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Irvingia gabonensis seed extracts protect against lead-induced testicular damage in Wistar rats

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Lead acetate exposure is known to cause testicular injury, adversely affecting sperm parameters and histological integrity. This study sought to assess the preventive effects of the ethanol extract of *Irvingia gabonensis* seed (EEIG) and its ethyl acetate fraction (EAF) against lead acetate-induced testicular damage in male Wistar rats. Forty-five male Wistar rats (8–10 weeks old, weighing 150–170 g) were randomly allocated into nine groups (n = 5 each). Group I (control) was administered distilled water for a duration of 56 days. Group II was exposed to lead acetate (60 mg/kg) for 28 days only. Groups III–V received lead acetate for the first 28 days, followed by EEIG at 125, 250, or 500 mg/kg BW for the next 28 days. Groups VI–VIII were similarly pretreated with lead acetate and then administered EAF at 50, 100, or 200 mg/kg BW for the subsequent 28 days. Group IX received lead acetate for 28 days, followed by distilled water for the remainder of the period. Sperm parameters, namely, count, motility, live-dead ratio, and abnormal sperm morphology, were assessed. Testicular and epididymal tissues were analysed histologically. Data were analysed utilizing one-way ANOVA with significance eastablished at p <0.05. Lead acetate exposure significantly reduced sperm motility and increased abnormal sperm morphology compared to the control group (p <0.05). Treatment with EEIG and EAF improved sperm parameters, with EAF showing superior effects in restoring sperm motility, reducing abnormal sperm cells, and improving histological integrity compared to the lead-only group. This study suggests that the ethanol extract of *I. gabonensis* seeds and its ethyl acetate fraction mitigate lead acetate-induced testicular toxicity, highlighting their potential as therapeutic agents against testicular damage.

Keywords: *Irvingia gabonesis*, Lead acetate, Male infertility, Sperm quality, Testicular injury

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Introduction

Male infertility represents a significant global health challenge, affecting approximately 7% of men worldwide[1–3]. It contributes substantially to the growing incidence of reproductive disorders and is a major factor in the increasing rates of infertility among couples[3]. The causes of male infertility are multifaceted, encompassing genetic, physiological, and environmental factors. Environmental toxicants, such as heavy metals, pesticides, and industrial chemicals, have emerged as critical contributors to reproductive dysfunction[4]. Lead exposure, in particular, is a well-documented environmental toxicant that impairs sperm production and function, leading to decreased fertility. Lead toxicity can disrupt hormonal balance, generate oxidative stress, and damage testicular tissues, thereby compromising sperm quality and overall reproductive health. In addition to lead, other environmental pollutants, such as endocrine-disrupting chemicals, have been linked to adverse effects on male reproductive parameters[5]. The increasing prevalence of environmental contaminants, coupled with lifestyle factors such as poor diet and stress, stresses the pressing need for complete strategies to confront and alleviate the effects of these factors on male fertility. Efforts to understand and combat the global burden of male infertility require a multi-disciplinary approach, integrating research, public health initiatives, and policy interventions to improve reproductive outcomes and overall male health.

Lead exposure continues to be an important health issue owing to its pervasive environmental and industrial prevalence[5]. Lead acetate, a common compound containing lead, is particularly notorious for its harmful effects on the reproductive system.

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This heavy metal disrupts testicular function, leading to oxidative stress, inflammation, and cellular damage in the testes. Research has shown that chronic exposure to lead acetate results in a significant decline in sperm quality, including reduced motility, lower sperm count, and an increase in abnormal sperm morphology[6]. The impact on sperm quality is of particular concern since it directly correlates with male fertility, increasing the risk of infertility and complications in reproductive health. The mechanisms underlying lead toxicity involve the generation of free radicals and the disruption of hormonal balance, which collectively compromise the integrity and functionality of sperm cells[7].

Irvingia gabonesis (ex O'Rorke Aubry-Lecomte) Baill is a native plant of West Africa that is also referred to as an African mango or bush mango and is renowned for its culinary and medicinal uses. In Nigeria and other parts of Africa, its seeds are a staple in traditional dishes, such as "ogbono soup", a popular and nutrient-rich food. Beyond its culinary uses, it has been used in traditional medicine for a very long time particularly for managing various health conditions, including male infertility[8,9]. Flavonoids, saponins, and phenolic acids are among the many bioactive substances found in this plant's seeds that are thought to have anti-inflammatory and antioxidant qualities. These substances could be responsible for the plant's well-known advantages in enhancing reproductive health[9,10].

Sperm quality is a pivotal factor in male reproductive health and is closely linked to fertility outcomes. Poor sperm quality, characterised by reduced motility, increased levels of abnormal sperm cells, and decreased sperm count, can severely affect a man's ability to conceive. Such impairments in sperm parameters are often associated with various reproductive disorders and infertility issues[3,11,12].

The bioactive substances found in *I. gabonensis*, including as flavonoids, phenolic acids, and saponins, have been the subject of much research because of their strong anti-inflammatory and antioxidant capabilities. It has been demonstrated that these substances neutralize reactive oxygen species (ROS) and lessen oxidative stress, which is a major factor behind testicular damage caused by lead. Its ability to prevent oxidative damage was highlighted by a study that showed, for example, that its extract dramatically decreased oxidative stress indicators in animal models[13]. Additionally, the seed may have antitumor properties[8].

According to earlier *in vitro* research, *I. gabonensis* extracts can shield cells from oxidative damage by decreasing lipid peroxidation and increasing the activity of antioxidant enzymes like catalase and superoxide dismutase[13]. *In vivo* studies have further supported these findings, with evidence that its supplementation improves sperm quality and testicular function in animal models exposed to environmental toxins. For example, Ojo *et al.*, reported that *I. gabonensis* extract ameliorated cadmium-induced testicular toxicity in rats by restoring sperm parameters and reducing testicular oxidative stress[14].

Lead acetate is known to induce testicular damage through oxidative stress, inflammation, and hormonal imbalance. Given the well-documented antioxidant and anti-inflammatory properties of *I. gabonensis*, it is scientifically plausible that its extracts could mitigate these effects. Emejulu *et al.*, demonstrated that *I. gabonensis* extract protected against hepatorenal toxicity induced by heavy metals, further supporting its potential to counteract lead-induced damage[15]. While the antioxidant and protective effects of it have been demonstrated in other models of toxicity, there is a rarity of research on its specific effects on lead-induced testicular injury.

The specific goal of this study is to look at how well *I. gabonensis* seed ethanol extract and its ethyl acetate fraction protect male Wistar rats' testes from damage caused by lead acetate. The work will assess their ability to improve sperm parameters—count, motility, viability, and morphology—to provide empirical evidence for their potential as therapeutic agents in mitigating lead-induced reproductive toxicity.

Materials and Methods

Plant sample collection and analysis

Mr. G. A. Ademoriryo, a taxonomist in the Department of Botany at Obafemi Awolowo University in Ile-Ife, identified fresh fruits and leaves of I. gabonensis that had been plucked at Ilora, Afijio Local Government, Oyo State, Nigeria. Specimens were then placed in the herbarium for reference after being given a voucher number (IFE-17976).

After being carefully cleaned and allowed to air dry for 14 days at room temperature, the seeds were hand extracted from the fruit pulp. An electric blender was then used to grind them into a fine powder. For 72 hours, the 1 kg of powdered seeds was macerated in 5 L of 70% ethanol while being shaken occasionally.

Whatman No. 1 filter paper was then used to filter the combination. The ethanol extract (EEIG; yield 7.46% w/w) was obtained by utilizing a rotary evaporator to condense the filtrate to dryness at 40°C under reduced pressure.

50 g of the ethanol extract was suspended in 500 mL of distilled water for the ethyl acetate fraction (EAF), which was then separated in a separatory funnel using 500 mL × 3 volumes of ethyl acetate. The EAF was obtained by pooling the ethyl acetate layers, drying them over anhydrous sodium sulfate, and then evaporating them at 40°C under decreased pressure. Before being used, EEIG and EAF were both kept in a refrigerator in airtight containers[8,9].

Animal use and experimental design

Forty-five male Wistar rats, weighing 140±10 g and aged between 8 and 10 weeks, were acquired from the Animal House at Obafemi Awolowo University's College of Health Sciences in Ile-Ife, Nigeria. The rodents were kept in standard cages with a 12-hour light/dark cycle, normal temperatures, and unlimited access to water and standard rodent chow. To make sure the animals would adjust to the lab environment, they were acclimated for two weeks prior to the experiment starting. A computerized weighing balance was used to record each animal's body weight once a week. The % change in weight from the starting body weight was then computed. After then, the rats were split up into nine groups (n=5) as shown in Table 1.

Ethical consideration

The Health Research Ethics Committee (HREC), Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria, provided ethical clearance for this work (HREC number IPH/OAU/12/1881). The Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) contains national and international rules for the care and use of laboratory animals, which were followed in all animal-related procedures. The landowner in Ilora, Afijio Local Government, Oyo State, Nigeria, granted permission to collect Irvingia gabonensis.

Treatment of animals

Drug administration

A 60 mg/mL stock solution was made by dissolving lead acetate, which was purchased from BDH Chemicals Ltd., in distilled water and then given orally. Using a cannula, I. gabonensis ethanol extract (EEIG) and its ethyl acetate fraction (EAF) were also given orally.

Collection of organs

At the end of the administration period, animals were anesthetised by intraperitoneal injection of ketamine (75 mg/kg BW), selected for its rapid onset, reliability, and common use in rodent studies. Adequate anaesthesia was confirmed by the absence of reflexes (e.g., pedal withdrawal), the rats were then cervically dislocated and sacrificed. The testes and epididymides were then excised, trimmed of fat, and weighed to determine percentage weight change and relative organ weight.

Sperm analysis

The cauda epididymis was meticulously dissected and minced in 1 milliliter of warm (37°C) physiological saline in order to assess sperm motility. A drop of the suspension was put on a microscope slide that had been warmed up beforehand, covered with a cover slip, and viewed at ×400 magnification using a light microscope. The

Table 1 — Experimental Protocol

S. No.	Groups	Treatment
1	I. Control	1 mL/Kg BW distilled water
2	II. Lead Acetate	60 mg/Kg BW LA
3	III. EEIG 125	60 mg/Kg BW LA, followed by 125 mg/Kg BW EEIG
4	IV. EEIG 250	60 mg/Kg BW LA, followed by 250 mg/Kg BW EEIG
5	V. EEIG 500	60 mg/Kg BW LA, followed by 500 mg/Kg BW EEIG
6	VI. EAF 50	60 mg/Kg BW LA, followed by 50 mg/Kg BW EAF
7	VII. EAF 100	60 mg/Kg BW LA, followed by 100 mg/Kg BW EAF
8	VIII. EAF 200	60 mg/Kg BW LA, followed by 200 mg/Kg BW EAF
9	IX. Recovery	60 mg/Kg BW LA, followed by 1 mL/Kg BW distilled water

Footnote: BW = Body weight; LA = Lead acetate; EEIG = Ethanol extract of *Irvingia gabonensis*; EAF = Ethyl acetate fraction of *Irvingia gabonensis*.

fraction of progressively motile sperm cells was used to represent motility.

To assess sperm vitality, the eosin–nigrosin staining method was employed. Smears were made and allowed to air dry after a tiny aliquot of the sperm solution was combined with the stain. While dead spermatozoa absorbed the eosin stain and showed pink, live spermatozoa did not and appeared white. A total of 400 sperm cells were counted per slide, and viability was expressed as the percentage of live spermatozoa.

For sperm morphology, smears prepared from the eosin–nigrosin stain were examined under oil immersion at ×1000 magnification. Morphological abnormalities, including head defects, mid-piece defects, and tail defects, were recorded, and the results were expressed as the percentage of abnormal sperm cells out of 400 counted cells.

Total sperm count was obtained by homogenising the cauda epididymis in 5 mL of physiological saline, followed by dilution and counting using an improved Neubauer hemocytometer. The number of spermatozoa per millilitre of suspension was calculated and recorded[6].

Statistical analysis

GraphPad Prism 5.03's one-way analysis of variance (ANOVA) was used to analyze the data. The post-hoc Newman-Keuls tool was used. P values were deemed significant if they were less than 0.05. The data was displayed as Mean ± S.E.M.

Results

Effect of fractions of IG on percentage body weight change

There was a significant decrease ($p = 0.04$) in the percentage body weight of group II rats relative to groups I and III. In contrast, there were no significant differences in groups IV, V, VI, VII, VIII, and IX (Fig. 1a).

Effect of fractions of IG on relative testicular weight

There was a significant decrease ($p = 0.018$) in the relative testicular weight of groups II, III, IV, V, VI, VII, VIII, and IX compared to group I. This was reversed (but not significantly) in groups III, IV, V, VI, VII, and VIII compared to group II. Group IX showed no sign of recovery (Fig. 1b).

Effect of fractions of IG on relative epididymal weight

There was no significant difference ($p = 0.0849$) in the relative weight of the epididymis in groups II, III, IV, V, VI, VII, VIII, and IX compared with group I (Fig. 1c).

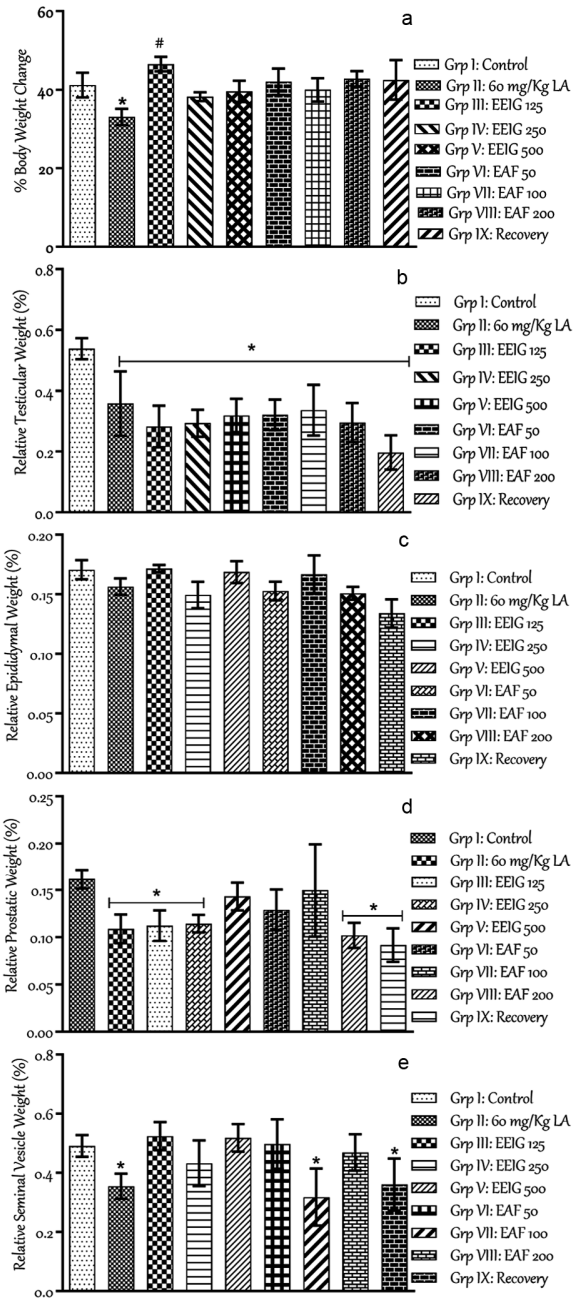

Fig. 1 — Effect of EEIGEAF on body and reproductive organ weights of rats following lead acetate–induced toxicity. a) Percentage body-weight change, b) relative testicular weight, c) relative epididymal weight, d) relative prostatic weight, and e) relative seminal vesicle weight. Data are presented as mean ± SEM (n = 5). * = significantly different from the control group ($p < 0.05$); # = significantly different from group II ($p < 0.05$).

Effect of fractions of IG on relative prostatic weight

There was a significant decrease ($p = 0.4795$) in the relative weight of the prostate in groups II, III, IV, VIII and IX compared to group I (Fig. 1d).

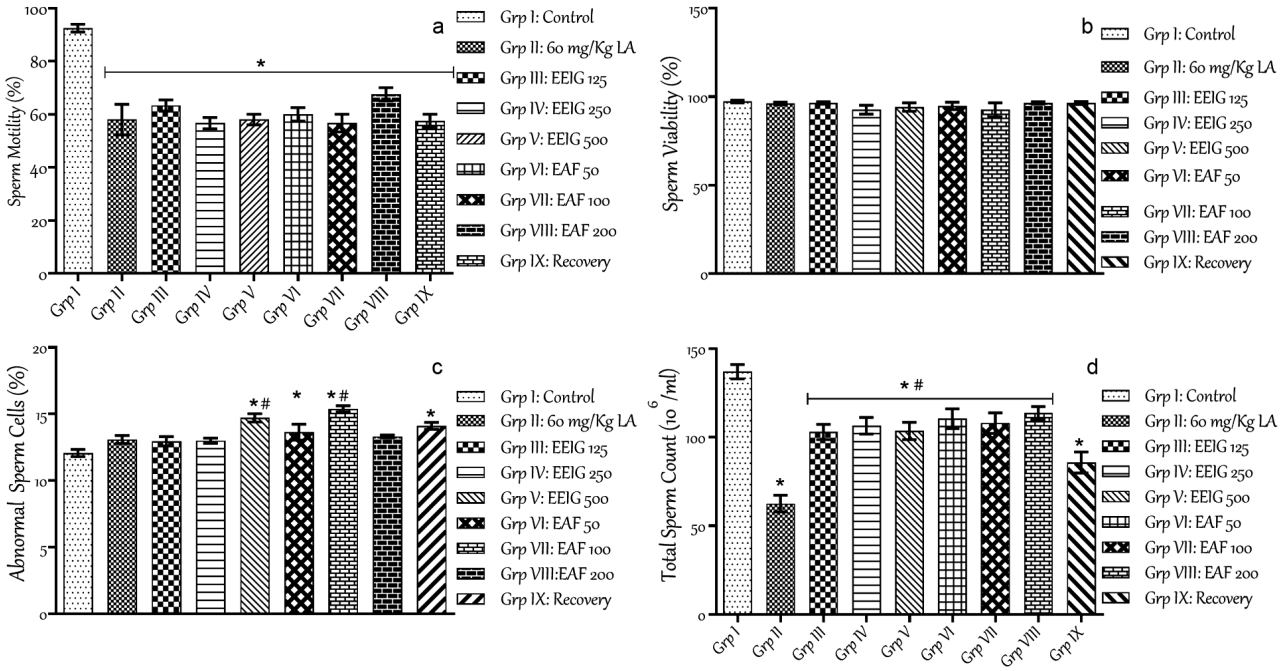

Fig. 2 — Effect of EEIG and EAF on sperm quality parameters of rats following lead acetate–induced toxicity. a) Sperm motility, b) sperm viability, c) percentage of abnormal sperm cells, and d) total sperm count. Data are presented as mean ± SEM (n = 5). * = significantly different from the control group ($p < 0.05$); # = significantly different from group II ($p < 0.05$).

Effect of fractions of IG on relative seminal vesicle weight

There was a significant decrease ($f = 1.160$, $p = 0.04$) in the relative weight of the seminal vesicle in groups II, VII, and IX relative to group I. Groups III, IV, V, and VI showed no significant difference when compared to group I (Fig. 1e).

Effect of fractions of IG on sperm motility

There was a significant decrease ($p = 0.0001$) in sperm motility in groups II, III, IV, V, VI, VII, VIII, and IX compared to group I. However, this was slightly reversed in group VIII compared to other groups. No significant change in groups II, III, IV, V, VI, VII, and IX compared to group II (Fig. 2a).

Effect of fractions of IG on sperm viability

There was no significant difference ($p = 0.5426$) in sperm viability of groups II, III, IV, V, VI, VII, VIII, and IX compared with group I (Fig. 2b).

Effect of EEIG and EAF on sperm morphology

There was a significant increase ($p = 0.0001$) in the percentage of abnormal sperm cells in groups V and VII, compared to group I. In groups V and VII, the percentage of abnormal sperm cells significantly increased relative to group II (Fig. 2c).

Effect of fractions of IG on total sperm count

There was a significant reduction ($p = 0.0001$) in total sperm count in groups II, III, IV, V, VI, VII, VIII, and IX compared to group I. However, EEIG and EAF significantly increased total sperm count in groups III, IV, V, VI, and VII compared to group II (Fig. 2d).

Effect of fractions of IG on testicular and epididymal histology

Histological examination of the testes in the control group (Fig. 3-I) revealed normal seminiferous tubules with intact germinal epithelium, active spermatogenesis, and abundant spermatozoa in the lumen. Lead acetate-treated rats (Fig. 3-II) exhibited marked degeneration of the germinal epithelium, widened interstitial spaces, vacuolation, and depleted luminal spermatozoa, indicating a severe disruption of spermatogenesis. Treatment with EEIG and EAF, particularly at higher doses (Fig. 3 - VI, VIII), demonstrated a progressive restoration of seminiferous tubule architecture, a reduction in interstitial space, and an increase in luminal sperm density compared to the lead-only group. The epididymal histology of control rats exhibited dense luminal spermatozoa, whereas lead-treated rats showed sparsely populated lumina. EEIG and EAF treatments improved epididymal sperm density, with

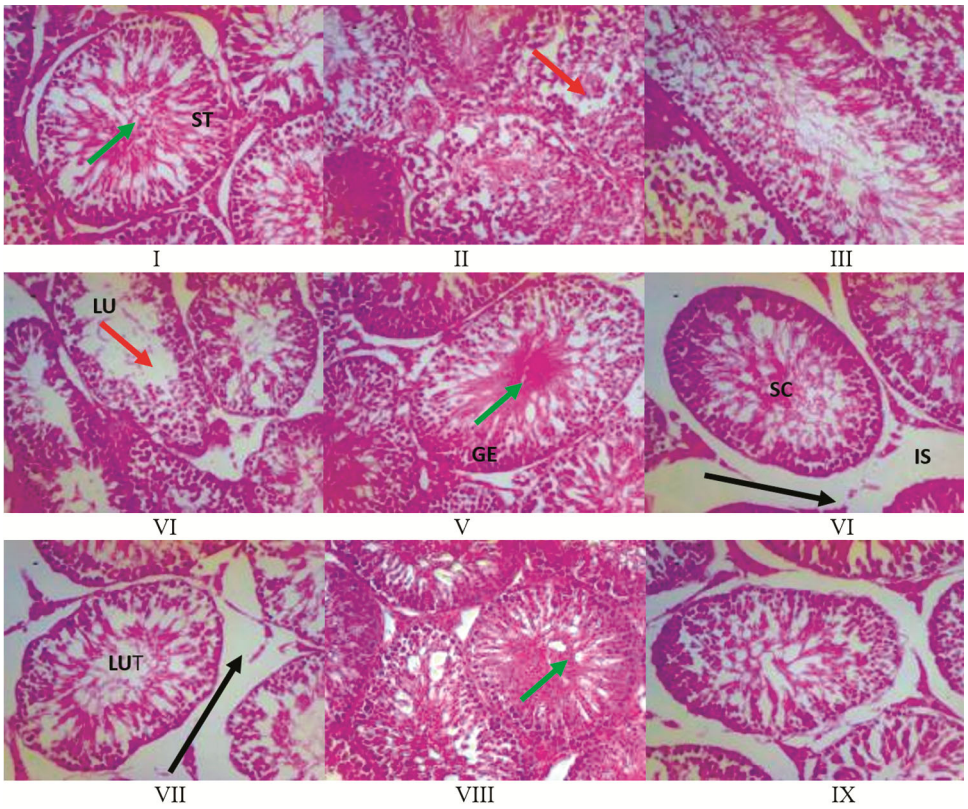

Fig. 3 — Representative photomicrographs of testes of treated rats (H & E × 400).

the 200 mg/kg EAF group exhibiting near-normal histological architecture. These observations corroborate the quantitative sperm analysis, confirming the protective effects of I. gabonensis extracts against lead-induced testicular and epididymal damage (Fig. 4).

Discussion

Several functional foods and plant nutraceuticals, including *I. gabonensis*, are known for their antioxidant properties and roles in metal detoxification and physiological regulation[16]. This study aimed to investigate how *I. gabonesis* alleviates lead acetate-induced reproductive toxicity in Wistar rats.

Administration of lead acetate resulted in a notable decrease in body weight, corroborating previous findings[17,18], which indicated that lead decreases body weight and feed efficiency. This weight loss is attributed to disrupted metabolic processes and imbalanced electrolyte homeostasis, particularly affecting zinc status, which is crucial for many metabolic functions. Additionally, lead exposure significantly reduced the relative weights of the testes, prostate, and seminal vesicles, possibly due to necrosis, apoptosis, and impaired spermatogenesis[6]. Lead's interference with trace element absorption and zinc-dependent enzymes contributes to decreased organ weights and poor nutrient absorption, further impacting growth and reproductive health[19].

Conversely, treatment with EEIG and EAF improved body and organ weights compared to the lead acetate group. This improvement is linked to the high nutritional value of its seeds, which are rich in moisture, lipids, proteins, and carbohydrates, essential for growth and health[9,20]. Rats treated with lead acetate alone experienced gastrointestinal distress, including watery stools and dehydration, aligning with reports of gastrointestinal issues caused by lead[21]. In contrast, rats treated with *I. gabonesis* extracts exhibited normal stool consistency, indicating anti-diarrheagenic effects and better fluid conservation, potentially due to balanced zinc metabolism and reduced gastrointestinal motility. This suggests that *I. gabonesis* could counteract lead-induced gastrointestinal disturbances and support overall health and growth.

Spermatogenesis involves a series of testicular events concerned with the synthesis and maturation of sperm cells. The progression of spermatogenesis can

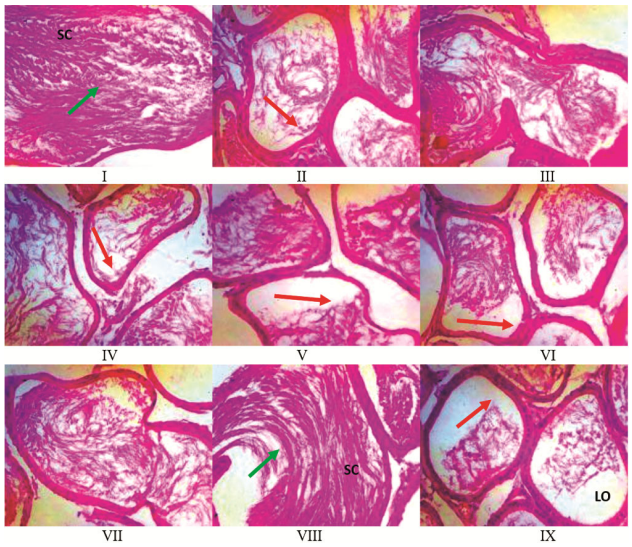

Fig. 4 — Representative photomicrographs of epididymis of treated rats (H & E × 400 Magnification).

Legend: Red arrow – lumen with fewer sperm cells; Black arrow – widened interstitial space; Green arrow – lumen with many sperm cells; ST – Seminiferous Tubule; LU – Lumen; IS – Interstitial Space; GE – Germinal Epithelium; SC – Sperm Cells; LO – Lobule.

be assessed *in vitro* by evaluating sperm motility, sperm viability, total sperm count, and sperm morphology. This study revealed a significant decrease in epididymal sperm motility and total sperm concentration in rats treated with PbAc. The histopathology of the epididymis of rats treated with PbAc only revealed that the lobules were very depleted, containing scanty sperm cells. Similarly, there was an increased percentage of sperm cells with abnormal morphology. These findings suggest that the processes involved in spermatogenesis have been significantly altered, resulting in a poor quality and reduced quantity of sperm cells stored in the epididymis. These results are consistent with previously published results[22–24].

Critical steps in the steroidogenic pathway are known to be negatively impacted by ROS. Increased ROS levels cause lipid peroxidation and membrane degradation, which impair sperm motility, deactivate glycolytic enzymes, and harm acrosomal membranes, rendering the sperm cell inoperable[25–27]. Due to their high polyunsaturated fatty acid content, spermatozoa are particularly vulnerable to reactive oxygen species[28]. According to reports, the main way that lead is hazardous is by interfering with the hypothalamic regulation of pituitary hormone release, which in turn affects spermatogenesis. Three elements are associated with reproductive potential: the quantity, quality, and availability of sperm. The testis of rats exposed to lead showed signs of disarray and interruption of spermatogenesis in the lumen of seminiferous tubules. Additionally, it has been noted that in albino rats given lead acetate, the cells of the seminiferous tubules—the location of spermatogenesis—display degenerative characteristics such as heterochromic nuclei, uneven basal lamina, and vacuolization[29] and exposure to lead acetate reduced sperm density and sperm activity, and increased sperm malformation in mice testis[21] From this study, both EEIG and EAF showed improved sperm quality suggesting that the plant was able to mitigate the effect of oxidative stress as earlier reported [9,30].

The production of rete testis fluid, a particular milieu in the lumen of the seminiferous tubules, sperm release, androgen metabolism, and different stages of spermatogenic cells are all mediated by sertoli cells. Higher dosages of PbAc have been shown to disrupt the Sertoli cell-based blood test is barrier, allowing the metals to enter the testicular tissues, particularly the seminiferous tubules[31]. The earliest morphological damage is typically shown in ultrastructural abnormalities, especially in Sertoli cells. In cases of prolonged exposure, this may potentially disrupt the homeostasis of sperm production and all spermatogenesis processes[32]. In this study, exposure to PbAc may have caused damage to the Sertoli cells, thereby significantly decreasing the total sperm concentration and leading to a marked increase in the percentage of abnormal sperm cells in the PbAc-treated group. However, a significant reversal of these parameters after administration of EEIG and EAF strongly suggests that 500 mg/Kg EEIG and especially 200 mg/kg of EAF can improve the compromised integrity of the blood-testis barrier and the Sertoli cells. It is also possible that certain compounds in the extracts are capable of neutralising Pb ions.

The limitations of this study include the absence of a standard positive control, restricted direct comparison of *I. gabonensis* effects with established therapeutic agents, and the study was conducted exclusively in male Wistar rats, which may limit extrapolation of findings to humans or other animal models.

Conclusion

This study demonstrates that lead acetate significantly impairs reproductive health in Wistar rats, as evidenced by reduced body weight, decreased organ weights, and compromised sperm parameters

such as motility, viability, and morphology. Lead acetate's adverse effects are attributed to its disruption of metabolic and hormonal balances, leading to decreased testicular weight and altered spermatogenesis. The observed gastrointestinal distress and weight loss further highlight the broad impact of lead toxicity on physiological functions, including nutrient absorption and metabolic regulation. Treatment with EEIG and EAF showed promising improvements in these parameters. The EEIG and EAF effectively mitigated lead-induced damage by enhancing body and organ weights, improving stool consistency, and restoring sperm quality. The findings suggest that *I. gabonesis* has potential as a therapeutic agent to counteract lead-induced reproductive toxicity, likely through its antioxidant properties and ability to support normal metabolic and hormonal functions. These results highlight the potential of *I. gabonesis* in managing metal-induced reproductive dysfunctions and offer a basis for further research into its mechanisms and broader applications.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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