ORIGINAL ARTICLE

Histopathological Degeneration of Spermatogenesis and Histomorphometric Alterations of the Testicular Microanatomy of Male Wistar Rats after Oral Lead Intoxication

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Abstract:

Background: Lead is a heavy metal known to exert pathological effects on the male reproductive organs. *Aim and Objectives:* To study the degeneration of spermatogenesis and histomorphometric alterations of the testicular microanatomy of male Wistar rats after oral lead intoxication. *Material and Methods:* Eighty male Wistar rats were divided into four groups (1- 4) and further subdivided into 4 subgroups (A, B, C, and D) each containing five (n=5) rats depending on dosage and duration (3, 6, 9 and 12 weeks) of treatment. Group 1 received 1 ml of distilled water daily. Group 2 received 0.5% lead daily, Group 3 received 1.0% of lead daily and Group 4 received 1.5% of lead daily respectively. Testicular weights, histomorphometry and histological analysis using Toluidine Blue were observed. *Results:* It showed that significant reductions ($P<0.05$) in testicular weights in the treatment groups administered with lead for periods of 3-9 weeks as compared to the control while a significant increase ($P<0.05$) was observed in the group administered for 12 weeks. Histomorphometric analysis showed significant reductions ($P<0.05$) in Seminiferous tubule diameter, length of germinal epithelium and cross sectional in the treatment groups administered for periods of 3-12 weeks while significant increase ($P<0.05$) in lumen diameter was observed in the treatment groups compared to the control. Histopathological analysis revealed that Lead influenced the testicular cytoarchitecture by disrupting the processes of spermatogenesis in the treatment groups as compared to control. *Conclusion:* The study showed that different dosages of lead over a period of time had gonadotoxic effects and tendencies to alter testicular profiles by disrupting spermatogenesis.

Keywords: Lead**,** Testies**,** Gonadotoxic, Histomorphometry, Spermatogenesis

Introduction:

Lead is a ubiquitous industrial and environmental pollutant that has been detected in every facet of bioenvironmental system [1]. An extreme toxic substance like lead affects health of individuals. Both children and adults suffer from lead poisoning effects, but it's more prevalent during childhood [2]. Lead toxicity induced a significant increase in apoptotic cell death in the seminiferous tubules of young growing rats [3]. Its disruptive nature has been implicated in spermatogenesis, lowered enzyme activities in the testis and testicular histo-architecture [4]. Reports by Gulvik [5] stated that it caused spermiation delay during spermatogenesis and warranted the release of immature testicular tubular spermatogenic cells.

The deleterious effects of lead on the reproductive system are due to its multifactorial ways of exerting its effects on many pathways. For example, it has the ability to mimic calcium and

substitute for it in cation channels such as the catSper channel [6]. The sudden appearance of round spermatids coincides with the activities of catSper gene expression at three weeks of age in the testis of growing mice [7]. In men, the disruption of the processes of spermatogenesis at any stage of cell differentiation can damage sperm DNA, increase abnormal sperm count, decrease the total sperm count and impair the stability of sperm chromatin [8].

Numerous reports have shown an association between impaired sperm motility and lead concentrations in seminal fluid [9]. In addition, lead accumulation could cause hormonal imbalance by directly affecting the neuroendocrine system, disrupting the secretion of androgens from Inhibin B from Sertoli cells or Leydig cells [10]. It is important to note that lead could pass through blood brain barrier and placental barrier. In bones its half life is about 35 years and this toxicity remains an urgent public health concern [11] due to its environmental pervasiveness and the awareness about its side effects [12].

Therefore, it was on this basis that we decided to study the fertility indices by investigating the histopathological degeneration of spermatogenesis and histomorphometric alterations of the testicular microanatomy of male Wistar rats after oral lead intoxication.

Ethical approval:

This experiment was carried out in conformity with the rules and guidelines of the Animal Ethics Committee of the Babcock University Ilishan, Ogun State. The research was approved to be in compliance with the Institutional Animal Care and Use committee (IACUC).

Animal Grouping and Treatments:

Eighty weaned male Wistar rats were divided into four groups (1, 2, 3 & 4) and further subdivided into 4 subgroups each containing five (n=5) rats depending on the dosage and duration of treatment. Group 1 (1A, 1B, 1C, 1D) served as control. This group received 1 ml of distilled water daily. Group 2 (2A, 2B, 2C, 2D) received 0.5% lead acetate for 3, 6, 9, and 12 weeks, Group 3 (3A, 3B, 3C, 3D) received 1.0% lead acetate for 3, 6, 9, and 12 weeks, Group 4 (4A, 4B, 4C, 4D) received 1.5% lead acetate for 3, 6, 9, and 12 weeks respectively. Treatment doses for lead adopted in this study were administered using an orogastric cannula and the experiment was based on evidence related to previously reported studies of its reproductive toxicity on Wistar rats. All animas were fasted overnight and body weight measured weekly.

Preparation of Treatment Solution:

Five hundred gram of lead acetate manufactured by BDH Chemicals Ltd England was purchased from Yomi- Esthony Company, Ilorin. Qualitative confirmation was done by diluting lead (Pb) with nitric acid to form solution of the lead acetate. The solution of lead acetate was reacted with excess concentrated ammonia (NH_3), dilute acetic acid and potassium chromate (K_2CrO_3). This formed a yellow precipitate as a confirmatory solution. For each administration, lead acetate was weighed (in g) and then dissolved in the appropriate ml of water to obtain the required concentration (in g/100 mL).

Sample Collection and Processing:

On completion of treatments, rats for histological analysis were euthanized using 20 mg/kg body weight of ketamine intra-peritoneally. Special stain such as Toluidine Blue was used to outline the morphological changes in the rat testis.

Light Microscopy:

For light microscopic studies, the testis sections on glass slides were captured using Olympus

binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0 MP Amscope Camera (Amscope Inc, Irvine California, USA).

Statistical Analysis:

Data collected were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's (HSD) multiple comparison test with the aid of SPSS (V20; Armonk, NY: IBM Corp USA). Data were presented as Mean ± Standard Error of Mean (SEM). P value ($p<0.05$) was considered statistically significant. All graphs were drawn using the GraphPad Prism v.6 (GraphPad Software Inc., USA).

Results:

Testicular Weights:

According to Fig. 1, for the rats administered lead for a 3 weeks period of time, a significant reduction ($p<0.05$) was observed in the Group D administered with 1.5% lead acetate when compared to the control group and other treatment groups. For the rats administered for a 6 and 9 weeks period of time, significant reductions ($p<0.05$) were observed in Groups C and D that was given 1.0% and 1.5% of lead acetate when compared to the control while significant increase ($p<0.05$) in Groups B, C and D were observed when compared to the control for a period 12 weeks of lead acetate administration.

Testiscular Histomorphometry:

(2A) Seminiferous tubule diameter:

According to Fig. 2A, for the rats administered with lead for a 3 weeks period of time, a significant reduction ($p<0.05$) was observed in Group D administered with 1.5% lead acetate when compared to the control group while significant decrease ($p<0.05$) in Groups B, C and D were observed when compared to the control group for a period 6, 9 and 12 weeks of lead acetate administration.

(2B) Germinal epithelial length:

According to Fig. 2B, for the rats administered with lead for a 3 weeks period of time, a significant reduction ($p<0.05$) was observed in

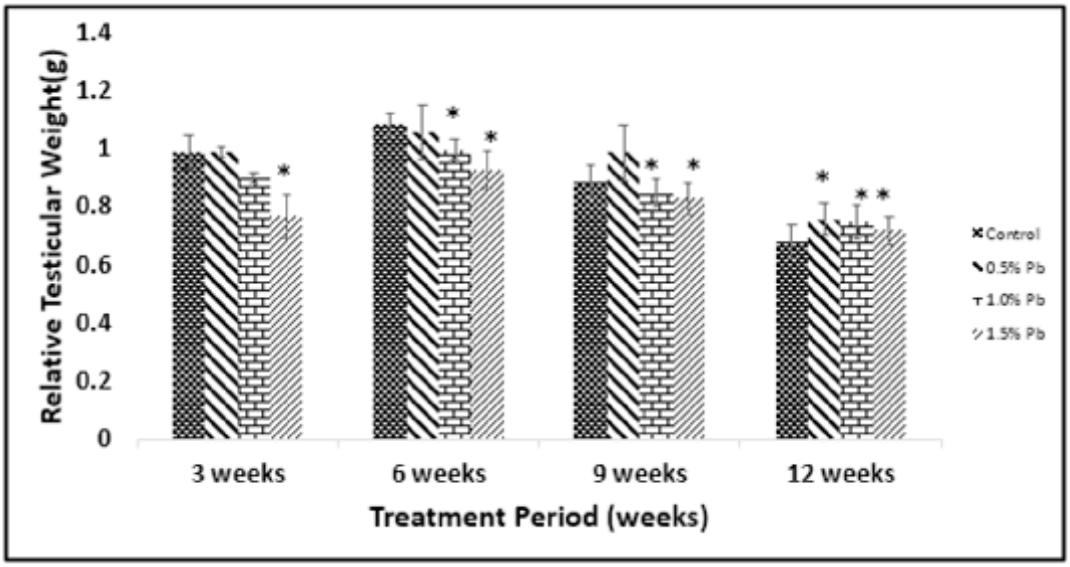

Fig. 1: Showing the Testicular Weights of Animals Treated with Varying Doses and Duration of Lead Acetate *($p<0.05$) - Significantly Different Compared to Control Group.

Group D only that was administered 1.5% lead acetate when compared to the control group. For the rats administered for a 6 and 9 weeks period of time, significant reductions ($p<0.05$) were observed in Groups C and D that was given 1.0% and 1.5% of lead acetate when compared to the control group while significant decrease ($p<0.05$) in Groups B, C and D were observed when compared to the control group for a period 12 weeks of lead acetate administration.

(2C) Lumen Diameter:

According to Fig. 2C, significant increase ($p<0.05$) in lumen diameter was observed in Group D only for the rats administered with lead for a 3 and 9 weeks period of time when compared to the control group while Groups B and C administered 0.5% and 1.0% of lead acetate recorded a non-significant reduction ($p>0.05$) when compared to the control group.

(2D) Tubular Cross Sectional Area:

According to Fig. 2D, for the rats administered with lead for a 3 weeks period of time, a significant reduction ($p<0.05$) was observed in the Group D administered 1.5% lead acetate when compared to the control group. A significant decrease ($p<0.05$) in Groups B-D was observed when compared to the control group for a period 6 weeks of lead acetate administration while a significant decrease ($p<0.05$) in Groups C and D was observed when compared to the control group for a period 9 and 12 weeks of lead acetate administration respectively.

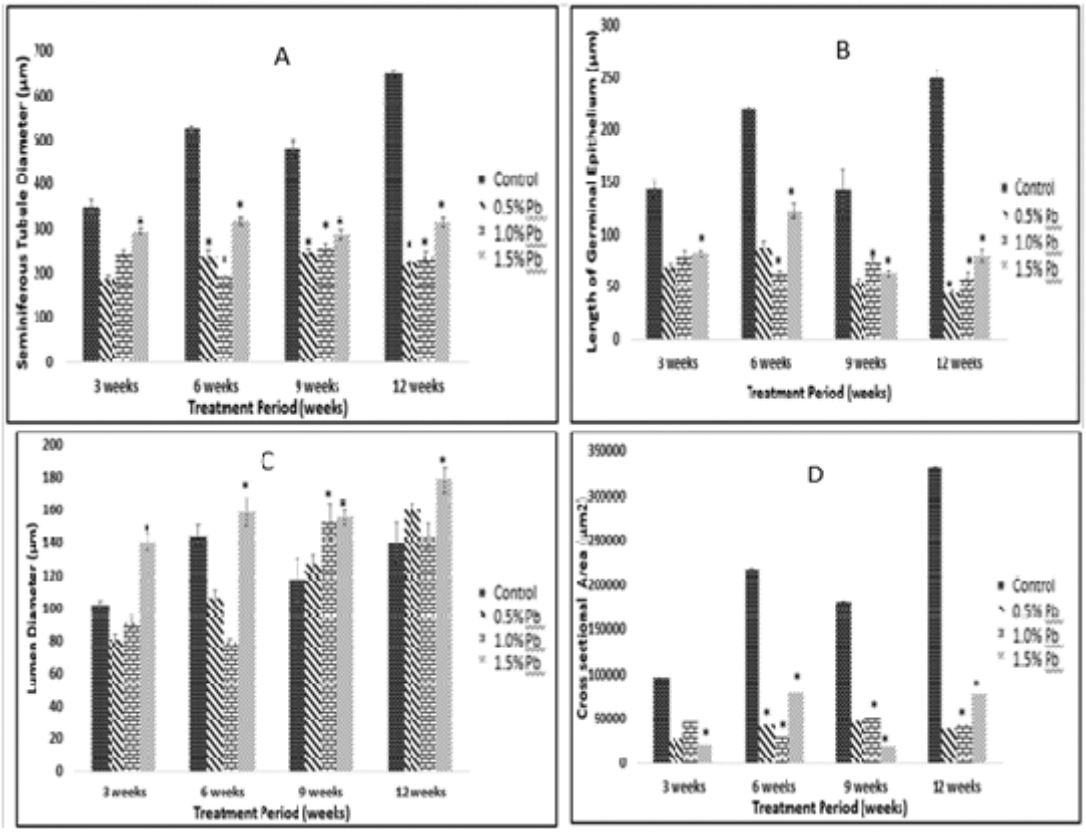

Fig. 2: Showing the Testicular Histomorphometry of Animals Treated with Varying Doses and Duration of Lead Acetate *($p<0.05$) - Significantly Different Compared to Control Group.

Histological Analysis:

Testicular cytoarchitecture of the control groups were in normal arrays. Groups given 0.5% and 1.0% lead showed degenerated seminiferous epithelium, reduced spermatogenic cells, widened lumen, absence of sertoli cells and thickened basement membrane while the group administered with 1.5% lead acetate for 3 and 6 weeks showed degenerated basement membrane and seminiferous tubules, vacuolation, widened lumen, little or no spermatogenic cells and reduced Leydig cells.

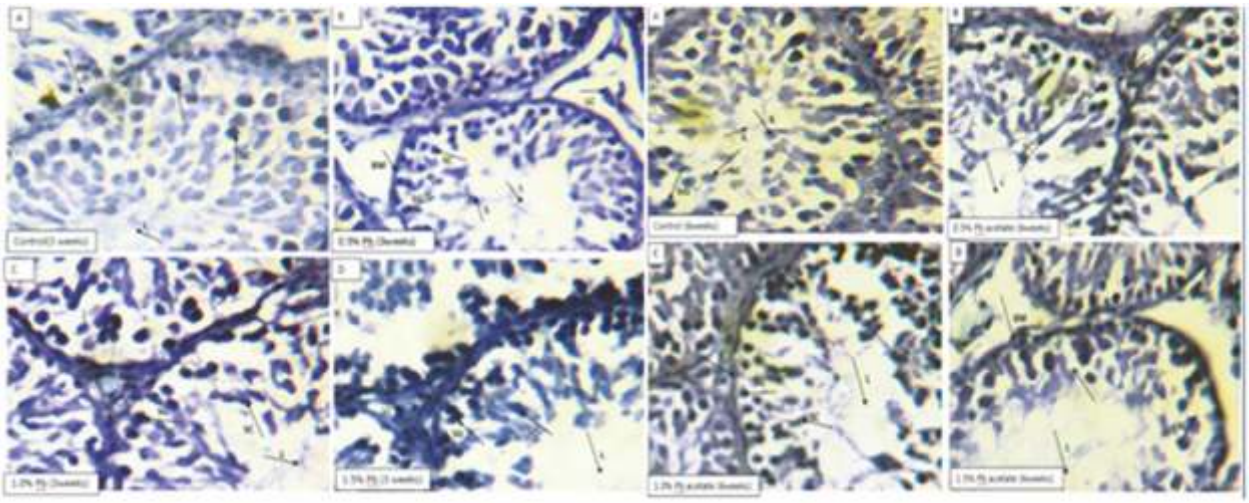

Fig. 3A: (3 and 6 weeks): General Structure of the Testes of Animals Treated with Varying Doses and Duration of Lead Acetate. SG-Spermatogonia I-Interstital space, BM-Basement Membrane, SC-Spermatocytes, L-Lumen. LC-Leydig cells, S-Spermatids. Toluidine Blue Stain. Mag.× 400.

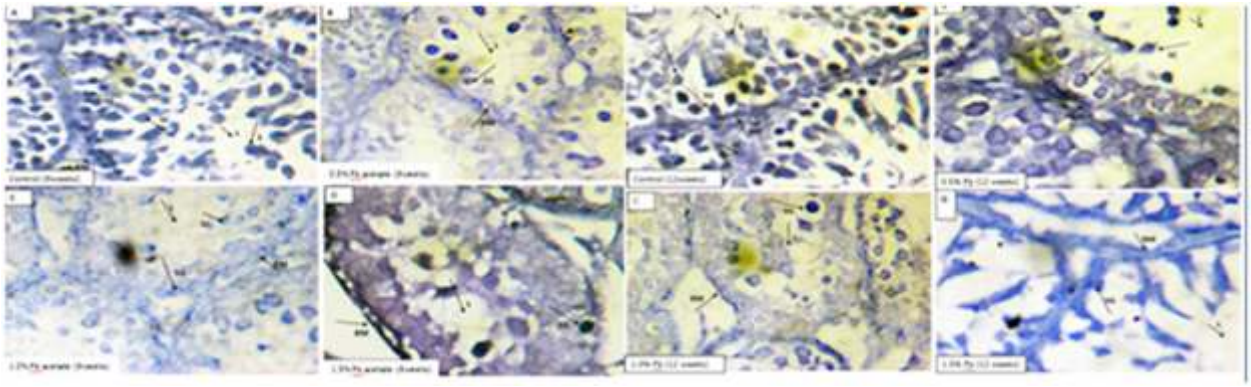

Fig. 3B: (9 & 12 weeks): General Structure of the Testes of Animals Treated with Varying Doses and Duration of Lead Acetate. SG-Spermatogonia I-Interstitial space, BM-Basement Membrane, SC- Spermatocytes, L-Lumen. LC-Leydig cells, S-Spermatids. Toluidine Blue stain. Mag.× 400.

Testicular cytoarchitecture of the control groups were in normal arrays. Groups given 0.5% and 1.0% lead showed a severe degenerated elongated seminiferous epithelium, very few spermatogenic cells, widened lumen, absence of Sertoli cells and degenerated basement membrane while the group administered with 1.5% lead acetate for 9 and 12 weeks showed degenerated basement membrane, seminiferous epithelium and tubule, severe vacuolation, presence of apoptotic cells, widened lumen, absence of spermatogenic cells and Leydig cells.

Discussion:

Lead acetate reduces testicular weight:

The decrease in the relative testicular weight over a shorter treatment period after (3 and 6 weeks) showed the devastating effects of lead consumption (Fig. 1). This can be corroborated by findings of Acharya *et al.* [13] who reported that testes weights significantly declined in lead-treated mice compared to respective control groups. Ahmad *et al.* [14] also reported the significant decrease in testicular weight of adult albino male rats in lead-treated group while the significant increase observed at 9 and 12 weeks of treatment is suggestive of the accumulation of lead in the testicular soft tissues thereby leading to atrophy. However, our results were in line with Graca *et al.* [15] who reported that lead causes increase in mean testis weight in adult rats.

Lead disrupts testicular histomorphometry:

The marked significant decrease ($p<0.05$) in seminiferous tubule diameter observed in the treatment groups for a period 6, 9 and 12 weeks of lead acetate administration showed the deleterious effects of lead acetate on a dose dependent manner. It further highlighted that lead degenerated the seminiferous tubules by shrinking its size (Fig. 2A). This was corroborated by reports by [16] and [17] who reported that lead acetate caused severe decrease in the diameter and epithelial thickness of rat seminiferous tubules.

Lead acetate induced histomorphometric changes in the form of reduced length of germinal epithelium and testicular cross sectional area; these reductions which occurred on a dose dependent manner had negative impacts on germinal cells of the testis as reported in this study. These histomorphometric changes were apparent in the histopathology due to the sole reason that spermatogenic cells were either reduced or non-existent for groups that received lead over a period of 9 and 12 weeks. The increase in lumen diameter corroborated with results observed in the histology for groups that received lead over a period 9 and 12 weeks. It means that the stages of spermatogenesis were highly truncated leading to reduced surface area for spermatogenic cells to properly proliferate.

Histopathological changes after lead acetate administration:

Spermatogenesis is a complex biological process of cellular transformation that produces male haploid germ cells from diploid spermatogonial stem cells. This process has been simplified morphologically as stages and phases of spermatogenesis, which progress through precisely timed and highly organized cycles. This cycle is essential for continuous sperm production, which is dependent upon numerous factors, both intrinsic (sertoli and germ cells) and extrinsic (androgens) [18]. However, the degenerative changes observed (degenerated basement membrane, seminiferous epithelium and tubules, severe vacuolation,

presence of apoptotic cells, widened lumen, absence of spermatogenic cells and Leydig cells) in this study appeared to be obvious at 9 and 12 weeks period of administration. These changes may be largely due to the capacity of lead to mimic calcium channel and substitute it with many of the fundamental cellular processes that are calcium dependent. The cell membrane is permeated by lead through voltage-dependent or other types of calcium channels. Lead exposure leads to the disruption of cation transport and cellular energetics through catSper channels in the testicular tissue and these may be responsible for altering the germinal epithelium of the testis and finally processes of spermatogenesis (spermatogonia to spermatocytes) [19].

Further examination revealed thickness of the basement membrane which is most marked in groups treated for periods of 9 and 12 weeks could be secondary to tubular shrinkage in degenerated seminiferous tubules. However, these might have an impact on the processes of differentiation and growth of germ cells in lead treated groups. Following the established mechanism of oxidative stress, reports showed that lead induced apoptosis of the germinal cells which was reported by Gorbel *et al.* [20] as a possible mechanism for loss of germinal epithelium. These apoptotic changes (Fig. 3B) observed in this study were apparent in groups treated with 1.5% of lead acetate over a period of 12 weeks, thereby bringing to the fore the deleterious effects it has on sexual reproduction and finally male fertility. These results agree with the findings of Giuliani *et al.* [21] and Makhlouf *et al.* [22] who proved that lead exposure induced testicular injury represented by apoptotic changes in most of the germ cells.

Findings from the histopathological study corroborated reports by Bokara *et al.* [1] who observed significant reduction in type A spermatogonia after lead toxicity, therefore, associating it with the decrease of germ cell populations. In another study, complete arrest of spermatogonia was seen in lead treated rats [23]. These findings were in agreement with [24] who reported that lead exposure caused progressive vascular, tubular and interstitial testicular damage. The results of this present study highlighted the association between lead intoxication and reduced male fertility on a dose and duration dependent levels.

Conclusion:

In conclusion, the links between the duration and dosage of lead acetate as a precursor to various degrees of gonadotoxicity and anti-spermatogenicity have been explained lucidly. Therefore, lead acetate administration have the tendencies to alter testicular profiles thereby disrupting spermatogenesis in rats and reducing male sexual function considering the observed effects it had on testicular weight, histological and histomorphometric presentations of the testis. However appropriate personal protection for workers should be encouraged and used.

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