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Oral lead acetate elicits multi-axis testicular toxicity with incomplete post-exposure recovery in male wistar rats

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Despite established knowledge of lead's adverse effects on male reproduction, the extent and persistence of testicular injury following oral exposure to lead acetate (PbAc), a common environmental and occupational contaminant, remain incompletely characterized. A systematic investigation is required to understand the simultaneous impact on endocrine function, oxidative balance, inflammatory status, genomic integrity, and apoptotic pathways, and critically, to determine whether such damage is reversible upon exposure cessation. This study investigated testicular toxicity induced by oral lead acetate (PbAc) focusing on injury, inflammation, oxidative stress, DNA damage, apoptosis, and endocrine disruption in male Wistar rats.Thirty rats were randomized (n=10/group): control (distilled water), PbAc (60 mg/kg, 28 days), and recovery (PbAc 60 mg/kg for 28 days followed by 28 days distilled water). Endpoints included follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone; superoxide dismutase (SOD) activity and malondialdehyde (MDA) for oxidative status; 8-hydroxy-2′-deoxyguanosine (8-OHdG) for genotoxicity; tumor necrosis factor-alpha (TNF-α) for inflammation; B-cell lymphoma 2 (Bcl-2) and caspase-3 for apoptosis; plus, testicular and epididymal histology. One-way analysis of variance (ANOVA) with Tukey's post hoc test determined significance (P<0.05). PbAc caused endocrine disruption (reduced FSH, LH, testosterone), oxidative imbalance (decreased SOD, increased MDA), increased 8-OHdG and TNF-α, pro-apoptotic signaling (decreased Bcl-2, increased caspase-3), and histological injury. Key finding: after 28 days without further exposure, several toxic effects persisted (elevated MDA and 8-OHdG; depressed gonadotropins; altered Bcl-2), indicating incomplete spontaneous recovery. Oral PbAc elicits multi-axis testicular toxicity that does not fully resolve after exposure cessation, underscoring the need for preventive and therapeutic strategies.

Keywords: Lead acetate, testicular toxicity, oxidative stress, apoptosis, endocrine disruption, post-exposure recovery

Introduction

Lead, a widely spread environmental contaminant, presents substantial hazards to both human health and wildlife. Lead exposure in humans mostly occurs by ingestion of contaminated food, water, and inhalation of polluted air[1]. Industrial activity, lead-based paints, and the use of lead petrol are significant factors contributing to the presence of lead in the environment[2,3].

The impact of lead toxicity on vulnerable populations, such as children and pregnant women, is a significant issue. Lead exposure in children is linked to cognitive deficits, learning challenges, and behavioural disorders[4,5]. Even minimal levels of lead exposure can result in enduring cognitive effects, causing a decline in academic performance and a higher incidence of attention-deficit/hyperactivity disorder[6]. Pregnant women exposed to lead can pass the toxic metal to their developing fetuses, leading to impaired brain development and adverse birth outcomes[7].

The adverse effects of lead are not limited to humans but also extend to wildlife and the environment. Lead poisoning in birds and mammals, such as eagles and condors, has been well-documented, often resulting from ingestion of lead-containing ammunition fragments or lead-based paint chips [8]. Lead exposure in wildlife not only

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threatens their survival but also raises concerns about the potential impacts on ecosystems.

Lead acetate has attracted interest among the several lead compounds because of its notable solubility and bioavailability. Lead acetate is commonly utilized in research environments to cause lead poisoning in experimental animal models[9]. Oral administration of lead acetate is a common method to investigate the toxic effects of lead exposure, particularly on reproductive and neurological functions. Studying the effects of lead acetate can provide valuable insights into lead's mechanisms of toxicity and help identify potential therapeutic strategies to mitigate its adverse effects[10].

The environment experienced a considerable increase in lead contamination throughout the early 20th century due to the advent of leaded petrol and lead-based paint. Leaded petrol, which was extensively utilized in automobiles for many years, caused the emission of lead particles into the atmosphere, resulting in the pollution of land and water[3]. The ban on leaded gasoline in many countries has substantially reduced environmental lead levels, leading to improvements in public health[11].

The detrimental health impacts of lead are mainly ascribed to its capacity to disrupt enzymatic and cellular mechanisms. Lead causes disturbances in the balance of calcium and iron levels in the body, affects the production of neurotransmitters, and produces reactive oxygen species, resulting in oxidative stress and harm to different cellular components[12]. Additionally, lead can cross the blood-brain barrier and interfere with brain development, affecting cognitive function and behavior, particularly in children.

Regarding the influence of lead on male reproductive health, it is worth noting that the testes exhibit a heightened susceptibility to harmful substances. Exposure to lead has been linked to decreased levels of testosterone, impaired production of sperm, and an increase in damage to sperm DNA[13,14]. The occurrence of lead-induced testicular damage is believed to be primarily caused by oxidative stress and apoptosis[15].

This study systematically quantified lead-induced testicular toxicity across endocrine (FSH, LH, testosterone), oxidative (SOD, MDA), genotoxic (8-OHdG), inflammatory (TNF-α), and apoptotic (Bcl-2, caspase-3) axes after 28 days of oral PbAc exposure in male Wistar rats. Uniquely, we also evaluated spontaneous post-exposure recovery by extending observation for an additional 28 days without any intervention. Unlike previous studies that primarily characterize lead-induced testicular injury during active exposure, the present study uniquely integrates endocrine, oxidative, genotoxic, inflammatory, and apoptotic endpoints to elucidate the molecular mechanisms underlying incomplete spontaneous recovery following exposure cessation.

Methodology

Animal Experimentation and Grouping

Male Wistar rats (n=30) weighing 200–250 grams were obtained from the animal breeding unit of the Animal House, College of Health Sciences, Obafemi Awolowo University (OAU), Ile-Ife. The rats were acclimatized for one week before the start of the experiment and maintained on normal rat chow (standard laboratory diet; protein: 20–23%, fat: 5–6%, fiber: 3–5%) ad libitum throughout the study period. The commercial feed used was HI-PRO®, manufactured by Flowergate, located along Sagamu Expressway, Kajola Village, Ogun State, Nigeria.

Experimental design

Thirty adult male Wistar rats (200–220 g) were randomized into three groups (n=10/group):

- **Control:** distilled water, 28 days.
- **PbAc:** lead acetate (60 mg/kg/day orally), 28 days.
- **Recovery:** lead acetate (60 mg/kg/day orally) for 28 days, followed by distilled water alone for an additional 28 days to assess spontaneous post-exposure recovery without therapeutic intervention.

Dose selection

The PbAc dose (60 mg/kg/day) was chosen based on previous studies reporting consistent testicular, hepatic, and hematological toxicity at this level in rats, while avoiding high lethality[16,17]. Comparable doses (40–100 mg/kg) are frequently used in reproductive toxicology studies of Pb exposure. This dose therefore represents a toxicologically relevant exposure that reliably models sub-acute lead toxicity in rodents.

Animal Care and Ethics

The experiment was conducted in compliance with ethical norms to safeguard animal well-being and minimise distress. The guidelines set by international and national authorities for the proper care and use of laboratory animals were meticulously adhered to.

Additionally, ethical approval was acquired by the Health Research Ethics Committee at the Institute of Public Health, Obafemi Awolowo University, Ile-Ife, with the assigned HREC number IPH/OAU/12/1881.

Animal sacrifice

Twenty-four hours after the last drug administration, the rats were humanely euthanized using an intraperitoneal injection of ketamine (40 mg/kg) and xylazine (40 mg/kg)[18]. Following euthanasia, the testes and epididymides were carefully dissected, harvested, and processed for further analysis.

Chemicals and reagents

Lead acetate trihydrate ($Pb(CH_3COO)_2 \cdot 3H_2O$, ≥99% purity) was purchased from BDH chemicals Ltd. Poole England. Commercial ELISA kits for FSH, LH, testosterone, TNF-α, caspase-3, and 8-OHdG, Rabbit polyclonal anti-Bcl-2 antibody (IHC grade) with horseradish peroxidase (HRP)-conjugated secondary antibody were obtained Sigma Aldrich. All other reagents were of analytical grade.

Assessment of Inflammation

The levels of tumor necrosis factor-alpha (TNF-α)were measured in testicular tissue homogenates using ELISA kits.

Oxidative stress parameters

Testes were homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4). Supernatants were used to determine superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels as indices of antioxidant defense and lipid peroxidation, respectively, using standard spectrophotometric methods.

Detection of DNA Fragmentation

The presence of 8-hydroxydeoxyguanosine (8OHdG), a marker of DNA fragmentation, was assessed in testicular tissue sections using ELISA kits.

Analysis of Apoptosis

Immunohistochemistrywas employed to examine the expression of B-cell lymphoma-2 (Bcl-2), a key regulator of apoptosis, in the testes while Caspase-3 a proapoptotic protein was assayed using ELISA kit.Paraffin-embedded testicular sections were deparaffinized, rehydrated, and subjected to antigen retrieval, followed by incubation with a rabbit polyclonal anti-Bcl-2 primary antibody and an HRP-conjugated secondary antibody; immunoreactivity was visualized using diaminobenzidine as chromogen and counterstained with hematoxylin, after which staining intensity was quantified for statistical analysis as previously described.

Hormone analysis

At sacrifice, blood was collected via retro-orbital venous puncture under light anesthesia into plain tubes, allowed to clot, and centrifuged at 3000 rpm for 10 min to obtain serum. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were quantified using commercially available rat ELISA kits, following the manufacturer's instructions. Absorbance was measured at 450 nm on a microplate reader.

Evaluation of Testicularand EpidydimalInjury

At the end of the experimental period, all animals were euthanized, the testes and epididymis were collected for histopathological examination. Histopathological assessment of testicular and epididymal sections was performed by a blinded observer using established qualitative criteria, including seminiferous tubular integrity, germinal epithelial thickness, presence and organization of spermatogenic cell layers, luminal sperm content, interstitial cell density, and epididymal epithelial integrity; observations were compared across groups to identify treatment-related alterations. Tissues were fixed in 10% neutral buffered formalin and processed for paraffin embedding. Sections were stained with hematoxylin and eosin (H&E) to examine morphological alterations.

Statistical Analysis

Data were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Results are expressed as mean ± SEM. For each ANOVA, F values represent the ratio of variance between groups to variance within groups, with associated degrees of freedom reported. Differences were considered significant at $P<0.05$.

Ethical approval

The international and national guides for the care and use of laboratory animals were duly followed and ethical clearance was obtained from Health Research Ethics Committee, Institute of Public Health, Obafemi Awolowo University, Ile-Ife with HREC number IPH/OAU/12/1881

Results

Hormonal Assay

The hormonal assay revealed that oral administration of lead acetate significantly disrupted the endocrine profile of male Wistar rats, particularly affecting the hypothalamic-pituitary-gonadal (HPG) axis. There was a marked decrease in serum follicle-stimulating hormone (FSH) levels (Fig. 1a) in both the lead-exposed group (Group II) and the recovery group (Group III) compared with the control (Group I), indicating impaired Sertoli cell function and potential disruption in spermatogenesis (F = 2.172, P = 0.049). Similarly, luteinizing hormone (LH) levels were significantly reduced (Fig. 1b) in Groups II and III relative to the control (F = 2.517, P = 0.027), suggesting compromised Leydig cell stimulation, which is essential for testosterone synthesis. Testosterone concentration was also significantly decreased (Fig. 1c) in Group II compared with Group I (F = 3.632, P = 0.0034), highlighting the direct testicular toxicity of lead acetate on steroidogenic function. Although Group III received a 28-day recovery period post-exposure, the persistent reduction in FSH and LH levels implies incomplete endocrine restoration and possible long-term impairment of reproductive hormonal balance. These findings suggest that lead acetate exerts its reproductive toxicity partly through endocrine disruption, potentially leading to subfertility or infertility in exposed males. Persisting suppression of gonadotropins after exposure cessation demonstrates limited endocrine recovery.

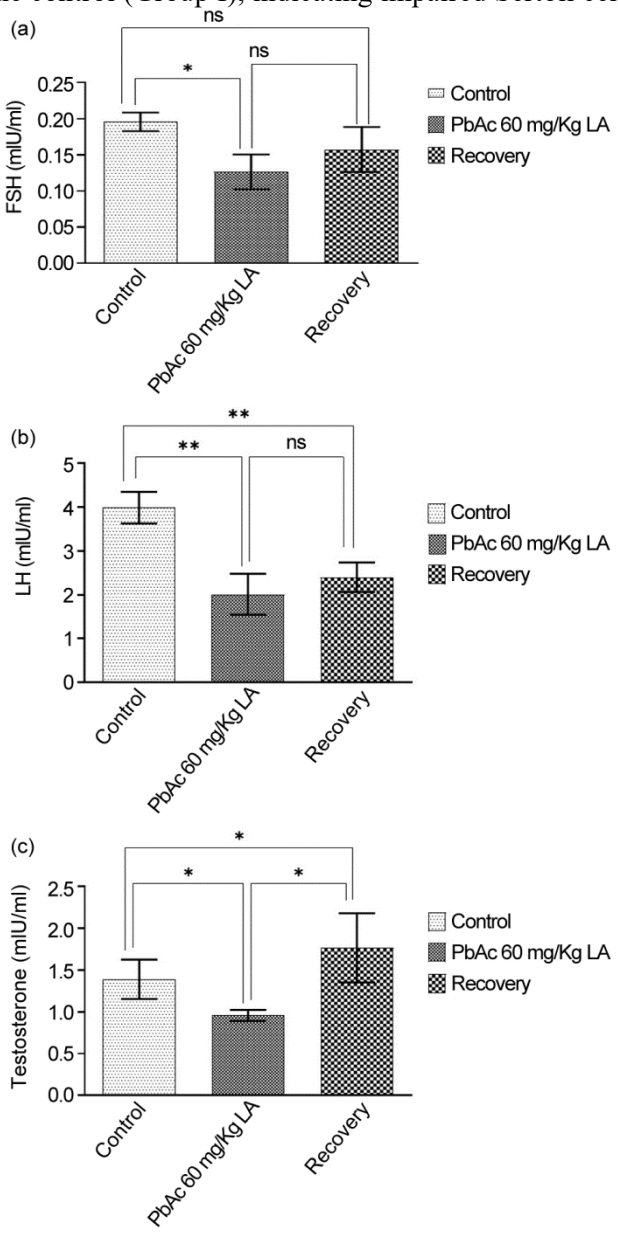

Fig. 1 — Effect PbAc on (a) Follicle Simulating Hormone, (b)Luteinizing Hormone, and (c)Serum Testosterone concentration in Male Wistar rats.

Oxidative stress assay

The oxidative stress assay results demonstrated that lead acetate exposure induced significant oxidative imbalance in the testicular tissue of male Wistar rats. Specifically, there was a significant reduction in the activity of superoxide dismutase (SOD), an essential antioxidant enzyme, in the lead-treated group (Group II) compared to the control (Group I), indicating a compromised antioxidant defense mechanism (F = 4.165, P = 0.0013; Fig. 2a). This decrease in SOD activity suggests an impaired ability to neutralize superoxide radicals, thereby predisposing the testicular tissue to oxidative damage. Furthermore, there was a significant increase in the concentration of malondialdehyde (MDA), a lipid peroxidation marker, in both the lead-treated and recovery groups (Groups II and III) relative to the control group (F = 49.21, P = 0.0001; Fig. 2b). The elevated MDA levels reflect enhanced lipid peroxidation and cellular membrane damage, underscoring the heightened oxidative burden induced by lead acetate. The sustained elevation of MDA in the recovery group suggests that the oxidative insult persisted even after cessation of lead exposure, indicating the potential for prolonged testicular oxidative stress and insufficient endogenous recovery. These findings highlight oxidative stress as a central mechanism in lead-induced testicular toxicity. MDA remained elevated in the recovery group, indicating incomplete resolution.

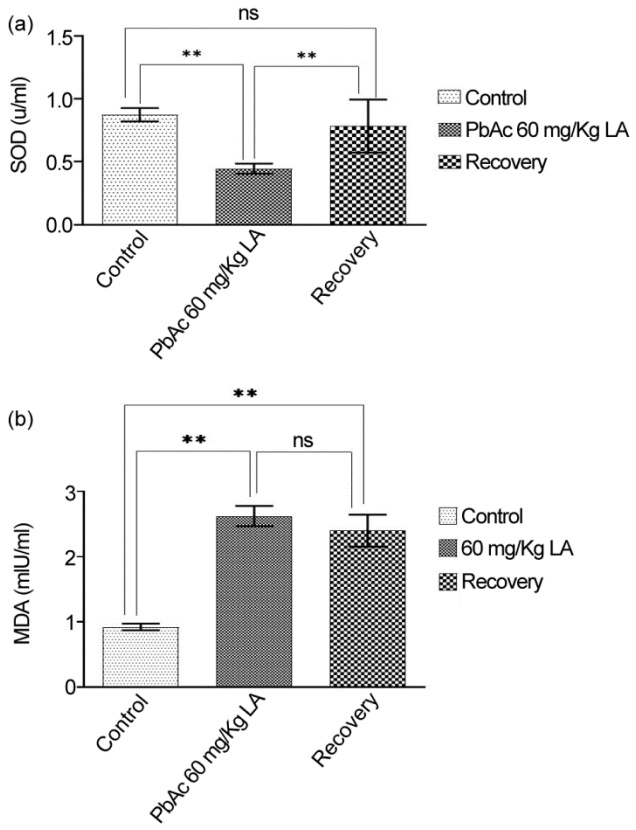

Fig. 2 — Effect PbAc on (a) Super oxide dismutase, and (b) Malondialdehyde in Male Wistar rats.

DNA fragmentation, Inflammation and apoptosis evaluation

The evaluation of DNA fragmentation, inflammation, and apoptosis further revealed the profound testicular toxicity induced by oral lead acetate administration. There was a significant elevation in the testicular concentration of 8-hydroxy-2'-deoxyguanosine (8OHdG), a well-established biomarker of oxidative DNA damage, in both the lead-exposed (Group II) and recovery (Group III) groups compared to the control (Group I), indicating genotoxic insult and impaired genomic integrity (F = 5.934, P = 0.0001; Fig. 3a). Similarly, testicular levels of tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, were significantly increased in Groups II and III relative to the control, suggesting an active inflammatory response triggered by lead-induced cellular stress (F = 4.476, P = 0.0008; Fig. 3b). This inflammatory response may contribute to testicular degeneration and spermatogenic failure. Testicular Bcl-2 expression was quantitatively assessed by image-based analysis of immune-histochemical staining intensity, and statistical analysis revealed a significant reduction in Bcl-2 expression in the PbAc-treated and recovery groups compared with

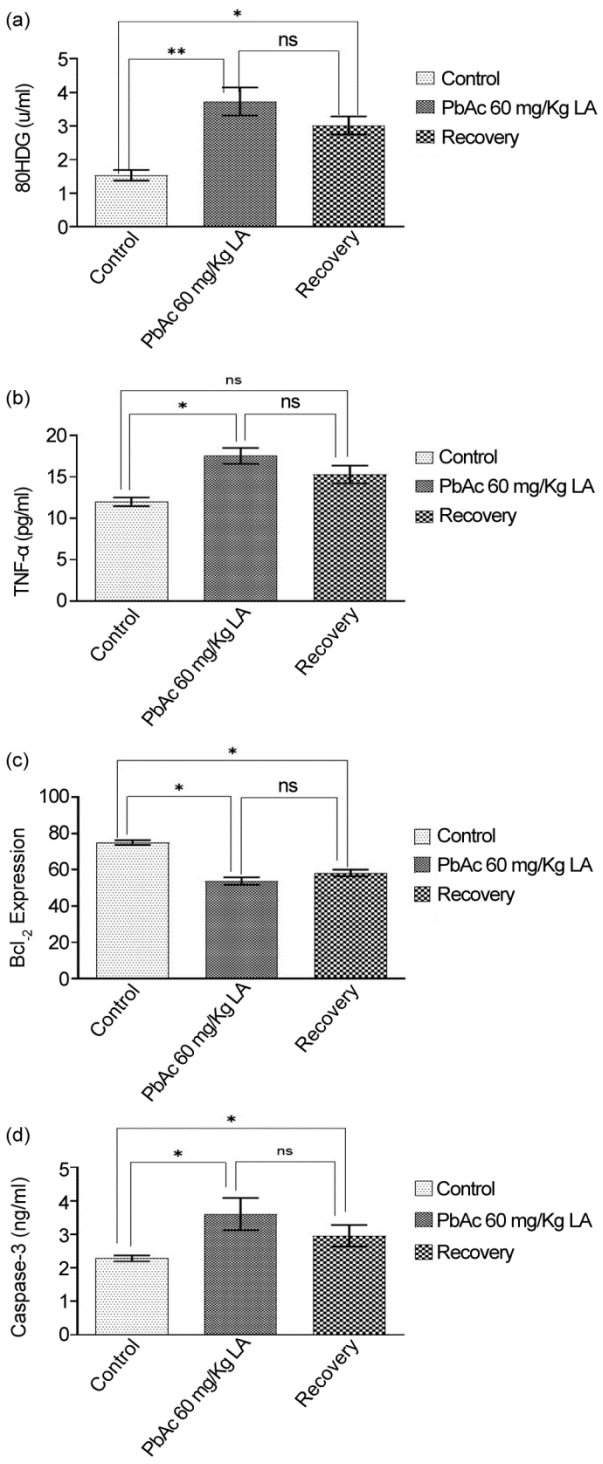

Fig. 3 — Effect PbAc on (a) 80HdG, (b)TNF-α, (c)Bcl-2,and (d) Caspase-3 in Male Wistar rats.

controls (Fig. 3c). B-cell lymphoma 2 (Bcl-2), an anti-apoptotic protein crucial for cellular survival, was significantly downregulated in both Groups II and III compared to the control (F = 31.35, P< 0.0001; Figs. 3c

and 6), reflecting a shift toward pro-apoptotic signaling. In line with this, caspase-3, a key executioner of apoptosis, was markedly elevated in Group II compared to Group I (F = 7.321, P = 0.0001; Fig. 3d), indicating activation of the apoptotic cascade. The persistence of altered 8OHdG, TNF-α, and Bcl-2 levels in the recovery group underscores the lingering effects of lead acetate on testicular cellular health. These findings demonstrate that lead acetate induces testicular injury through intertwined mechanisms involving DNA damage, inflammation, and programmed cell death.Incomplete recovery of Bcl-2 expression suggests lasting pro-apoptotic signaling.

Histopathological study of testis

In-group I, histology of testis revealed healthy seminiferous tubules (ST) with very thick germinal epithelium (GE) and lumen (LU) containing healthy and mature spermatozoa (SP). The interstitium is compact and contains considerable populations of interstitial (IS) cells. Group II showed distorted histoarchitecture. Seminiferous has indistinct boundaries (blue arrow), the concentric arrangements of the germ cells series are disrupted leaving a scattered populations of germ cells without specific arrangements with clogged and undefined lumina. Maturing spermatids are highly limited in number. Group III showed sign of testicular atrophy as evident by widened interstitia (black arrow) but healthy and reduced numbers of seminiferous tubules. The tubules appear normal with the spermatogenic wave intact and mature spermatozoa present (Fig. 4).

Histopathological study of epididymis

In group I epididymis, lobules (LU) show healthy epithelia and high sperm content suprifying prolific sperm production and healthy/progressive spermatogenesis. The interstitium (IS) though is a little less compact. Group II revealed good epithelial linings,

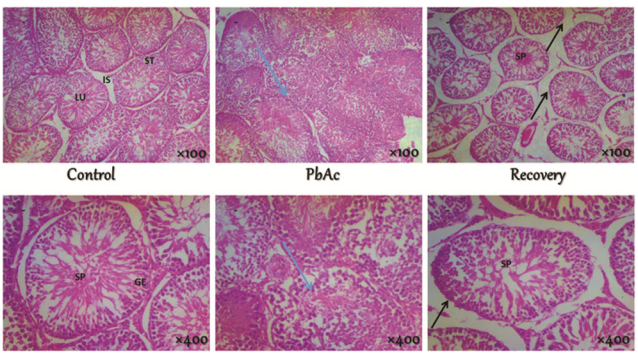

Fig. 4 — Representative photomicrographs of testes of treated rats.

compact interstisium and good histoarchitecture. The lobules are very depleted containing scanty populations of spermatozoa relative to group I, clear evidence of perturbations in spermatogenesis. Group III has well-arranged histoarchitecture, there are no signs of recovery in spermatogenesis as most of the lobules are near empty (Green arrow). The population of spermatozoa within the lobules is worse relative to group II (Fig. 5).

Immunohistochemistry of testicular Bcl-2

There was a downregulation of testicular expression of Bcl-2 in groups II and III compared to group I (Fig. 6).

Discussion

This study provides integrative evidence that oral PbAc exposure disrupts multiple axes of testicular health, including oxidative stress, endocrine balance, apoptosis, DNA integrity, and inflammatory status. Beyond confirming well-established toxic effects of lead, our key contribution is demonstrating that the persistence of elevated MDA and 8-OHdG levels alongside sustained suppression of gonadotropins and

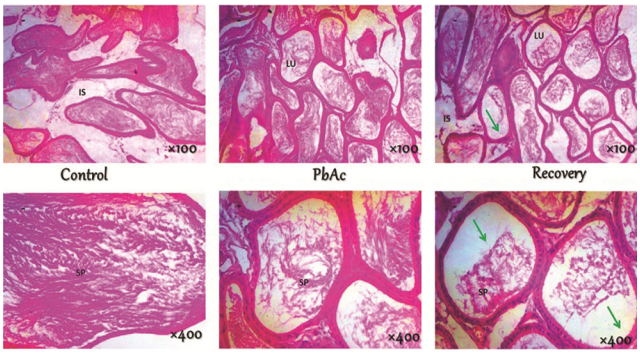

Fig. 5 — Representative photomicrographs of epididymis of treated rats

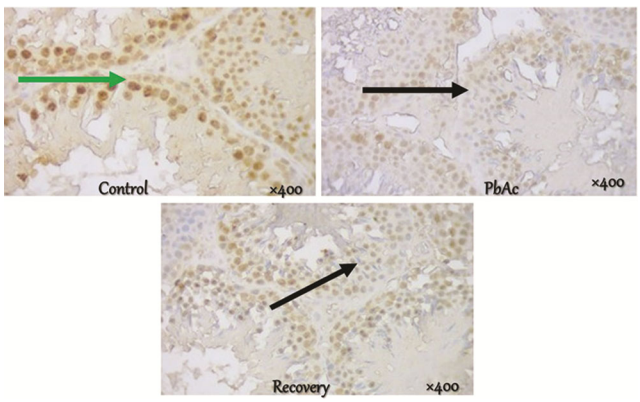

Fig 6 — Representative photomicrographs of testis of treated rats showing expression of Bcl-2.

downregulation of Bcl-2 expression after a 28-day recovery period provides mechanistic evidence that spontaneous restoration of testicular homeostasis following lead exposure is markedly limited.

The decrease in hormone levels caused by exposure to Pb is due to the dysfunction of the hypothalamus-pituitary-gonadal axis. Exposure to Pb causes the degradation of the gonadotrophic cells in the pituitary gland[19] and triggers apoptotic signals in the Leydig cells[20]. In addition, Pb hampers the formation of steroidogenic enzymes in Leydig cells, leading to a decrease in the secretion of testosterone[21]. Luteinizing hormone plays a crucial role in stimulating the release of testosterone from Leydig cells in the testes. An optimal level of testosterone is necessary for the proper structure and functioning of the reproductive organs, as well as for the maintenance of the structure and function of the male accessory glands[22]. The decrease in testosterone levels in rats exposed to lead poisoning can be attributed to a diminished sensitivity of Leydig cells to luteinizing hormone (LH), inhibition of enzymes involved in the production of hormones and LH, a decrease in the number of LH binding sites in Leydig cells, or a reduction in the synthesis and release of LH from the anterior pituitary gland[23,24]. The latter is the most probable scenario, given there was a significant decrease in the blood concentration of LH in the groups that were treated with PbAc. Lead exposure could potentially have significant implications for reproductive function, as it appears to specifically affect spermatogenesis and Leydig cells. The clear decrease in the weight of the seminal vesicle and prostate in group II, which was treated with PbAc alone, is another indication of a lowered plasma testosterone level. This is because the secretory function of the seminal vesicle and prostate relies on androgens and is highly responsive to the levels of testosterone in circulation. FSH secretion is essential for the proper development of sperm cells, since it promotes the growth, maturation, and activity of Sertoli cells. These cells release signals that are crucial for the onset and continuation of germ cell production[20,23].

The Leydig cells in the testes secrete testosterone in response to luteinizing hormone (LH) stimulation and regulate the process of spermatogenesis in the seminiferous tubules. FSH and testosterone promote spermatogenesis. The decrease in serum concentrations of sex hormones can be linked to the harm inflicted on Leydig cells by oxidative stress induced by lead exposure.

The toxic effects of Pb have been associated with its capacity to trigger apoptosis[24,25]. Research has shown that lead-induced cytotoxicity is linked to apoptosis, as demonstrated in various experimental studies involving different parts of the rat's body, such as the brain, testis, fibroblasts, lung, and retinal rod cells. Lead-induced apoptosis has been investigated exclusively in cerebellar neurons, rat hippocampus[26], and rod-photoreceptors within neuronal cells. The study found that lead acetate had a significant impact on the biomarkers of cellular apoptosis in the testis of rats. Specifically, it affected the levels of caspase-3, a protein that promotes apoptosis, and Bcl-2, a protein that inhibits apoptosis[27].

Lead exposure resulted in a notable elevation in testicular caspase-3 expression, suggesting that it triggers apoptosis in the rat testis, leading to an increase in testicular cell death. Apoptosis is a natural biological process that involves the targeted elimination of specific cells. Apoptosis, which acts as a counterforce to cell growth, has a role in regulating the number of cells in testicular tissue and eliminating unnecessary or damaged cells. However, an excessive amount of apoptosis, as observed in this study, can lead to the impairment of male reproductive function[28].

When intracellular cysteine proteases are active, they cleave their substrates at specific aspartic acid residues. These proteases are known as caspases, which stands for Cysteine Aspartylspecific Proteases[29]. The proteases exist as inactive zymogens in nearly all mammalian cells, but can be activated by undergoing proteolytic processing at conserved aspartic acid (Asp) residues, leading to their active states. Upon activation, the zymogen pro-proteins undergo cleavage, resulting in the formation of the active enzymes' large (20 kd) and small (10 kd) subunits. This process usually involves the removal of an N-terminal prodomain from the processed polypeptide chain[30].

Tumour necrosis factor (TNF) family receptors activate caspases as a signalling mechanism, connecting ligand binding at the cell surface to trigger apoptosis. Another potential stimulator of caspase activity is the involvement of mitochondria, which release caspase activating proteins into the cytosol, so initiating apoptosis. Another clear indication of apoptosis in this study is the reduced number of

interstitial cells of Leydig in the testis of rats treated with PbAc, together with the erosion and expansion of interstitial gaps[31].

The B cell lymphoma-2 (Bcl-2) proteins are a crucial part of the apoptotic pathway. The Bcl-2 oncogene is a gene that inhibits cell death and specifically controls the process of apoptosis. Bcl-2 proteins are present in the mitochondrial membranes, nucleus, and endoplasmic reticulum of numerous cells in the body. The main function of Bcl-2 family members is to control apoptosis[32]. The dysregulation of apoptosis is a primary factor in the development of numerous illnesses. The Bcl-2 family of proteins plays a crucial role in controlling apoptosis, the programmed cell death. Dysfunctions in the activity of these proteins have been linked to several diseases such as cancer, neurological disorders, ischemia, and autoimmune diseases[33].

This study discovered that rats exposed to PbAc had a noteworthy rise in apoptotic activity, resulting in a reduction in Bcl-2 expression (Fig 6). Toshiyuki found that lead (Pb) exposure triggers apoptosis by increasing oxidative stress. This leads to mitochondrial malfunction and the release of cytochrome C, resulting in a decrease in Bcl-2 expression[34].

Exposure to PbAc substantially elevated the levels of the inflammatory marker TNF α in the tissue of the testicles. Studies have shown that oxidative stress triggers the activation of transcription factors, such as NF-κB, leading to the production of inflammatory markers[35,36]. PbAc induces a disruption in the redox balance in the tissue of the testes, resulting in the excessive production of inflammatory markers, including TNF-α and IL-1β. A recent study revealed a connection between oxidative stress and the occurrence of an inflammatory response after exposure to Pb. Previous studies observed a notable increase in the pro-inflammatory cytokine (TNF-α), linking this behaviour to the excessive production of reactive oxygen species (ROS). Lead (Pb) induces the phosphorylation of NF-κB, leading to the release and activation of pro-inflammatory molecules such as TNF-α, IL-1 β, and NOs_2[37,38].

Lead, a genotoxic metal, has been found to harm the structure of DNA either by directly producing oxygen free radicals or indirectly by changing the enzymes that repair DNA. The study revealed that the levels of 8OHdG, a marker indicating DNA fragmentation index (DFI) in germ cells of rats treated with PbAc, were significantly elevated compared to the control group[39]. Previous study reported similar findings, showing a notable rise in the amount of 8-OHdG in the hippocampus. This indicates that 8-OHdG has the potential to cause considerable DNA damage in various tissues[40].

It is crucial to acknowledge that DNA fragmentation in germinal cells is a natural occurrence that can happen during the process of spermatogenesis. Lead exposure has led to an elevated level of nuclear DNA fragmentation in germ cells, which can be attributed to an excessive production of reactive oxygen species (ROS). Exposure to lead stimulates the generation of reactive oxygen species (ROS) within cells, which can cause structural and genetic harm to the testicles[41]. ROS, or reactive oxygen species, are known to harm the polyunsaturated fatty acids found in phospholipids within cell membranes. This damage leads to a decline in cellular function and can also contribute to gene alterations. Reactive oxygen species can cause genetic changes, such as point mutations, by damaging DNA through oxidation. This damage, as evidenced by the presence of 8-OHdG, can lead to mutations in DNA bases, specifically G>T/C>A transversions[42].

The increased concentration of polyunsaturated fatty acids in testes makes them more susceptible to oxidative damage. Multiple studies have demonstrated that the normal functioning of the testes, namely the production of sperm (spermatogenesis), is partially affected due to mechanisms that depend on reactive oxygen species (ROS)[43]. Reactive oxygen species have been found to induce chromosomal abnormalities and gene changes, leading to defective sperm and/or a substantial decrease in sperm count. Lead-induced reactive oxygen species (ROS) can result in genetic changes in germ cells, leading to aberrant sperm production. Sperm is highly susceptible to oxidative damage due to its limited antioxidant activity[44].

Oxidative stress is one of several mechanisms that cause DNA fragmentation. Other mechanisms include germ cell apoptosis during spermatogenesis, defects in chromatin remodelling, and compaction during the spermiogenesis process[45]. Previous studies found that exposure to lead can cause damage to the DNA integrity of germ line, resulting in cellular death. Under typical circumstances, apoptosis is a natural biological mechanism that regulates the cell population in testicular tissue by destroying damaged cells and maintaining a specific number of cells. Nevertheless, an excessive amount of apoptosis can lead to a modification in the male reproductive

function. Additionally, there have been reports indicating that germinal DNA fragmentation is frequently more evident in spermatids. This confirms that PbAc specifically impacts spermiogenesis to a greater extent. This phenomenon can be elucidated through a chemical mechanism, since lead has the potential to disrupt the DNA of germ cells and impede the regular advancement of nuclear condensation, a critical stage in the spermiogenesis process[46–48].

Lead possesses the capacity to attach to human protamines during spermiogenesis, which modifies the stability of sperm chromatin and has the potential to impact the proper condensation of chromatin[49,50]. This modification causes a change in the order of DNA and potentially played a role in the observed rise in DNA fragmentation documented in his research.

The histopathological examination of testes exposed to lead revealed the presence of seminiferous tubule atrophy and notable changes in spermatogenesis. The epididymis exhibited disruption that impaired its capacity to store sperm cells. The findings align with previous research [49,51–54]. Lead has a high propensity to accumulate in the testis due to its great attraction for adipose tissue. Lead deposition can cause tissue harm or organ damage due to its precipitous formation. Testicular damage, characterised by inflammation, erosion of the seminiferous tubules and germinal epithelium, and vacuolization of the tubules, impairs the functioning of the testis in terms of spermatogenesis and steroidogenesis[52]. The study observed that the majority of seminiferous tubules and lobules of the epididymis had no sperm.

The decrease in sperm concentration seen in the treated rats is mostly due to a disruption in the several phases of spermatogenesis. Typically, spermatogenesis progresses in a continuous and uninterrupted manner. However, a decrease in the number of spermatids, which are the cells most affected, can disturb the process of spermatogenesis and result in the loss of spermatozoa in the seminiferous tubules. Alternatively, abnormalities in the basal membrane of the seminiferous tubules may result from their atrophy due to cellular degeneration or the contraction of their myoid cells. The observed alterations can be ascribed to a process of protein interaction[55–57].

Lead acetate administration has been found to reduce the activity of some enzymes, such as alkaline phosphatase, in the testes. This can lead to structural damage to the membrane of the seminiferous tubules[49]. In addition, the thickening observed in the basal membrane could be attributed to either an upsurge in collagen synthesis or a decline in collagen phagocytosis by fibroblasts. This is because lead toxicity can also disrupt the binding of collagen to phagocytic fibroblasts. Furthermore, conspicuous gaps were observed among the germ line cells, indicating a probable depletion of Sertoli cells. Based on our findings Pb induced the separation of Sertoli and germ cells in a laboratory setting. They hypothesise that this could be the underlying factor responsible for the observed disruptions in spermatogenesis in living organisms. Sertoli cells serve a dual function. Firstly, they safeguard the germ cells by acting as a blood-testis barrier, preventing harmful substances from the bloodstream from reaching them. Furthermore, in reaction to follicle-stimulating hormone (FSH) and testosterone, they release several proteins that regulate the process of sperm differentiation. Therefore, the toxic effect of lead on Sertoli cells may partially account for the observed interruption of spermatogenesis.

The impact of lead acetate on cellular damage is demonstrated by a reduction in the quantity of spermatogenic cells, including Leydig cells and Sertoli cells, within the seminiferous tubules. The seminiferous tubules are composed of spermatogenic cells. The presence of lead acetate resulted in a reduction in the size and dimensions of seminiferous tubules, as a consequence of the progressive harm inflicted on these cells. Lead acetate induced oxidative damage to lipids, proteins, and other substances in spermatogenic cells, resulting in cellular destruction[55].

The study found that rats treated with PbAc saw a notable rise in oxidant status, as seen by an increase in the quantity of malondialdehyde in their testicles. Malondialdehyde is a biomarker of lipid peroxidation. Additionally, the rats showed a decrease in antioxidant capacity, namely a downregulation of SOD activity. The disruption in the oxidation status is caused by the toxic effects of Pb, which leads to an increase in the creation of free radicals due to an imbalance between the generation of oxidants and the activities of antioxidants[52]. Pb has been documented to hinder the function of antioxidant enzymes that neutralise free radicals by attaching to SH-containing groups or metal cofactors of antioxidant enzymes[37]. Rats exposed to PbAc typically experience oxidative damage in their testicular tissue[35]. The overproduction of reactive oxygen species (ROS) in treated rats leads to lower levels of antioxidant enzymes and their

mRNA expression, which in turn compromises testicular function. Extended exposure to Pb leads to the production of free radicals and reactive oxygen species (ROS), which result in organ damage due to the toxic effects of oxidative stress and the weakening of the antioxidant defence system. Lead possesses the capacity to readily oxidise fatty acids, which are crucial constituents of cell membranes. When fatty acids undergo oxidation, they can induce harm to both cells and membranes[51].

The tissue MDA level is an essential diagnostic indicator used to assess oxidative stress. It is a byproduct of peroxidized polyunsaturated fatty acids (PUFA). Elevated MDA levels indicate a rise in lipid peroxidation. Studies have established that heavy metals have the ability to elevate MDA levels in rat tissues[58,59]. The current study observed an elevation in MDA levels in the group treated with PbAc, indicating the occurrence of lipid peroxidation caused by lead. The elevated concentration of MDA indicates the production of lipid peroxides, as well as the deterioration of membrane structure and function. The findings of this investigation align with the observed elevation of testicular MDA levels in rats treated with lead[60–62]

It has been identified that an elevated level of MDA in the testicles is associated with an increased percentage of sperm cells displaying defective morphology and dead sperm[63,64]. Cells have inherent defensive mechanisms to counteract the detrimental effects of reactive oxygen species (ROS). Superoxide dismutase (SOD) eliminates the superoxide radical by turning it into H2O2, which is then rapidly converted into water by either catalase (CAT) or glutathione peroxidase (GPx). In addition, glutathione peroxidase converts lipid hydroperoxides into alcohols. Suppression of any of these antioxidant enzymes can result in harmful consequences because of the buildup of superoxide radicals and hydrogen peroxide. IG extracts effectively reduced the MDA level in rats treated with lead acetate. The injection of IG in lead acetate treated rats resulted in the restoration of antioxidant enzyme activities, specifically superoxide dismutase, in the testis to their normal levels. The findings indicate that IG treatment leads to a decrease in Malondialdehyde (MDA) levels, suggesting that IG has the ability to scavenge reactive oxygen species (ROS)[45,65–67].

Limitations

The study's conclusions are constrained by the use of an animal model, which may not fully replicate human responses. A single dose and exposure period were tested, leaving longer-term or low-dose effects unexplored. Mechanistic pathways (e.g., specific signaling cascades) were not delineated. Future work should address these gaps to enhance translational relevance. Sperm functional parameters were not assessed in the present study because these endpoints have been extensively reported in our earlier work using the same lead acetate exposure model, where significant impairments in sperm count, motility, and morphology were demonstrated; accordingly, the current investigation was intentionally designed to extend those findings by focusing on endocrine, oxidative, genotoxic, inflammatory, and apoptotic mechanisms that may underlie incomplete post-exposure recovery.

Conclusion

This study demonstrates that oral lead acetate exposure elicits multi-axis testicular toxicity in male Wistar rats, confirming significant endocrine disruption, oxidative stress, genotoxicity, inflammation, and apoptosis during active exposure. Crucially, the key objective of assessing spontaneous post-exposure recovery was addressed, revealing that several toxic effects—including elevated oxidative damage (MDA), persistent genotoxicity (8-OHdG), suppressed gonadotropins, and altered apoptotic signaling (Bcl-2)—did not fully resolve after 28 days without further exposure. These findings confirm the initial hypothesis and underscore that lead-induced testicular injury is not only severe but also enduring, highlighting the critical need for preventive measures and therapeutic interventions to mitigate irreversible reproductive damage.

Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose Clinical Trial Number: Not applicable

Author Contributions

Oyedayo Phillips Akano conceptualized and designed the study. Material preparation, data collection, and analysis were performed by Oyedayo

Phillips Akano, Olumide Stephen Akinsomisoye, Opeyemi Adebola Adetunji, Bayo Olufunso Adeoye, and Ajayi Ayodeji Folorunsho. Olumide Stephen Akinsomisoye supervised the research. The first draft of the manuscript was written by Oyedayo Phillips Akano and Olumide Stephen Akinsomisoye. All authors contributed to data interpretation, critically reviewed the manuscript for intellectual content, and approved the final version for submission.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to institutional data protection policies and confidentiality agreements but are available from the corresponding author on reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Health Research Ethics Committee at the Institute of Public Health, Obafemi Awolowo University, Ile-Ife, with the assigned HREC number IPH/OAU/12/1881

Consent to participate

Not Applicable

Consent to publish

Not Applicab

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