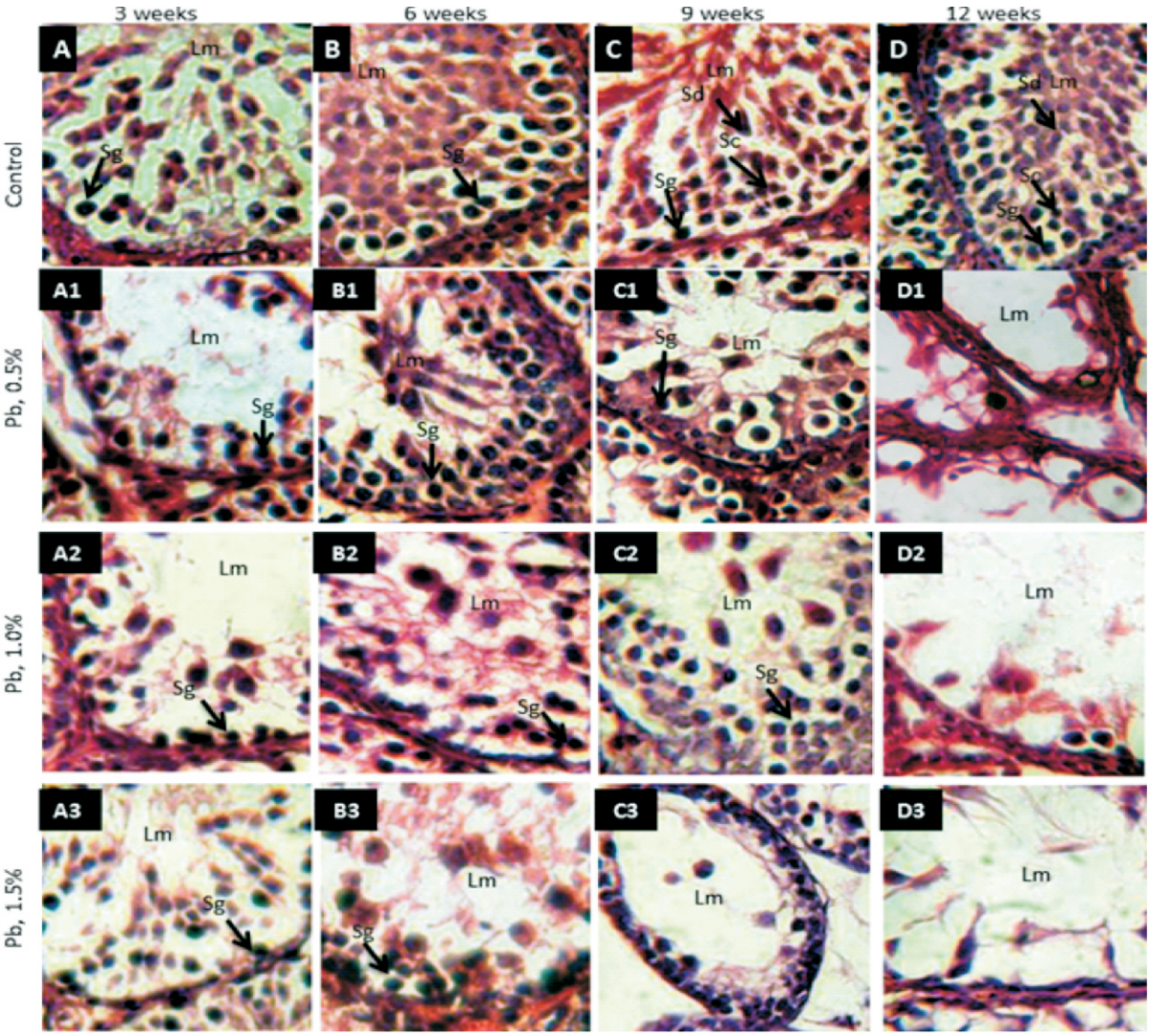


**Figure 2:** Representative light micrographs of sections of the seminiferous tubules of control rats and rats treated with increasing doses of Pb acetate for variable periods. The severity of testicular damage, indicated by loss of germ cells, depends on the doses and duration of Pb acetate administered. Testicular lesions are most pronounced in rats treated with lead acetate for 12 weeks (D1, D2, D3). Sg: spermatogonia; Sc: spermatocyte; Sd: spermatid; Lm: lumen. Haematoxylin and eosin stain, x400.

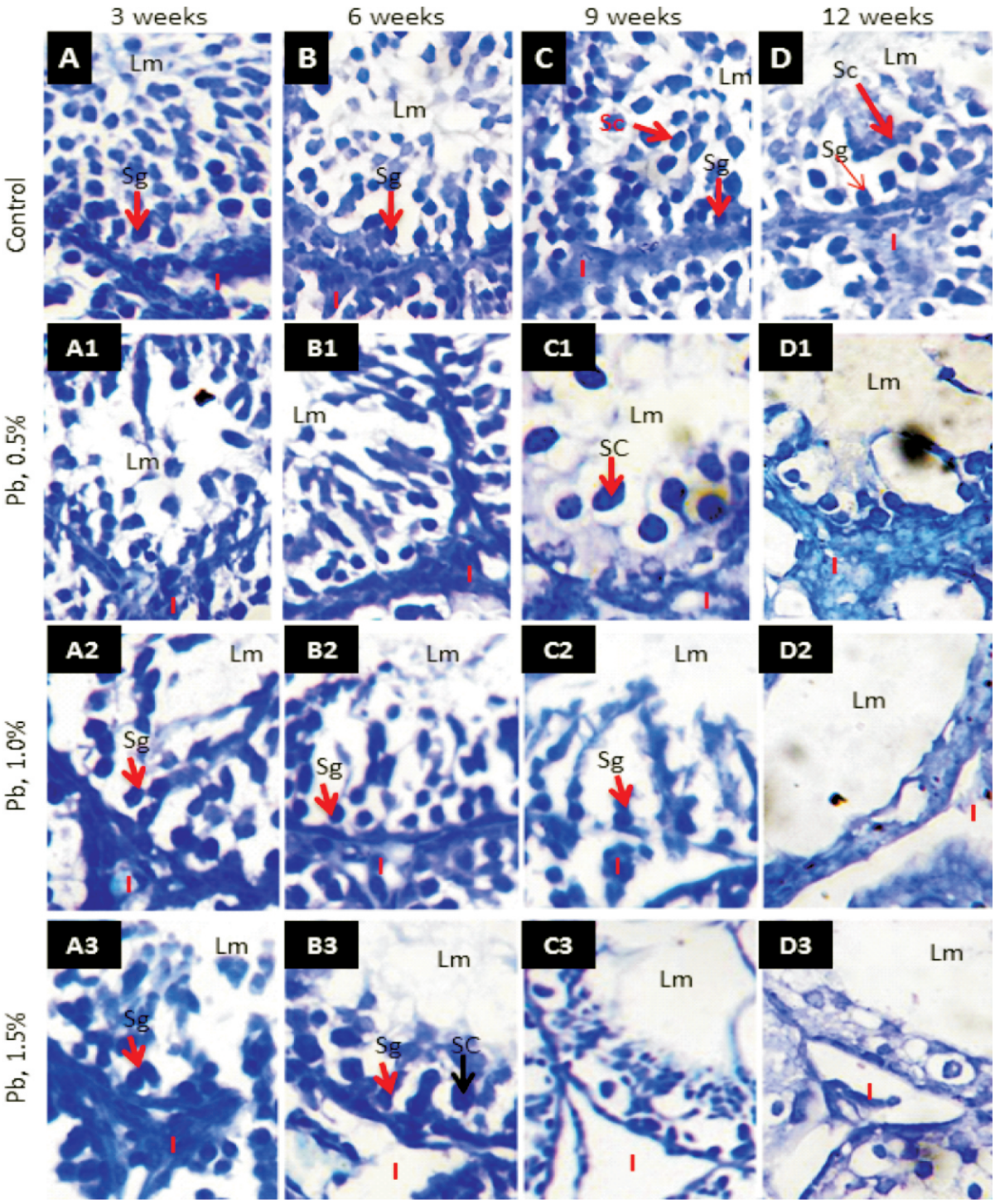


**Figure 3:** Representative light micrographs of sections of the testes of control rats and rats treated with Pb acetate, showing the interstitial tissue (I) and adjoining part of the seminiferous tubules. Dose- and duration-dependent loss of testicular interstitium and the associated cells of Leydig were induced by oral Pb acetate, especially in rats exposed for 12 weeks (D1, D2, D3). I: interstitium; Lm: lumen; Sg: spermatogonium; Sc: spermatocyte; SC: Sertoli cell. Toluidine blue stain; x400.

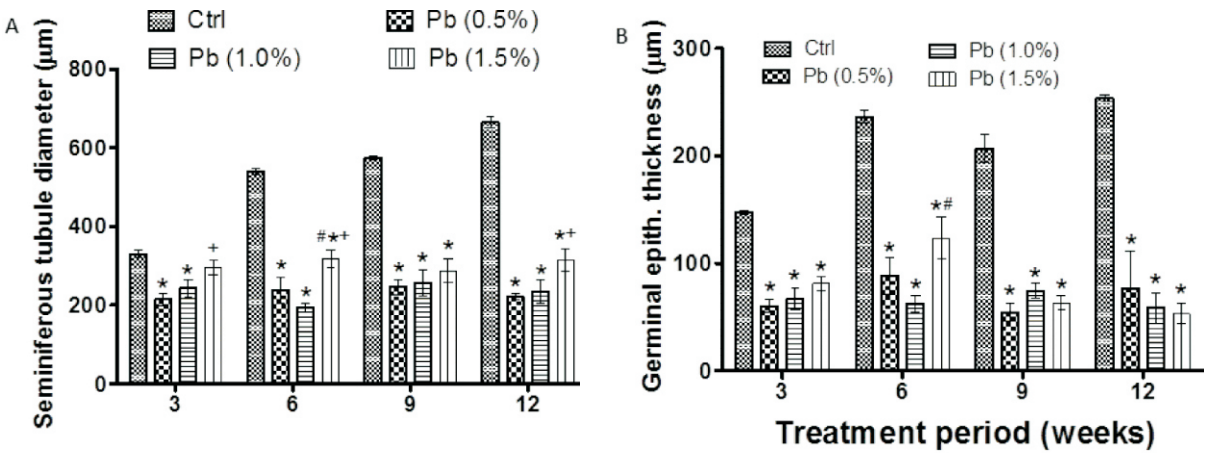


**Figure4:** Seminiferous tubule diameter (A) and germinal epithelium thickness (B) in Pb-intoxicated rats. Both parameters decreased significantly with increasing doses and duration of Pb acetate compared with the untreated controls. **P*<0.05 vs. controls, $^{+}$*P*<0.05 vs. Pb (0.5%), $^{\#}$*P*<0.05 vs. Pb (1.0%).

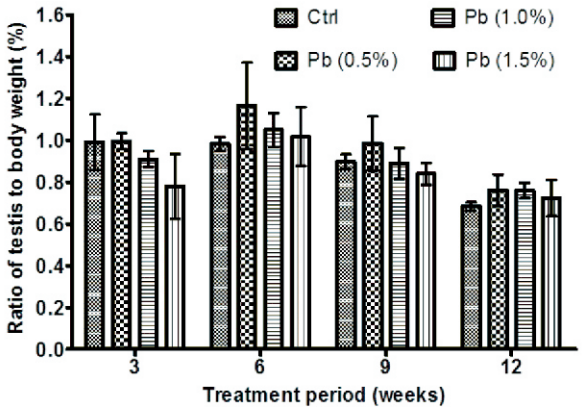


**Figure5:** Ratios of testicular to body weights in rats treated with oral Pb acetate at increasing doses and durations. The weights of the testicles relative to the total body weights did not differ significantly between the Pb-treated and control rats.

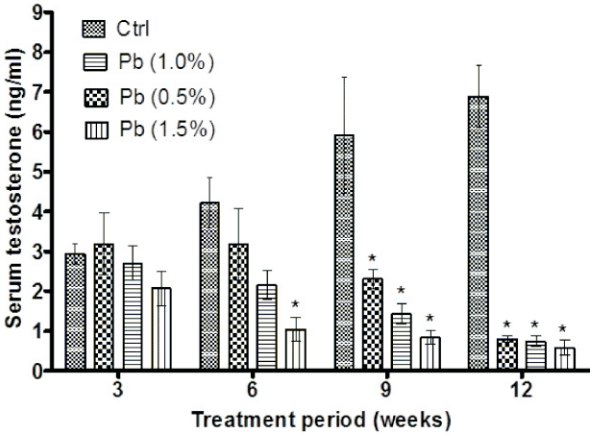


**Figure 6:** Serum testosterone in rats treated with orals doses of Pb acetate for variable durations. Serum testosterone concentrations are inversely proportional to the dose and duration of Pb acetate. **P*<0.05 vs. controls.

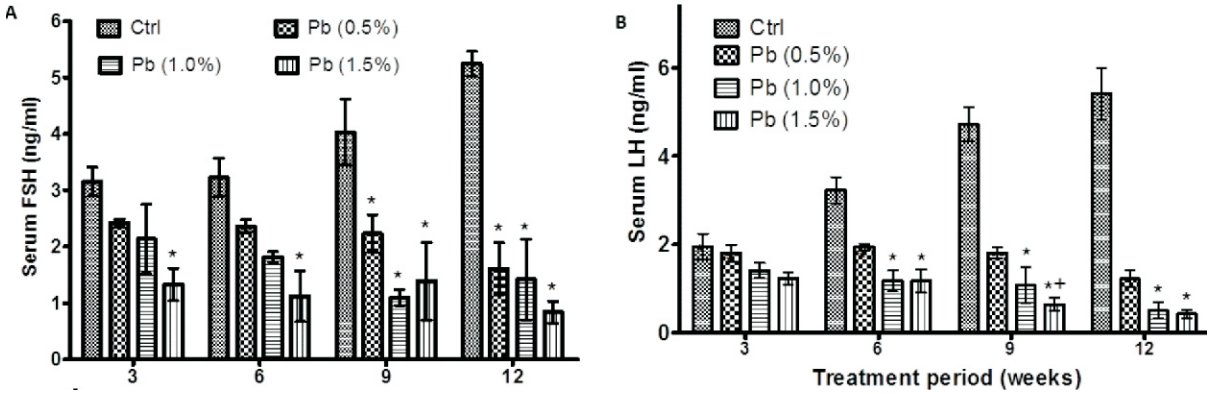


**Figure 7:** Analysis of serum FSH (A) and serum LH (B) in rats treated with oral Pb acetate at increasing doses and durations. The lowest serum levels of gonadotropins were recorded for rats receiving highest doses of Pb acetate for the longest durations. **P*<0.05 vs. controls, $^{+}$*P*<0.05 vs. Pb (0.5%).

**Caudal Epididymal Sperm Counts and Sperm Motility Decreased Following exposure to High Concentrations of Lead Acetate in Rats' Drinking Water**

In Wistar rats, high concentrations of oral Pb produced significant decreases ($P<0.05$) in caudal epididymal sperm density, depending on the dose and duration of Pb exposure (Fig. 1A). The lowest sperm count occurred at postnatal week 12 in rats receiving 1.5% Lead in their drinking water. Moreover, irrespective of the doses and duration of oral Pb treatment in Wistar rats, caudal epididymal sperm motility decreased significantly ($P<0.05$) compared with the controls (Fig. 1B).

**Oral Pb Intoxication Reduced Serum Gonadotropin and Testosterone Levels in Rats**

Significant decreases in serum gonadotropins (FSH and LH) and testosterone occurred only at weeks 9 and 12 of postnatal life in the Pb-exposed rats, irrespective of the doses of Pb administered (Fig. 6 and 7). At postnatal weeks 3 and 6, serum gonadotropin and testosterone levels did not differ significantly from the controls in most of the Pb-treated rats.

**DISCUSSION**

The effects of the dose and duration of oral treatment with Lead acetate on some male reproductive parameters are reported in the present study. Juvenile (recently-weaned) male Wistar rats were exposed daily to high oral doses of lead acetate and the effects of such exposure were studied at the early juvenile (age: 3 weeks), late juvenile (age: 6 weeks), peripubescent (age: 9 weeks) and early post-pubescent (age: 12 weeks) stages of postnatal development.

Our findings showed that the hormonal and morphological effects of exposure to lead are influenced by the doses and duration of exposure in the testes of Wistar rats. Exposure to high concentrations of lead impairs the steroidogenic function of the testicles as suggested by the significantly low serum testosterone (T) levels of in the exposed animals.

Such deleterious endocrine effects are associated with histological evidence of testicular interstitial cell loss . It is interesting to note that significant decreases in serum T were observed at the peripubescent (age: 9 week) and early post-pubescent (age: 12 weeks) stages, a period when histological evidence of interstitial tissue loss was also seen in the testicles . This suggests that long-term lead toxicity would most adversely impact the steroidogenic function of the testis at the period of reproductive maturity (peripubescent and early post-pubescent stages), and have adverse implications on the fertility potential of the organism. Previously, low serum T had been reported in men that worked in the lead smelting factory following variable periods of exposure to lead [13].

Moreover, oral lead disturbs the endocrine function of the hypophysis in our rat model. Specifically, Lead exposure in such rats significantly lowered serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) at the peripubescent (age: 9 weeks) and early post-pubescent (age: 12 weeks) stages, the same periods when serum T levels were significantly reduced . Thus, as previously reported in human male subjects [13], chronic exposure to lead disturbs the hypothalamo-hypophyseal-gonadal axis, with adverse effects on testicular steroidogenic function.

Furthermore, given the physiological importance of normal serum and testicular T levels to the progression of spermatogenesis in the seminiferous tubules, our findings of significantly low caudal epididymal sperm density (seen only at the early post-pubescent stage) might not be unconnected with low serum T. However, it is known that oral exposure to Pb could induce apoptotic death of germ cells in rodent [14]; lead could bind directly to DNA, thereby causing damage to germ cell nuclear material [15]. Moreover, low sperm count induced by chronic oral exposure to lead in the present study could be related to the ability of lead to worsen testicular oxidative stress, as previously reported in Wistar rats [16].

Meanwhile, in addition to reducing sperm density significantly at week 12 of age, oral lead severely impairs sperm motility at ages 6, 9 and 12 weeks, irrespective of the dose of lead administered. Thus, lead toxicity has more deleterious age-dependent effects on sperm motility than sperm density. This could be directly related to the ability of lead to impair calcium homeostasis as it is able to replace calcium ion in biological samples with ease [3], thereby altering such calcium-dependent cellular events as motility. Moreover, it has been reported that lead could perturb energy metabolism essential for cellular motile function [17].

In addition, our study also reports the effects of the doses (0.5%, 1%, and 1.5% of lead acetate in rats' drinking water) and duration of lead exposure on the histomorphometry of the seminiferous tubules and the cyto-architecture of these tubules. Among the stages of postnatal development studied in the Wistar rats (early juvenile, late juvenile, peripubescent and early post-pubescent), testicular germ cell loss was most evident at the early post-pubescent age (12 weeks old rats), in all the doses of lead administered. Moreover, the severity of testicular lesion was dose-dependent. Thus, testicular lesions induced by oral lead are influenced not only by the dose but also the duration of exposure, with the highest dose and longest duration producing the most severe testicular damage.

Morphometric analysis of the seminiferous tubules (in terms of their diameters and the heights of the germinal epithelium) showed attenuation of these parameters

irrespective of the dose and duration of oral lead treatment. Thus, at each major developmental milestone (i.e., early juvenile, late juvenile, peripubescent and early post-pubescent period), testicular damage (characterised by reduced seminiferous tubule diameter and diminished germinal epithelium) can be induced by oral lead exposure; and this is associated with significantly reduced caudal epididymal sperm density that is most evident in the early post-pubescent stage. These agree with recent findings in our laboratory [4], and thus suggest that the deleterious effects of lead on the testis are most pronounced at that developmental stage when an organism is most reproductively active (early post-pubescent period).

A combination of factors could explain the attenuation of seminiferous tubule diameter and reduced germinal epithelial height in our model, including lead-induced increased apoptotic germ cell death. This is because lead can bind to nuclear DNA to form Pb-DNA complex by electrostatic forces, thereby inducing damage to the DNA helical structure, followed by death of the cell [15]. In addition, lead could also induce germ cell death with the seminiferous tubule by exacerbating testicular oxidative stress [16,18].

Our findings in the present study showed that the deleterious effects of oral lead treatment on testicular micro-anatomy and steroidogenic function is dose-dependent and age-specific. Lead produces its most severe effects on the testis at the early post-pubescent stage of development by attenuating seminiferous tubule diameter, germinal epithelium height and caudal epididymal sperm density in rats. Moreover, sperm motility is impaired not only at the early post-pubescent age but also at the peripubescent (age: 9 weeks) and late juvenile (age: 6 weeks) periods in rats. The reduced titres obtained for serum T and gonadotropins suggest derangement of the hypothalamo-hypophyseal-gonadal axis, especially at the peripubescent and early post-pubescent periods. These hormonal perturbations would contribute significantly to the peculiar (deranged) testicular microanatomy seen in rats administered relatively high doses of lead. Such morphological changes are most severe at the peripubescent and early post-pubescent periods of postnatal development when active reproductive activity is just commencing, with grave consequences for male fertility.

## CONCLUSION

Findings in this study indicate that the adverse effects of juvenile oral lead intoxication on male reproductive hormones, testicular microanatomy and sperm parameters are age- and dose-specific; and are most pronounced in the peripubescent and early post-pubescent postnatal periods, when active reproductive activity is just commencing, with critical consequences for male fertility.

## REFERENCES


1. Medecins Sans Frontieres. *Lead poinsoning crisis in Zamfara state Northern Nigeria.* Nigeria2012.
2. Eduardo B, Ricardo HB. Ecotoxicity and tesicular damage (Environmental chemical pollution). A review. *Int J Morphol.* 2008;26(4):833-840.
3. Shirran SL, Barran PE. The use of ESI-MS to probe the binding of divalent cations to clamodulin. *J Am Soc Mass Spectrom.* 2009;20(6):1159-1171.
4. Akinola OB, Oyewopo AO, Biliaminu A, Aremu SA, Afolayan ST, Sanni GO. Testicular histomorphometry and semen quality of adult Wistar rats following juvenile oral lead intoxication. *European Journal of Anatomy.* 2015;19(1):65-72.
5. Priya PH, Reddy PS. Effect of restraint stress on lead-induced male reproductive toxicity in rats. *J Exp Zool A Ecol Genet Physiol.* 2012;317(7):455-465.
6. Cernochova D, Kamarad V. Toxic effect of lead of mice testicles after its administration with drinking water. *Acta Univ Palacki Olomuc Fac Med.* 1992;133:9-13.
7. Ng TP, Goh HH, Ng YL, et al. Male endocrine functions in workers with moderate exposure to lead. *Br J Ind Med.* 1991;48(7):485-491.
8. Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the Care and Use of Laboratory Animals.* Washington, DC2011.
9. Nathan E, Huang HF, Pogach L, Giglio W, Bogden JD, Seebode J. Lead acetate does not impair secretion of Sertoli cell function marker proteins in the adult Sprague Dawley rat. *Arch Environ Health.* 1992;47(5):370-375.
10. Oyewopo AO, Togun VA. Effect of temperature on motility and concentration of male Sprague-Dawley rat epididymal spermatozoa. *Sci Focus.* 2005;10:35-40.
11. Gower DB. Analysis of androgens and their derivatives. In: Makin HLJ, Gower DB, eds. *Steroid Analysis*. New York: Springer; 2010:457-558.
12. Bancroft JD, Stevens A. *Theory and Practice of Histological Techniques*. Second ed. New York: Churchill Livingstone; 1982.
13. Braunstein GD, Dahlgren J, Loriaux DL. Hypogonadism in chronically lead-poisoned men. *Infertility.* 1978;1(1):33-51.
14. Wang C, Zhang Y, Liang J, Shan G, Wang Y, Shi Q. Impacts of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in testis. *Clin Chim Acta.* 2006;370(1-2):82-88.
15. Zhang H, Zhang M, Liu R, Chen Y. Assessing the mechanism of DNA damage induced by lead through direct and indirect interactions. *J Photochem Photobiol B.* 2014;136:46-53.
16. Kumar BA, Reddy AG, Kumar PR, Reddy YR, Rao TM, Haritha C. Protective role of N-Acetyl L-Cysteine against reproductive toxicity due to interaction of lead and cadmium in male Wistar rats. *J Nat Sci Biol Med.* 2013;4(2):414-419.
17. Naha N, Manna B. Mechanism of lead induced effects on human spermatozoa after occupational exposure. *Kathmandu Univ Med J.* 2007;5(1):85-94.
18. Marchlewicz M, Wiszniewska B, Gonet B, et al. Increased lipid peroxidation and ascorbic Acid utilization in testis and epididymis of rats chronically exposed to lead. *Biometals.* 2007;20(1):13-19.