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Original Research Article

Effect of Oral Administration of Lead Acetate Exposure on the Histology of the Testis and Testicular Sperm Concentration in Wistar Albino Rats

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Abstract: Lead is a heavy metal found in all environmental components such as earth crust, air and water.. The present study evaluates the effect of lead acetate on the histology of the testis and testicular sperm concentration in Wistar Albino rats. Twenty four matured male albino rats were weighed and divided into four groups I, II, III and IV, each comprising of six animals. Group I served as control while groups II, III and IV received 50mg/kg, 100mg/kg and 150mg/kg body weight lead acetate solution orally for 21 days respectively. At the end of the experiment, animals were sacrificed tissues processed for testicular histology. Administration of 150mg/kg and 200mg/kg of lead acetate solution caused decreased in testicular sperm concentration that was significant at $p < 0.05$ with an increase in coiling and head detachment that was dose dependent. Histological observation showed thickening and detachment of the basement membrane, degeneration of the germ cells, and degeneration of the cells of the spermatogenic series including sertoli cells. There were also interstitial oedema, congestion of blood vessel, karyolysis and degeneration of Leydig cells. Therefore this study concludes that lead acetate adversely affect the testis by producing various degrees of histopathological changes as dosage increases.
Keywords: lead acetate, exposure, testis.

INTRODUCTION

Man is exposed to various types of environmental contaminants at different stages of his lifespan, majority of them harmful. One of the oldest harmful agents known to mankind is lead. It came into use very early in the history of civilization and its poisonous effects were soon discovered [1]. Classically two distinct categories of lead poisoning exist in modern era: the occupational and the pediatrics. The former has generally been associated with workers employed in specific types of manufacturing processes while the later has been recognized primarily as a problem of large urban centre. Lead is a heavy, soft metal which occur in the form of ores, with a specific gravity of 11:34 and atomic weight of 207.21. It is bluish in colour and tarnishes to dull gray [2].

In adult normal daily intake of lead is 0.3 mg but daily intake of 3.5 mg taken for a few months' results in toxicity [3]. A blood lead value of 80 mg or above is considered as an unequivocal instance of lead poisoning [4]. The maximum tolerable level is 0.05mg/L water and the World Health Organization (WHO) health guideline for lead in potable or drinking water is 0.01mg/L (WHO, 1993). In addition to being a by-product of mixing activities natural sources of atmospheric lead include soil erosion by wind, volcanic dust, forest fire, sea salt and decay of radon gas [5] [6].

The non-biodegradable nature of lead is the prime reason for its prolong persistence in the environment. Human environmental exposure is often through leaded gasoline, industrial process such as lead melting and coal combustion, battery recycling, grids and bearings, pencils, some body creams, lead-containing paint, food stored in lead can liners, food stored in ceramic jars, or contaminated water through pipes cast in lead or soldered using lead and plants grown in lead-rich soil [7]. Inhalation of lead particulates is a primary route of occupational lead exposure. Lead is absorbed rapidly through the lungs when inhaled. Up to 70% of the inhaled lead is absorbed depending on the particle size. On the other hand, oral ingestion is a primary form of exposure in the

general population [7]. In the ambient air lead can be in the form of vapors, very fine particles and organic halogens such as lead bromide, lead acetate and lead chloride. Divalent lead compounds derived from these environmental sources accumulate either rapidly or gradually and compete with proteins that bind to zinc at molecular level [8]. For instance more than 99% of blood lead content is stored in erythrocytes bound to zinc- dependent delta-aminolerulinic acid dehydrogenase (ALAD). ALAD is an essential enzyme in heme synthesis that is potentially inhibited by lead [9].

MATERIALS AND METHOD

This research was conducted in the Department of Human Anatomy University of Maiduguri, to observe effect of lead acetate on the histology of the testis and testicular sperm concentration in Wistar Albino rats.

Materials

Analytical grades of lead acetate (BDH Chemical Limited), distilled water, animal cages, drinkers, animal feed, syringe, intubation tubes, electronic weighing balance, triple beam balance, Bouins fluid, specimen bottles, EDTA sterile test tubes, beakers, pipette, glass slides, cover-slips, heamocytometer and reagent, centrifuge, microscope, absolute alcohol, xylene, paraffin wax, haematoxylin and eosine stains, mountant, glycerol and microtome.

Animals

Mature male albino rats weighing between 150 to 220 grams were obtained from the laboratory animal holdings, Department of Animal Science, University of Jos, Nigeria. The study was conducted on 24 albino rats. The animals were maintained on pellet diet and water ad libitum. The rats were housed 6 per cage and allowed to acclimatize to existing climatic condition in the animal house for the period of 14 days before the commencement of administration of lead acetate solution. Animals were kept in well ventilated cages and housing with the average temperature of 27 ± 20C. The lighting consists of natural day light: darkness rhythm.

Experimental Design

After acclimatization 24 rats were divided into four groups I, II, III and IV. Each group comprising of six rats each, which was grouped according to their body weight.

Group I was the control group. The rats were given normal feed and water ad libitum for the period of the experiment.

Group II was the low dose group. The rats were administered lead acetate solution by oro gastric intubation at a dose of 50mg/kg once daily for the period of 21days in addition to normal feed and water ad libitum.

Group III was the medium dose. The animals were administered lead acetate solution by oro gastric intubation at a dose 100mg/kg once daily for 21 days in addition to normal feed and water ad libitum.

Group IV was the high dose. The rats were administered lead acetate solution by orogastric intubation at a dose of 150mg/kg once daily for 21 days in addition to normal feed and water ad libitum.

Preparation of Solution

A stock solution of lead acetate salt was prepared by dissolving 1gm of lead acetate in 20ml of deionized water. From the stock solution low dose group was administered 50mg/kg per body weight while medium and high dose received 100mg/kg and 150mg/kg per body weight, respectively.

Organ Body Ratio

The testes were removed from the scrotum along with the epididymis and the testes weigh using the digital balance. Weights of left and right testes were recorded. Organ body ratio was obtained by dividing total weight of testes by body weight of the rats under investigation. Weights of rats were obtained using the triple arm balance.

Estimation of Sperm Count

Sperm counts were estimated by the use of haemocytometer. Testes that were removed were crushed in normal saline solution. The crushed mixture was pipetted and used to feed the haemocytometer. The number of sperm head were counted and recorded per each quadrant and multiplied by a standard factor to obtain the total sperm volume.

Tissue Processing for Paraffin Section

To determine the effect of lead on the histology of the testis, the organs were fixed in bouins fluid. They were then cut into smaller sizes. The tissues were dehydrated in graded series of ethanol (30, 50, 75, 95 and 100%) in ascending order, cleared in xylene, and infiltrated in molten paraffin. The specimens were embedded in fresh wax and allowed to solidify and form block. Paraffin sections between 3-5mm thick were cut using a rotary microtome. The sections were placed on a glass slide and stained with haematoxylin and eosin mounted on the glass slide. The sections were dried overnight at oven temperature between 35 to 40C

[10]. Photomicrographs of the tissue sections were taken using photomicroscope.

Statistical Analysis

Data obtained from this research work were subjected to one way analysis of variance (ANOVA), using statistical software package SPSS version 20. The results obtained were expressed as mean ± standard deviation. Differences between means were based on a significance level of $p<0.05$ [11].

RESULTS

Table-1: Effect of Lead Acetate on Body Weight of Mature Male Rats

Dose mg/kg N=6	Weight of Rats on Day 1 (g)	Weight of Rats on Day 21 (g)	Percentage Weight Gain/Loss
0.00	146.10 ± 9.12	172.40 ± 17.60*	+18.02%
50.00	160.50 ± 11.80	164.20 ± 15.90	+2.32%
100.00	184.40 ± 8.20	179.50 ± 17.90	-2.66%
150.00	201.00 ± 9.20	196.6 ± 9.00	-2.63%

Values are Mean ± S.D. *= $p<0.05$. (Comparison relative to control) N=number of rats per group

Effect of Lead Acetate Exposure on Body Weight

The changes in the mean body weight of animals treated with lead acetate, and their control are presented in Table-1. The mean body weight of rats administered 50mg/kg of lead acetate, increased by 2.32% compared to the mean starting weight. In the group administered 100mg/kg the weight reduced by 2.66% compared to the starting weight. In the group administered with 150mg/kg the body weight decreased by 2.63%. The increase in weight was significant ($p<0.05$) in the control group.

Table-2: Effect of Lead Acetate on Organ to Body Weight Ratio of Mature Male Rats

Dose mg/kg N=6	Mean Weight of Left Testes (g)	Mean Weight of Right Testes (g)	Weight of Rats on Day 21 (g)	Average Organ to Body Weight Ratio
0.00	1.19 ± 0.17	0.95 ± 0.47	172.40 ± 17.60	0.01
50.00	1.07 ± 0.20	1.03 ± 0.25	164.20 ± 15.90	0.01
100.00	1.12 ± 0.17	1.04 ± 0.19	179.50 ± 17.90	0.01
150.00	1.24 ± 0.26	1.24 ± 0.26	196.60 ± 9.00	0.01

Values are Mean ± S.D. *= $p<0.05$. (Comparison relative to control) N= number of rats per group.

Effect of Lead Acetate Exposure on Organ to Body Weight Ratio

Changes in the mean organ to body ratio of the testis of animals treated with lead acetate and their control are presented in Table-2. The changes show that as dosage increases organ to body ratio decreases. For the control the mean was 0.013, for 50mg/kg treated group it was 0.012, for 100mg/kg it was 0.012 and for 150mg/kg it was 0.0118.

Table-3: Effect of Lead Acetate on Testicular Sperm Concentration

Dose mg/kg N=6	Mean weight of paired testes Day 21 (g)	Testicular Sperm Conc. million/mil
0.00	2.31 ± 0.33	$4.14 \times 10^6 \pm 47749$
50.00	2.10 ± 0.44	$4.08 \times 10^6 \pm 47749$
100.00	2.16 ±0.35	$3.26 \times 10^6 \pm 56569$*
150.00	2.32 ± 0.41	$3.07 \times 10^6 \pm 135351$*

Values are Mean ± S.D. *= $p<0.05$. (Comparison relative to control). N= number of rats per group.

Effect of Lead Acetate Exposure on Testicular Sperm Concentration

The present study shows that as dosage increases sperm count decreases (Table-3). The sperm count was 4.14×10^6 for the control group animals, 4.08×10^6 for the rats treated with 50mg/kg, 3.26×10^6 for the rats administered 100mg/kg and 3.07×10^6 for those administered 150mg/kg. The difference in sperm count of 3.26×10^6 and 3.07×10^6 were statistically significant between the rats administered 100mg/kg and 150mg/kg lead acetate solution respectively, compared to the control, but was not significant in rats

administered 50mg/kg. The sperm cells in the control group were normal with their head, tail and acrosome normal. While in the animals treated with lead acetate solution the sperm cells showed detached tails from the head, coiled tails and abnormal and absence of acrosome as dosage increases.

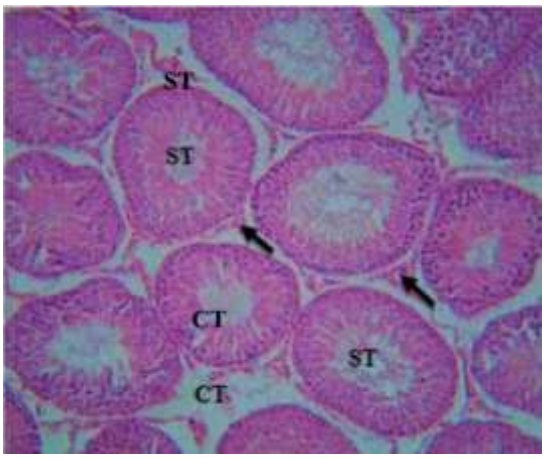

Fig. 1 photomicrograph of control rat testis showing normal seminiferous tubules (ST), blood vessels (arrow) and interstitial connective tissue (CT) H&E x100.

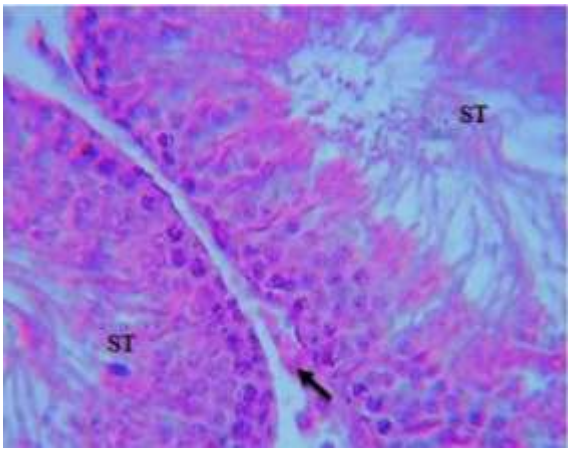

Fig. 2: Photomicrograph of control rat testis showing normalseminiferous tubules with turf of spermatids in the lumen (ST) and interstitial connective tissue (arrow) H&E x400.

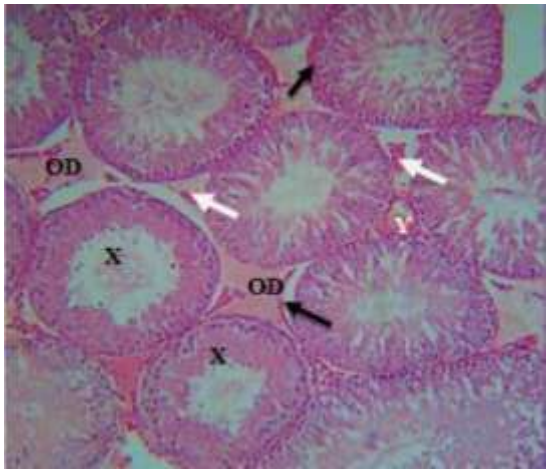

Fig-3: Photomicrograph of rat testis treated with 50mg/kg showing interstitial oedema (OD), focal degeneration of the germ cells of the seminiferous tubules (X) detachment and thickening of basement membrane (arrows), congestion of blood vessels (Y) and scanty Leydig cells (white arrows) H&E x100.

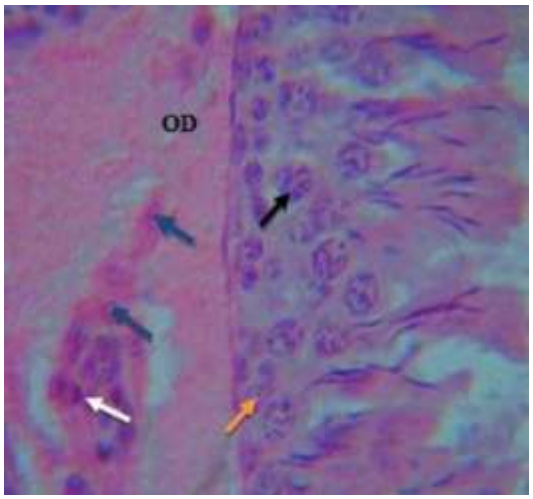

Fig-4: Photomicrograph of rat testis treated with of 50mg/kg of lead acetate showing interstitial oedema (OD) Leydig cells undergoing karyorrhexis(blue arrows), clumping of Leydig cells (white arrows), primary spermatocytes undergoing karyolysis (black arrows) and spared sartoli cells (orange arrow)H&E x400.

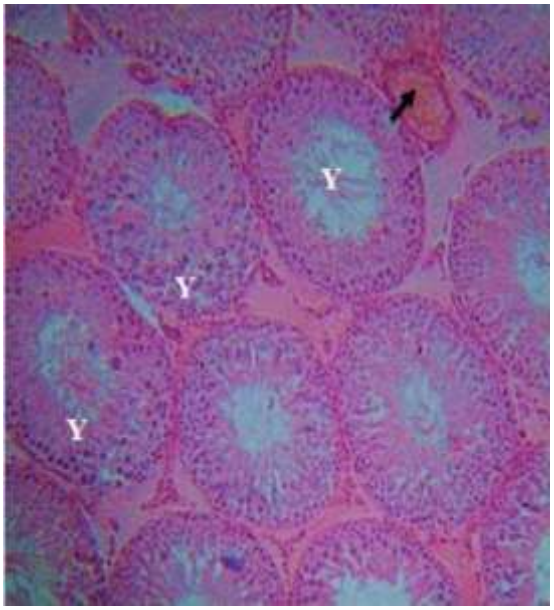

Fig-5: Photomicrograph of rat testis treated with 100mg/kg lead acetate showing focal deterioration of germ cells (Y) and congestion of blood vessel (arrows) H&E x100

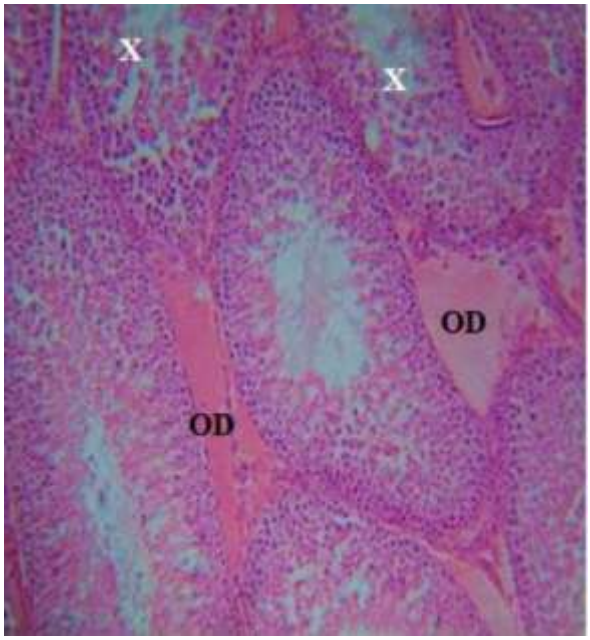

Fig-6: Photomicrograph of rat testis treated with 100mg/kg lead acetate showing focal interstitial oedema (OD) focal deterioration of the germ cells of the seminiferous tubules (X) H&E x200

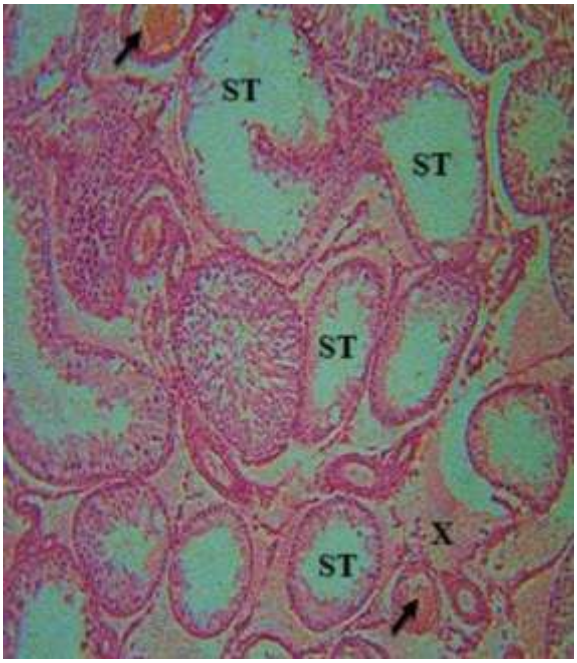

Fig-7: Photomicrograph of rat testis treated with 150mg/kg lead acetate showing focal seminiferous tubular eruption (ST), empty spaces between germ cells in most of the seminiferous tubules (ST) interstitial oedema (X) and congestion of blood vessels(arrows) H&E x100.

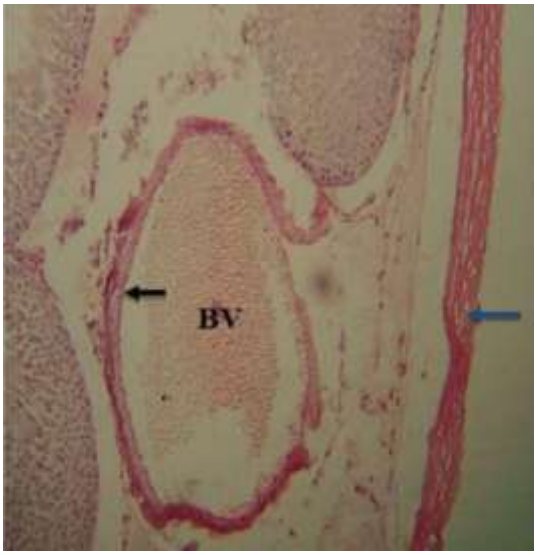

Fig-8: Photomicrograph of rat testis treated with 150mg/kg lead acetate showing focal enlarged congested blood vessel (BV),deterioration of the endothelial lining of the blood vessel (arrow) and thick albuginea (blue arrow) H&E x200.

Histological Evaluation of the Testis

Light microscopy examination of semi-thin sections from the rat testis of control group revealed normal seminiferous tubules lined by stratified epithelium and showed the following spermatogenic series of cells: spermatogonia, spermatocytes, spermatids and tails of spermatozoa in the lumen of seminiferous tubules (Figures 1, and 2). In the interstitial connective tissue space the Leydig cells were also seen clumping around the blood vessels (Figures 1, and 2), with the effect being dose-dependent. The rats administered with 50mg/kg of lead acetate caused oedema, congestion of blood vessels, degeneration and deterioration of germ cells of the seminiferous tubules, thickening and detachment of the basement membrane, degeneration of cells of the spermatogenic series, primary spermatocytes undergoing karyorrhexis and Leydig cells undergoing karyolysis (Figures 3 and 4). The rats administered 100mg/kg lead acetate caused focal interstitial oedema, blood vessel congestion, degeneration and deterioration of germ cells of the seminiferous tubules, thickening of basement membrane, degeneration of spermatogonia, Sertoli cells, spermatozoa and Leydig cells (Figures 5 and 6).The rats given 150mg/kg lead acetate caused focal seminiferous tubular degeneration, empty spaces in the seminiferous tubules, sever degeneration of the interstitial cells of Leydig, interstitial oedema, focal enlarge congested blood vessel and degeneration of the endothelial lining of blood vessel, thicken albuinea and thickening of the basement membrane (Figure 7 and 8).

DISCUSSION

Oral administration of lead acetate at doses of 50mg/kg and 100mg/kg to the rats was observed to have caused an increase of 2.66% and 2.63% in the mean body weight of the rats respectively while administration of 150mg/kg caused a decrease in body weight of 2.63% though both decrease in body weight were not statistically significant (Table 4.1). Ahmad *et al.;* in 2003 reported that oral administration of 0.25ml/100g body weight of lead acetate once daily for 30days caused a considerable degree of weight loss this report was not in agreement with this present study [1]. Sidhu and Nehru (2004) also reported that oral administration of 50ml/kg of lead acetate for a period of 8 weeks caused a significant decrease in the body weight of the treated rats [12]. Similarly, El-Sayed (2004) reported significant loss of weight in rats after 6 months of oral lead dosing in tap water containing 125mg/100ml of lead acetate [13], while Kamruzzaman in 2006 and Haque *et al*.; in 2006 observed similar findings in rats at exposure concentrations of 400mg/kg bwt and 200mg/kg bwt, respectively [14, 15]. Khan *et al;* in 2008, however, recorded a high decrease of up to 36.4% in the mean weight of mice after 42 days treatment with 100mg/kg bwt of lead [16]. In the present study where different concentrations of lead acetate were administered orally to mature Wistar albino rats for 21days, the effects on organ to body weight ratio were calculated (Table 4.2). The average organ to body weight ratio, is a better way to assess the damage to the testes in relation to the body. When 50mg/kg lead acetate solution was administered the average organ to body weight ratio was 0.01, when administered 100mg/kg body weight of lead acetate the mean organ to body ratio was 0.01 and when administered 150mg/kg it was 0.01. The pvalue of the treated group was $p>0.05$ which was statistically not significant. Ahmad *et al.;* in 2003 observed that oral administration of 0.25ml/100g body weight for 28days resulted in significant ($p<0.05$) decrease in organ to body ratio of the testes in albino rats [1]. This result was not in agreement with this present study. In this study where different concentrations of lead acetate solution were administered orally to adult albino rats for 21days, it shows that as dosage increases sperm count decreases. The difference in sperm count of 3.26×10^6 and 3.07×10^6 were statistically significant between the rats administered 100mg/kg and 150mg/kg lead acetate solution respectively, compared to the control. Similar observations were reported by [17, 18].

In the present study, histopathological changes were observed in the photomicrographs of lead acetate treated animals. In the rats treated with 50mg/kg, in the interstitial connective tissue, there was interstitial oedema, congestion of blood vessels, scanty Leydig cells undergoing karyolysis, degeneration of germ cells of the seminiferous tubules, thickening and detachment of the basement membrane, and primary spermatocytes undergoing karyorrhexis. Similar observations were made by Lancranjan *et al.;* in 1975, Thomas and Brogan, 1983, Ahmad *et al.,* 2003 and Makhlouf *et al.,* in 2008 [19, 20, 1, 21]. In the rats treated with 100mg/kg there was interstitial focal oedema, blood vessel congestion, degeneration of the Leydig cells. There was focal deterioration of germ cells of the seminiferous tubules, thickening of basement membrane, degeneration of the spermatogonia, spermatozoa and the sertoli cells. In the animals treated with 150mg/kg there were focal seminiferous tubular degeneration, empty seminiferous tubules and spaces between germ cells, interstitial oedema, blood vessel congestion and degeneration of endothelial lining, thicken albuginea, sever degeneration of the interstitial cells of Leydig and focal seminiferous tubular degeneration. In previous work treated with 40mg/kg

the basement membrane of the low dose treated group 40mg/kg basement membrane was normal but in those administered 80mg/kg there was thickening and disruption of the basement membrane. The cause of the thickening was increased amount of collagenous fibers resulting from either over production of collagen fibers by fibroblast or decrease in the rate of collagen phagocytosis [22]. Lead toxicity might cause disruption of collagen binding to phagocytic fibroblast. This tendency towards fibrosis may be one of the possible explanations for the shrinkage of seminiferous tubules and irregularities in the basal lamina [1]. It could be secondary to tubular shrinkage or as a result of contraction of myoid cell. At some places reduced thickness of germinal epithelium and their detachment from the basal lamina was seen along with empty spaces between them [23, 24].From this study the lumina of the seminiferous tubules in at a dose of 75mg/kg treated rats, were empty. Similar findings had been reported by Buaas *et al.;* in 2004 and Sawhnay *et al.,* in 2005 [25, 26]. Batra *et al.;* in 2001, Antnio *et al.;* in 2004, Makhlouf *et al.;* in 2008, also reported the empty lumen and immature germ cells in the lumen [27, 21]. Ahmed *et al.;* in 2012 observed that these changes were marked in high dose treated group his findings was parallel to that described by Makhlauf *et al.;* in 2008 [28, 21]. There was intertubular space increase because of shrinkage of seminiferous tubules along with edema, vacuolation and dilated capillaries [22]. Massanyi *et al.,* in 2007 also noticed dilated blood capillaries in the interstitium, while Al-Attar, (2011) saw interstitial edema [23, 24, 29].

CONCLUSION

This study concludes that lead acetate exposure at doses of 50mg/kg, 100mg/kg and 150mg/kg caused histopathological changes such as interstitial and intra-seminiferous tubular oedema, congestion and degeneration of endothelial lining of blood vessels, seminiferous tubular eruption and degeneration, thickening and detachment of the basement membrane of the seminiferous tubules, thickening of albuginea, degeneration of cells of the spermatogenic series including the interstitial cells Leydig, decrease in sperm count and detachment of the head from the tails of sperm cells and coiling of the tails of the spermatozoa including abnormal acrosome. It also caused decreased in testicular sperm concentration which was dose dependant.

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