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Research Article

Therapeutic effects of *Irvingia gabonensis* extracts in mitigating lead-induced reproductive damage in male Wistar rats: Restoration of hormonal and oxidative markers

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Abstract

This study investigated the impact of ethanol extract (EEIG) and ethyl acetate fraction (EAF) of *Irvingia gabonensis* seeds on reproductive hormones, oxidative, inflammatory and apoptotic markers in Wistar rats subjected to lead acetate-induced reproductive damage. The research sought to elucidate the methods by which *I. gabonensis* mitigates male reproductive toxicity. Forty-five male Wistar rats, aged 8 to 10 weeks, were categorised into nine groups as follows: Group I was administered 1 ml/kg of distilled water (control) for 56 days; Group II was administered 60 mg/kg of lead acetate for 28 days. Groups III, IV, and V were administered 60 mg/kg of lead acetate during the initial 28 days, followed by 125 mg/kg, 250 mg/kg, and 500 mg/kg of EEIG, respectively, for an additional 28 days. Groups VI, VII, and VIII were administered 60 mg/kg of lead acetate for 28 days, thereafter receiving 50 mg/kg, 100 mg/kg, and 200 mg/kg of EAF, respectively, for an additional 28 days. Group IX was administered 60 mg/kg of lead acetate for 28 days, thereafter receiving 1 ml/kg of distilled water. All therapies were delivered orally. Measured parameters encompassed FSH, LH, testosterone, SOD, MDA, 8OHdG, TNF-α, Caspase-3 and Bcl-2, utilizing established methodologies. The results indicated substantial decreases in FSH, LH, testosterone, SOD, Bcl-2 levels in the lead acetate group, accompanied by elevated levels of MDA, 8OHdG, TNF-α, and Caspase 3. The administration of EEIG and EAF counteracted these alterations, with increased dosages demonstrating enhanced restoration of hormone levels and reduced oxidative and inflammatory indicators. This study demonstrates that *Irvingia gabonensis* possesses antioxidative, anti-inflammatory, and anti-apoptotic properties, effectively mitigating lead acetate-induced reproductive toxicity in male Wistar rats. Both ethanol extract and ethyl acetate fraction showed dose-dependent efficacy in restoring reproductive hormones, reducing oxidative stress, and modulating inflammatory and apoptotic markers. These findings suggest *I. gabonensis* as a potential natural therapy for male reproductive damage caused by environmental toxins, warranting further exploration of its clinical relevance and mechanisms.

1. Introduction

Reproductive toxicity is a major health issue, with exposure to environmental toxins such as heavy metals increasingly associated with negative reproductive effects in both humans and animals [1]. Lead, a widespread environmental pollutant, has notably detrimental effects on male reproductive health. Chronic lead exposure is linked to hormonal abnormalities, oxidative stress, and inflammation in

reproductive organs, resulting in diminished fertility [2]. The adverse consequences have stimulated comprehensive investigation into possible therapies or ameliorative substances that might alleviate lead-induced reproductive damage. Recent years have seen a focus on natural chemicals and plant-based medicines for their antioxidant, anti-inflammatory, and hormone-regulating capabilities, which may aid in restoring reproductive function following toxicant exposure.

A notable contender is *Irvingia gabonensis*, sometimes referred to as African mango, a tree indigenous to West and Central Africa that has been extensively researched for its therapeutic and nutritional attributes [3, 4]. Historically employed in African medicine for many diseases, extracts from *Irvingia gabonensis* have demonstrated antioxidant, anti-inflammatory, and hepatoprotective properties in both in vitro and in animal research [5]. The medicinal advantages of *Irvingia gabonensis* are mostly ascribed to its bioactive constituents, including flavonoids, phenols, and carotenoids, all recognized for their antioxidative and protective attributes [3, 6]. Nevertheless, despite its extensive use, limited research has investigated the impact of *I. gabonensis* on reproductive health, especially concerning heavy metal toxicity. Various components of the plant, including bark, leaf, and seed, have been utilized in the treatment and management of several illnesses [7].

Lead exposure, mostly via polluted water, food, or industrial settings, presents a significant threat to human health, with enduring effects on several biological systems. Chronic lead exposure in males has been linked to reproductive dysfunction, characterized by diminished libido, poor spermatogenesis, and decreased testosterone levels [8]. The mechanisms of lead-induced reproductive damage encompass hormonal imbalance, oxidative stress, and heightened inflammatory responses in the testicular and epididymal tissues. Lead can impede essential enzymes implicated in steroidogenesis, the synthesis of testosterone and other androgens. Consequently, testosterone and LH levels may diminish, disturbing the intricate hormonal equilibrium vital for sustaining male reproductive function [9, 10].

Oxidative stress, characterized by an imbalance between the formation of reactive oxygen species (ROS) and antioxidant defenses, is pivotal in lead poisoning. Lead exposure increases reactive oxygen species production in reproductive organs, surpassing intrinsic antioxidant defenses and resulting in cellular damage [11]. This oxidative damage may present as lipid peroxidation, protein oxidation, and DNA damage, leading to cellular malfunction or apoptosis. Malondialdehyde (MDA), a consequence of lipid peroxidation, is frequently assessed as a biomarker for oxidative stress, whereas SOD activity functions as a measure of antioxidant capability [11]. Besides oxidative stress, lead exposure activates inflammatory pathways, resulting in elevated levels of pro-inflammatory cytokines, such as TNF-α, in the testes. This inflammation aggravates tissue damage, leading to the emergence of reproductive disorders and diminished fertility [12].

Lead toxicity is a major contributor to male reproductive dysfunction, mediated by disruptions in key molecular and biochemical markers [13,14]. Hormonal imbalances, such as decreased levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, are hallmarks of lead-induced reproductive toxicity [15]. Oxidative stress plays a critical role, evident through reduced antioxidant enzyme activity, such as superoxide dismutase (SOD), and elevated malondialdehyde (MDA) levels, which signify lipid peroxidation [16]. DNA damage, marked by increased 8-hydroxy-2'-deoxyguanosine (8OHdG) [17], further exacerbates cellular dysfunction. Inflammatory responses, driven by elevated tumor necrosis factor-alpha (TNF-α) [18], and apoptotic pathways, marked by increased caspase-3 and reduced Bcl-2 levels [19, 20], underscore the multifaceted nature of lead-induced reproductive damage. Understanding the modulation of these markers is essential for developing effective interventions.

Researchers are exploring natural substances with antioxidant, anti-inflammatory, and hormone-regulating capabilities as possible therapies for reproductive damage caused by heavy metals. *I. gabonensis* has abundant bioactive components, including flavonoids and phenolic acids, which have

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potent antioxidant properties that may mitigate oxidative stress and inflammation in reproductive organs. Ethanol and ethyl acetate extractions of *I. gabonensis* are recognized for their substantial concentration of bioactive components, perhaps enhancing their efficacy in alleviating lead-induced reproductive damage.

This study evaluates the effects of ethanol extract (EEIG) and ethyl acetate fraction (EAF) of *Irvingia gabonensis* on hormone levels, oxidative stress markers, DNA damage, and inflammation in male Wistar rats exposed to lead acetate. Key metrics include FSH, LH, testosterone, SOD, MDA, 8OHdG, TNF-α, and Caspase-3, to assess reproductive, antioxidant, and anti-inflammatory benefits.

2. Materials and methods

2.1 Plant preparation

Fresh fruits and leaves of *Irvingia gabonensis* were gathered at Ilora in Oyo State, Nigeria, and identified by a taxonomist from Obafemi Awolowo University, where a herbarium specimen was preserved. The seeds of the fruit (Fig 1) were deshelled, desiccated, and pulverized, thereafter undergone ethanol extraction. The resultant solution was concentrated using a rotary evaporator and vacuum oven. Three fractions were separated using solvents of ascending polarity (n-hexane, ethyl acetate, and ethanol) using vacuum liquid chromatography, resulting in n-hexane (50%), ethyl acetate (23%), and ethanol (27%) fractions [3]. The crude extract and ethyl acetate fraction (EAF) were formulated into stock solutions for further analysis.

Figure 1. Fresh seeds of *Irvingia gabonensis*

2.2 Phytochemical analysis

The phytochemical analysis of the extract revealed many secondary metabolites, such as anthraquinones, alkaloids, phenols, tannins, saponins, flavonoids, steroids, terpenoids, and glycosides, by conventional qualitative assays. A quantitative study was performed to assess the overall content of alkaloids, flavonoids, saponins, terpenoids, phenolics, and tannins, enhancing the comprehension of the plant's antioxidant and anti-inflammatory capabilities [3].

2.3 Animal use

Forty-five male Wistar rats, aged 8 to 10 weeks and weighing between 150 and 170 g, were allocated into nine groups (Table 1), each subjected to distinct treatments for a duration of 56 days.

Table 1. Experimental design

No.	Groups	Treatment
I.	Control	1 ml/Kg BW distilled water for 56 days
II.	Lead Acetate	60 mg/Kg BW LA for 28 days
III.	EEIG 125	60 mg/Kg BW LA for 28 days, followed by 125 mg/Kg BW EEIG for 28 days
IV.	EEIG 250	60 mg/Kg BW LA for 28 days, followed by 250 mg/Kg BW EEIG for 28 days
V.	EEIG 500	60 mg/Kg BW LA for 28 days, followed by 500 mg/Kg BW EEIG for 28 days
VI.	EAF 50	60 mg/Kg BW LA for 28 days, followed by 50 mg/Kg BW EAF for 28 days
VII.	EAF 100	60 mg/Kg BW for 28 days, followed by 100 mg/Kg BW EAF for 28 days
VIII.	EAF 200	60 mg/Kg BW LA for 28 days, followed by 200 mg/Kg BW EAF for 28 days
IX.	Recovery	60 mg/Kg BW LA for 28 days followed by 1 ml/Kg BW distilled water for 28 days

LA = Lead Acetate; EEIG = ethanol extract of *Irvingia gabonensis*; EAF = ethyl acetate fraction of ethanol extract of *Irvingia gabonensis*

2.4 Drug administration

Lead acetate was formulated as a 60 mg/ml solution by dissolving 6 g in 100 ml of distilled water, with fresh stock solutions created routinely for uniform dosage. Lead acetate was orally delivered to the rats by an intubation technique. The ethanol extract (EEIG) and ethyl acetate fraction (EAF) of *Irvingia gabonensis* were delivered orally using a cannula.

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2.5 Animal Sacrifice

Upon conclusion of the experiment, the animals were euthanized via cervical dislocation for collecting sample. Blood was collected via cardiac puncture and subsequently processed to isolate serum, which was utilized to assess reproductive hormones including luteinizing hormone, follicle-stimulating hormone, and testosterone.

2.6 Tissue collection

Following euthanasia via cervical dislocation, tissues from the testes and epididymis were carefully dissected and preserved for further histological and biochemical analysis. Some parts of the tissues were immediately placed in ice for biochemical assays, while the remaining parts were fixed in formalin for subsequent examination to assess cellular changes and damage associated with lead acetate-induced toxicity.

2.7 Ethical approval

Ethical clearance was obtained from the Health Research Ethics Committee, Institute of Public Health, Obafemi Awolowo University, Ile-Ife with HREC number IPH/OAU/12/1881.

2.8 Biochemical assays

Biochemical tests were performed to assess oxidative stress indicators, inflammation, and reproductive hormones in testicular samples from both treated and control groups. Lipid peroxidation was quantified by assessing malondialdehyde (MDA) levels by the thiobarbituric acid (TBA) technique established by Ohkawa et al. (1979). The testicular tissues were homogenized, treated, and centrifuged, with MDA levels quantified by measuring absorbance at 532 nm. The activity of superoxide dismutase (SOD) was evaluated according to the technique established by McCord and Fridovich (1969), utilizing tissue homogenates combined with a Tris-HCl buffer, EDTA, and pyrogallol. The SOD activity was determined by the inhibition rate of pyrogallol autooxidation. Furthermore, testicular DNA damage was assessed by measuring 8-Hydroxydeoxy-guanosine (8-OHdG) using an ELISA, subsequent to tissue homogenization and supernatant extraction.

The inflammatory marker TNF-α was measured using an ELISA using rat-specific kits, subsequent to tissue homogenization and centrifugation. Numerous cleaning procedures were implemented on microwell strips to improve precision. Hormonal evaluations comprised follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, all quantified in serum using ELISA. Procedures for sample incubation and microplate reading were employed to ascertain optical densities and calculate hormone concentrations. FSH and LH levels were measured by including enzyme conjugate reagents and TMB solution, whereas testosterone levels required the addition of serum, enzyme, and biotin reagents into wells, followed by absorbance measurement at 450 nm for concentration assessment.

2.9 Histology and immunohistochemistry

Following the sacrifice of the rats, the left testes and epididymis were carefully excised, weighed, and fixed in 10% Neutral Buffered Formalin. The tissues were then processed through dehydration, clearing, infiltration, and embedding steps using increasing concentrations of alcohol, xylene, and paraffin wax, respectively. Sections of 5 μm were cut and mounted on glass slides.

Immunohistochemistry for Bcl-2 was performed using the ImmPRESS™ HRP Polymer System (Vector Labs, USA). Paraffin-embedded tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval with a citrate solution. After blocking endogenous peroxidase and proteins, the sections were incubated with primary rabbit anti-Bcl-2 antibody, followed by ImmPRESS™ HRP Anti-Rabbit IgG Reagent. Staining was developed with DAB and counterstained with hematoxylin. Photomicrographs were taken at x400 magnification, and image analysis was done using Image J software to quantify positive immunoreactive cells.

2.10 Statistical Analysis

The data were analyzed with one-way analysis of variance (ANOVA) in GraphPad Prism 8. The Neuman-Keuls post hoc method was utilized. P values below 0.05 were deemed significant. Data is expressed as Mean ± S.E.M.

3. Results

3.1 Effect of fractions of IG on serum follicle stimulating hormone

There was a significant decrease in serum FSH levels

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in Group II (60 mg/kg lead acetate) compared to the control group (Group I) ($f = 2.172$, $p = 0.049$), as indicated by the asterisk (*). This decrease was also observed in Group IX (recovery group), which continued to show lower FSH levels after 28 days of recovery without treatment. However, treatment with both the EEIG and EAF fractions significantly reversed the FSH reduction, with varying efficacy depending on the dose and type of fraction. Specifically, the 125 mg EEIG (Group III) and 500 mg EEIG (Group V) significantly increased FSH levels compared to Group II, with the 500 mg dose showing a more pronounced effect. Similarly, all doses of EAF (50 mg, 100 mg, and 200 mg; Groups VI, VII, and VIII) significantly improved FSH levels compared to Group II, with Group VII (100 mg EAF) showing the highest restoration.

When comparing the two fractions, both EEIG and EAF fractions demonstrated a dose-dependent effect on restoring FSH levels. However, the EAF at 100 mg (Group VII) was more effective in reversing the decrease in FSH compared to the higher doses of EEIG (500 mg, Group V), suggesting that the EAF fraction may have a more potent effect on restoring FSH levels after lead acetate exposure. Interestingly, the recovery group (Group IX), which received only distilled water after the lead exposure, did not show significant improvement in FSH levels, indicating that the therapeutic effect of the fractions was necessary for significant hormonal restoration (Fig 2).

3.2 Effect of fractions of IG on serum luteinizing hormone

There was a significant decrease in serum LH levels in Groups II (60 mg/kg lead acetate), III (125 mg EEIG), IV (250 mg EEIG), VI (50 mg EAF), and IX (recovery group) compared to the control group (Group I), as indicated by the asterisk (*). The statistical analysis showed a significant overall effect ($F = 2.517$, $p = 0.027$), indicating that the lead acetate exposure and recovery period without treatment led to a significant reduction in LH levels.

However, treatment with 100 mg (Group VII) and 200 mg (Group VIII) doses of EAF significantly increased LH levels compared to Group II, as denoted by the hash sign (#). This suggests that the EAF fractions were effective in reversing the LH reduction caused by lead acetate exposure.

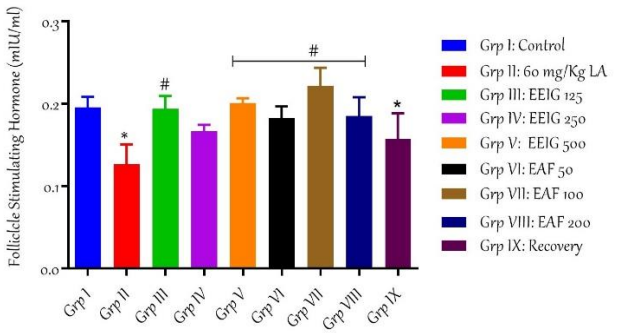

Figure 2. Effects of EEIG and EAF on concentration of follicle stimulating hormone in rats following lead acetate-induced toxicity (Results are expressed as Mean ± SEM, n = 5. * Indicates a significant difference from the control, whereas # denotes a significant difference from group II).

When comparing the two fractions, the EEIG treatments (Group III and Group V) did not significantly restore LH levels to the same extent as the EAF fractions. The 100 mg and 200 mg doses of EAF (Groups VII and VIII) exhibited more pronounced effects on LH restoration compared to the EEIG treatments. Additionally, the recovery group (Group IX), which received only distilled water after lead acetate exposure, did not show significant improvement in LH levels, highlighting the necessity of active treatment for LH restoration.

These results suggest that the EAF fraction of *Irvingia gabonensis* is more effective in mitigating lead acetate-induced disruptions in LH levels, with higher doses demonstrating a stronger therapeutic effect (Fig. 3).

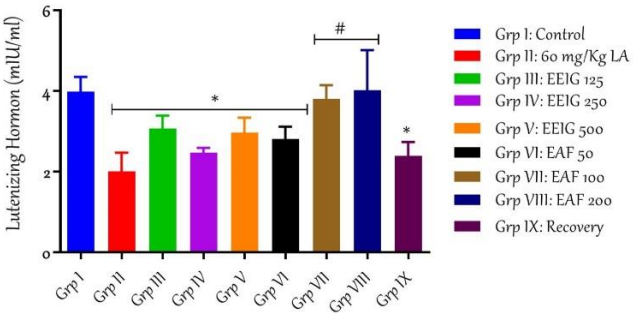

Figure 3. Effects of EEIG and EAF on concentration of luteinizing hormone in rats following lead acetate-induced toxicity. Results are expressed as Mean ± SEM, n = 5. * Indicates a significant difference from the control, whereas # denotes a significant difference from group II.

3.3 Effect of fractions of IG on serum testosterone

Fig. 4 shows the effects of ethanol extract (EEIG) and ethyl acetate fraction (EAF) of *Irvingia gabonensis* on

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serum testosterone levels in male rats following lead acetate-induced reproductive toxicity. There was a significant decrease in serum testosterone levels in Group II (60 mg/kg lead acetate) compared to the control group (Group I), as indicated by the asterisk (*). The statistical analysis revealed a significant overall effect (F = 3.632, p = 0.0034), indicating that lead acetate exposure significantly lowered testosterone levels.

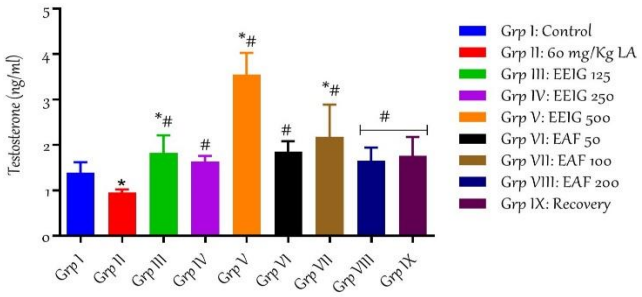

Figure 4. Effects of EEIG and EAF on concentration of testosterone in rats following lead acetate-induced toxicity. Results are expressed as Mean ± SEM, n = 5. * Indicates a significant difference from the control, whereas # denotes a significant difference from group II.

Groups III (125 mg EEIG), V (500 mg EEIG), and VII (100 mg EAF) showed a significant increase in testosterone compared to both Group I and Group II, as denoted by the asterisk (*). Moreover, Groups III, IV (250 mg EEIG), V, VI (50 mg EAF), VII, VIII (200 mg EAF), and IX (recovery group) also exhibited a significant increase in testosterone compared to Group II, as indicated by the hash sign (#).

These results suggest that both EEIG and EAF fractions were effective in reversing the testosterone reduction caused by lead acetate exposure. Notably, the 500 mg EEIG dose (Group V) had the most substantial effect on testosterone restoration, outperforming the other doses of EEIG and EAF. The recovery group (Group IX), which received only distilled water following lead acetate exposure, also showed a significant improvement in testosterone levels compared to Group II, highlighting the importance of active treatment for hormonal restoration (Fig. 4).

3.4 Effect of fractions of IG on testicular superoxide dismutase

There was a significant decrease (f = 4.165, p = 0.0013) in the testicular concentration of SOD in group II compared to group I. This was significantly reversed in groups III, IV, V and VIII. Groups V and VIII showed a significant increase in SOD relative to group I. (Fig. 5). When comparing the effects of EEIG and EAF, it appears that EEIG is more effective in increasing SOD levels, as it demonstrated a more significant reversal of the LA-induced decrease in SOD. This higher potency of EEIG could be attributed to its specific components or mechanisms of action, possibly indicating a stronger antioxidant effect or a more potent stimulation of SOD production. On the other hand, while EAF also exhibited a protective effect on SOD levels, the magnitude of the increase was less substantial than that of EEIG, suggesting that EAF might exert its effects through a different mechanism or has a lower antioxidant capacity.

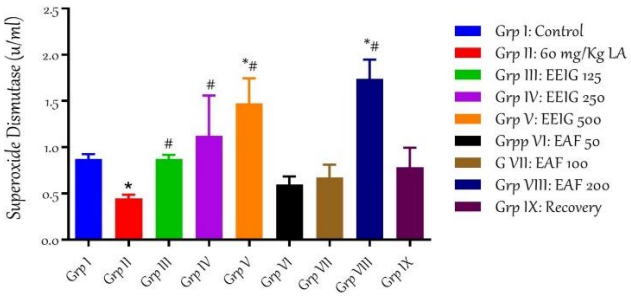

Figure 5. Effects of EEIG and EAF on concentration of superoxide dismutase in rats following lead acetate-induced toxicity

3.5 Effect of fractions of IG on testicular malondialdehyde

There was a significant increase (f = 49.21, p = 0.0001) in testicular concentration of MDA in groups II and IX compared to group I. However, this was significantly reversed in groups III, IV, V, VI, VII, and VIII (Fig. 6). EEIG appears to be more potent in decreasing MDA levels compared to EAF. Groups treated with EEIG showed a more significant reversal of the LA-induced increase in MDA. While EAF also showed an antioxidant effect on MDA, the magnitude of the decrease was less pronounced compared to EEIG.

3.6 Effect of fractions of IG on testicular 8-hydroxydeoxyguanosine (8-OHdG)

There was a significant increase (f = 5.934, p = 0.0001) in testicular concentration of 8OHdG in groups II, IV and IX compared to group I. This was significantly reversed in Groups III, IV, V, VI, VIII and VIII

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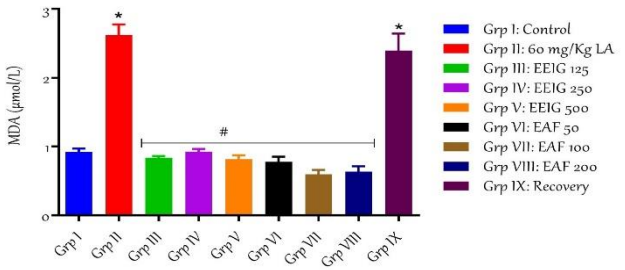

Figure 6. Effects of EEIG and EAF on concentration of MDA in rats following lead acetate-induced toxicity.
Results are expressed as Mean ± SEM, n = 5. * Indicates a significant difference from the control, whereas # denotes a significant difference from group II.

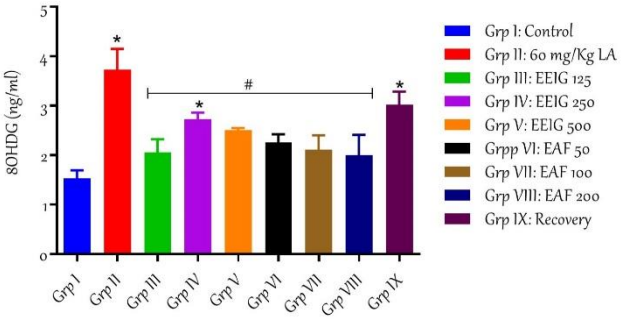

Figure 7. Effects of EEIG and EAF on concentration of 8OHdG in rats following lead acetate-induced toxicity.
Results are expressed as Mean ± SEM, n = 5. * Indicates a significant difference from the control, whereas # denotes a significant difference from group II.

compared to group II (Fig. 7). In this study, the effects of EEIG and EAF on 8-OHdG levels were assessed to determine their role in mitigating oxidative DNA damage caused by lead acetate (LA) exposure.
The control group (Group I) had baseline levels of 8-OHdG, showing no oxidative DNA damage. However, LA-treated Group (Group II) exhibited a significant increase in 8-OHdG levels, indicating that lead acetate exposure led to oxidative DNA damage.
The EEIG-treated groups (Groups III, IV, V) showed a notable decrease in 8-OHdG levels compared to the LA-treated group. This suggests that EEIG has a potent antioxidant effect, effectively reducing oxidative DNA damage induced by lead exposure. The reduction in 8-OHdG was more significant in these groups, highlighting EEIG's stronger protective properties.
Similarly, the EAF-treated groups (Groups VI, VII, VIII) demonstrated a decrease in 8-OHdG levels compared to the LA-treated group, but the decrease was less pronounced than that seen with EEIG. This suggests that while EAF also exhibits antioxidant activity, it may not be as effective as EEIG in reversing oxidative DNA damage.
The recovery group (Group IX) showed a significant increase in 8-OHdG levels compared to the control group, suggesting that even after the cessation of lead exposure, complete recovery from oxidative DNA damage was not achieved.
When comparing EEIG and EAF, it was evident that EEIG was more effective in reducing 8-OHdG levels, indicating that EEIG may possess stronger antioxidant properties or more potent bioactive components. On the other hand, while EAF also exhibited some antioxidant activity, its effect on reducing 8-OHdG was less significant than EEIG's.
Overall, both EEIG and EAF demonstrated protective effects against oxidative DNA damage caused by lead acetate, with EEIG showing a more substantial ability to reduce oxidative stress. This suggests that EEIG may offer a more potent therapeutic option for mitigating lead-induced reproductive toxicity.

3.7 Effect of fractions of IG on testicular tumor necrosis factor – α

There was a significant increase ($f = 4.476$, $p = 0.0008$) in testicular concentration of TNF-α in Groups II and IX compared to group I. This was however, significantly reversed by 200 mg EAF in group VIII compared to group II (Fig. 8).

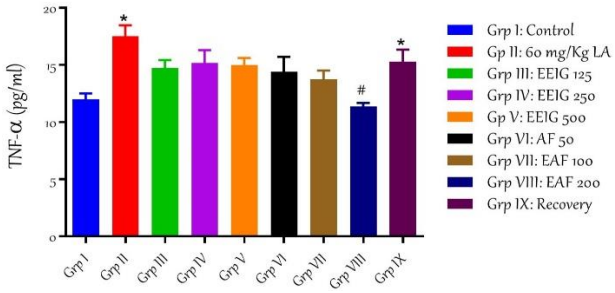

Figure 8. Effects of EEIG and EAF on concentration of TNF-α in rats following lead acetate-induced toxicity.
Results are expressed as Mean ± SEM, n = 5. * Indicates a significant difference from the control.

The Control Group (Group I) had baseline TNF-α levels, showing no inflammatory response. In contrast, the LA-treated Group (Group II) exhibited a significant increase in TNF-α, indicating that LA exposure induced inflammation.

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The EEIG-treated groups (Groups III, IV, V) showed a decrease in TNF-α levels compared to the LA-treated group, although this reduction was not statistically significant. This suggests that EEIG may have some anti-inflammatory effects, but its ability to reduce TNF-α levels is less pronounced compared to other groups.

Similarly, the EAF-treated groups (Groups VI, VII, VIII) also exhibited a decrease in TNF-α levels compared to the LA-treated group. However, only the 200 mg EAF group (Group VIII) showed a significant decrease in TNF-α levels, suggesting that EAF might have an anti-inflammatory effect at higher doses.

The recovery group (Group IX) showed a significant increase in TNF-α levels compared to the control group, suggesting that even after the cessation of LA exposure, complete recovery from inflammation was not achieved.

When comparing EEIG and EAF, it was observed that EEIG did not show a significant reduction in TNF-α levels, indicating that it might not be as effective in reducing inflammation as EAF. In contrast, EAF demonstrated a more pronounced anti-inflammatory effect, particularly at the 200 mg dose, indicating that higher doses of EAF may be more effective in modulating inflammation.

Overall, while both EEIG and EAF showed some potential to reduce inflammation, **EAF** appears to be more effective at higher doses. Further research is needed to explore the mechanisms by which EAF exerts its anti-inflammatory effects and to investigate other inflammatory markers that could provide a more comprehensive understanding of their impact on testicular health.

3.8 Effect of fractions of IG on testicular caspase-3

There was a significant increase ($f = 7.321$, $p = 0.0001$) in testicular Caspase-3 concentration in group II relative to control. This was significantly reversed by both EEIG and EAF in Groups III, IV, V, VI, VII, and VIII compared to group II (Fig. 9). The Control Group (Group I) had baseline Caspase-3 levels, indicating no apoptotic activity. However, the LA-treated Group (Group II) exhibited a significant increase in Caspase-3 levels, suggesting that LA exposure induced apoptosis.

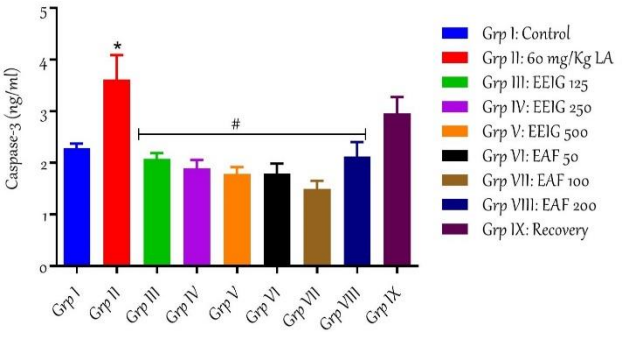

Figure 9. Effects of EEIG and EAF on concentration Caspase-3 in rats following lead acetate-induced toxicity. Results are expressed as Mean ± SEM, n = 5. * Indicates a significant difference from the control, whereas # denotes a significant difference from group II.

The EEIG-treated groups (Groups III, IV, V) showed a significant decrease in Caspase-3 levels compared to the LA-treated group, indicating that EEIG has a strong anti-apoptotic effect. This suggests that EEIG is effective in reducing apoptosis induced by LA.

The EAF-treated groups (Groups VI, VII, VIII) also showed a decrease in Caspase-3 levels compared to the LA-treated group, though the reduction was not as significant as observed in the EEIG-treated groups. This indicates that while EAF has some anti-apoptotic effects, it may be less potent than EEIG in reducing apoptosis.

The Recovery Group (Group IX) showed a significant increase in Caspase-3 levels compared to the control group, suggesting that complete recovery from apoptosis was not achieved even after cessation of LA exposure.

When comparing EEIG and EAF, EEIG appears to be more effective in decreasing Caspase-3 levels, showing a more significant reversal of LA-induced apoptosis. In contrast, EAF demonstrated a weaker effect, indicating that EEIG may be more potent in inhibiting apoptosis.

3.9 Effect of fractions of IG on expression of testicular Bcl – 2

Groups I, IV, V, VI, VII, and VIII showed normal expression of Bcl-2 (green arrow) in the testis compared to group II. There was a significant reduction in Bcl-2 expression (black arrow) in Groups II, III and IX compared to Groups I, IV, V, VI, VII, and VIII (Plate 1).

3.10 Quantification of Bcl-2

There was a significant decrease ($f = 31.35$, $p = < 0.0001$)

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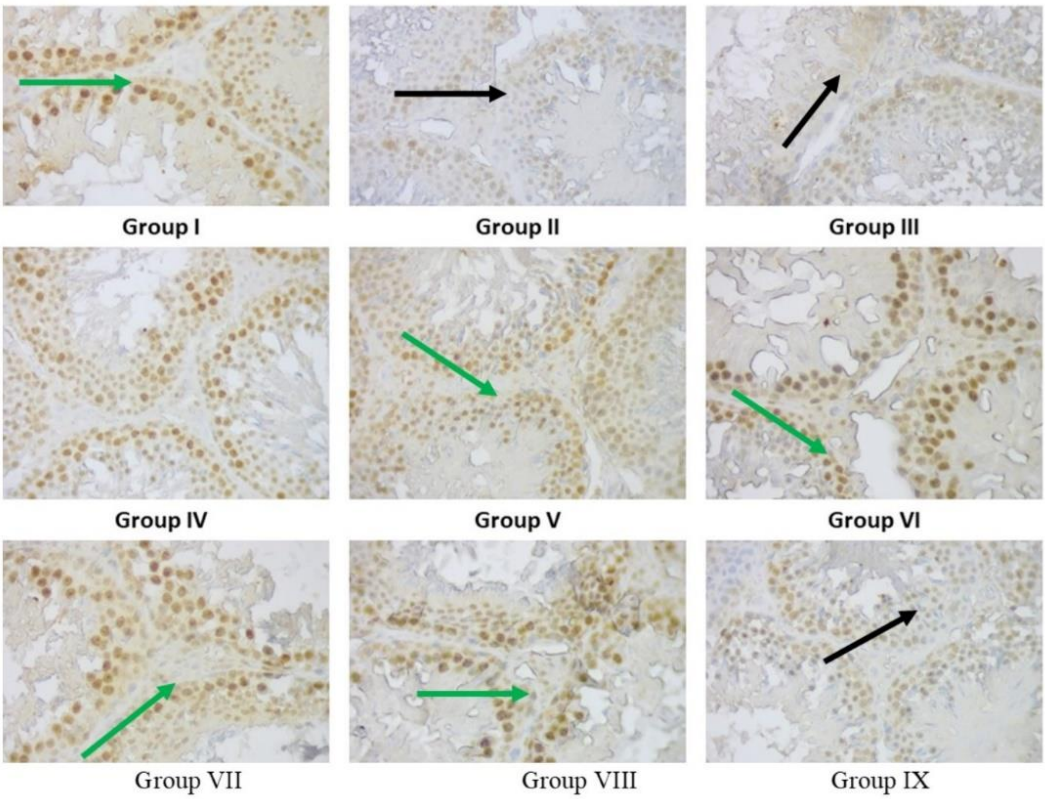

Plate 1. Representative photomicrographs of testis of treated rats showing expression of Bcl-2 (× 400) (Green arrow: area with appreciable Bcl-2 expression, Black arrow: area with reduced Bcl-2 expression).
(Grp I: Control, Grp II: 60mg/Kg LA, Grp III: EEIG 150, Grp IV: EEIG 250, Grp V: EEIG 500, Grp VI: EAF 50, Grp VII: EAF 100, Grp VIII: EAF 200, Grp IX: Recovery).

in testicular expression of Bcl-2 in Groups II, III and IX compared to group I. This was however reversed by 250 mg, 500 mg EEIG (Groups IV and V respectively), 50 mg, 100, and 200 mg EAF in Groups VI, VII and VIII respectively compared to group II (Fig. 10).

4. Discussion

This study demonstrated that exposure to lead acetate (PbAc) resulted in hormonal disturbance of the pituitary-gonadal axis, characterized by a significant decrease in serum FSH, LH, and testosterone levels (Figs 2-4). Hormonal decline due to Pb exposure is ascribed to the dysfunction of the hypothalamus-pituitary-gonadal axis. Lead exposure causes degradation of the gonadotrophic cells in the pituitary gland [21] and triggers apoptotic signals in the Leydig cells [22]. Moreover, Pb impedes the synthesis of steroidogenic enzymes in Leydig cells, leading to decreased testosterone release [21].

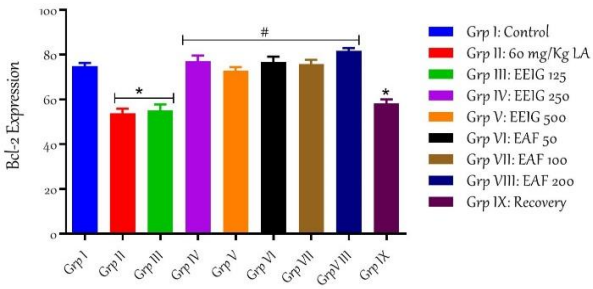

Figure 10. Quantification of expression of Bcl-2 in rats following lead acetate-induced toxicity

Luteinizing hormone is a crucial stimulant for testosterone release from Leydig cells in the testes. An adequate level of testosterone is crucial for the structural and functional integrity of reproductive organs and the maintenance of male accessory glands' structure and function. The diminished testosterone levels in Pb-intoxicated rats in this current study may be attributed to the decreased

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responsiveness of Leydig cells to LH, inhibition of enzymes essential for steroid hormonal biosynthesis, a reduction in the number of LH binding sites in Leydig cells or a decline in the synthesis and secretion of LH from the anterior pituitary [23]. The latter is very probable, given there was a significant reduction in blood LH levels in groups administered PbAc. These may ultimately have significant repercussions on reproductive function, as spermatogenesis and Leydig cells seem to be affected by lead exposure. Follicle-stimulating hormone (FSH) secretion is essential for optimal spermatogenesis, as it promotes the proliferation, maturation, and functionality of Sertoli cells, which in turn generate signals necessary for the initiation and sustenance of germ cells [24].

PbAc has been documented to induce pathogenic changes in Leydig cells, leading to androgen deprivation [25, 26]. The Leydig cells in the testes secrete testosterone upon stimulation by LH and regulate spermatogenesis in the seminiferous tubules. Spermatogenesis is augmented by follicle-stimulating hormone (FSH) and testosterone. The decrease in serum sex hormone levels in the lead only-treated group in the present study may be a result of oxidative stress-induced damage to Leydig cells resulting from Pb poisoning [25].

Treatment with EEIG and EAF resulted in an improved hormonal profile in the treated rats compared to group II. EEIG at 500 mg/kg significantly elevated serum testosterone levels. This increase could be due to the potential ability of EEIG to influence the hypothalamic-pituitary-gonadal axis, potentially by modulating the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are critical for testosterone production. EAF at 100 and 200 mg/kg also significantly elevated the levels of FSH, LH, and testosterone. The distinct differences in action and intensity between EEIG and EAF could stem from their varied chemical compositions. EEIG may contain higher concentrations of bioactive compounds like flavonoids and polyphenols, which could have a more potent effect on hormonal regulation. In contrast, EAF may have a different profile of bioactive components, contributing to its effectiveness, but with a less pronounced impact than EEIG. It would be useful for the authors to correlate these hormonal changes with the observed effects on FSH and LH. For example, improvements in testosterone levels may be a direct result of changes in FSH and LH, suggesting that the hormonal cascade triggered by EEIG and EAF impacts testosterone production through these intermediary hormones. This may occur via reducing the Pb^{2+} content in testicular tissue, mostly by displacement and inactivation, thus elevating serum levels of sex hormones. An essential way by which IG (Irvingia gabonensis) may function is by the donation of elements or molecules such as electrons, protons, or functional groups (e.g., hydroxyl or methyl groups). These molecules could play a role in antioxidant defense, enzymatic activities, or hormone regulation, contributing to the therapeutic effects observed in the study. For example, polyphenols and flavonoids present in IG are known to donate electrons and protons, which may neutralize free radicals, reduce oxidative stress, and thereby protect against cellular damage. Additionally, certain bioactive compounds in IG might interact with molecular targets involved in metabolic and hormonal regulation, thus enhancing the physiological processes involved in reproductive health [7]. These effects may also influence the pituitary gland and, therefore, the brain through the modulation of signaling pathways that regulate the hypothalamic-pituitary-gonadal (HPG) axis. Bioactive compounds in Irvingia gabonensis (IG) could act on the hypothalamus to stimulate the release of gonadotropin-releasing hormone (GnRH), which in turn promotes the pituitary gland to secrete gonadotropins, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropins are critical in regulating testicular function, stimulating Leydig cells in the testes to produce and secrete increased levels of testosterone. By enhancing this hormonal cascade, IG may support reproductive health and improve testosterone production. The presence of antioxidant phytochemicals, such as flavonoids in IG, may induce metal chelating activity, hence displacing Pb^{2+} from its binding site in the testis [3]. Toxic effects of Pb have been linked to the ability of

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this metal to induce apoptosis. Studies have indicated that apoptosis is associated with lead-induced cytotoxicity in several experimental studies, including rat brain, rat testis, rat fibroblasts, rat lung, and rat or mouse retinal rod cells [27–30]. In neuronal cells, lead-induced apoptosis has been studied only in cerebellar neurons, rat hippocampus, and rod – photoreceptors [31]. In this study, lead acetate significantly altered the biomarkers of cellular apoptosis – caspase-3 which is a pro apoptotic protein and Bcl-2, an anti-apoptotic protein – in the testis of rats [32].

Lead induced a significant increase in testicular caspase- 3 expression indicating that it provokes apoptosis in the rat testis, indicative of an increase in the death of testicular cells [32]. Apoptosis is a physiological process of selected cell deletion. As an antagonist of cell proliferation, apoptosis contributes to keeping the cell number in testicular tissue and helps to remove superfluous and damaged cells [33], but excessive apoptosis could cause destruction of male reproductive function as seen in this study. Lead acetate, increases the expressions of caspase-3, which induced apoptosis of germ cells [34]. One mechanism of activation of caspases is through tumor necrosis factor (TNF) family receptors, which use caspase activation as a signaling mechanism and connecting ligand binding at the cell surface to induce apoptosis. Another possible activator of caspase is the participation of mitochondria, by releasing caspase activating proteins into the cytosol, thereby triggering apoptosis.

EEIG and EAF significantly reduced the activation of caspase 3, suggesting that these fractions may inhibit the apoptotic process by blocking caspase activation. Irvingia gabonensis is characterized by a high concentration of pharmacologically active compounds that function as caspase inhibitors. These compounds may include flavonoids, polyphenols, and other bioactive molecules known for their antioxidant and anti-apoptotic properties.

An important component of the apoptotic pathway is the family of proteins commonly known as the B cell lymphoma-2 (Bcl-2). Bcl-2 oncogene is a generalized cell death suppressor gene that directly regulates apoptosis. Many cells in the body have Bcl-2 proteins in their mitochondrial membranes, nucleus and endoplasmic reticulum. The primary role of Bcl-2 family members is the regulation of apoptosis [35]. The disruption of the regulation of apoptosis is a causative event in many diseases. Since the Bcl-2 family of proteins is the key regulator of apoptosis, its dysfunction has been implicated in many diseases including cancer, neurodegenerative disorders, ischemia and autoimmune diseases [35].

This study found that PbAc intoxicated rats exhibited a significant increase in apoptotic activity, which was associated with a decrease in Bcl-2 expression. The reduced expression of Bcl-2 likely precipitated the increased apoptotic activity, as Bcl-2 is an anti-apoptotic protein that plays a critical role in inhibiting cell death. A decrease in Bcl-2 expression would disrupt the regulation of apoptosis, leading to heightened apoptotic activity. However, the administration of EEIG and EAF decreased apoptosis by decreasing oxidative stress, this is either by scavenging Pb ions and preventing their toxic effect or by scavenging free radicals. The expression of testicular Bcl-2 was however significantly increased following post administration of IG, this suggests that it is able to block the apoptotic reactions and further cell death and downregulate pro-apoptotic proteins (caspase–3). EEIG and EAF induced upregulation (increased expression) of the anti-apoptotic gene Bcl-2. This increased Bcl-2 expression suggests an enhanced ability of the system to protect cells from apoptosis and prevent cell death. [36].

Exposure to PbAc dramatically elevates the levels of the inflammatory marker TNF α in testicular tissue. Oxidative stress has been shown to activate transcription factors, including NF-κB, leading to the production of inflammatory markers [12]. PbAc induces a disruption in the redox equilibrium within testicular tissue, directly resulting in the upregulation of inflammatory markers, including TNF-α and IL-1β. EEIG and EAF mitigated inflammation through antioxidant mechanisms, either by neutralizing reactive oxygen species (ROS) or by activating the Nfe-2l2 pathway. Prior research has shown that IG demonstrates anti-inflammatory effects by suppressing mRNA expression of TNF-α, IL-1β, IL-6, and cyclooxygenase-2, as well as downregulating Nos2 expression and reducing its product, nitric oxide, in macrophages exposed to

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lipopolysaccharide [37]. In this scenario, IG suppressed the excessive generation of TNF-α through its reactive oxygen species (ROS) scavenging capacity by neutralizing free radicals and oxidative stress. By reducing oxidative damage, IG likely inhibited the activation of inflammatory pathways that lead to increased TNF-α production. This antioxidant activity helps to modulate the inflammatory response, preventing the overproduction of pro-inflammatory cytokines like TNF-α.

Genotoxic agents such as lead have been documented to compromise DNA integrity either directly by generating reactive oxygen species or indirectly by modifying the enzymes involved in DNA repair [38]. This study revealed that 8OHdG, a measure of DNA fragmentation index (DFI), was considerably elevated in the germ cells of PbAc-treated rats compared to the control group.

DNA fragmentation in germinal cells is a natural occurrence that may transpire during spermatogenesis. Lead exposure has led to an increase in nuclear DNA fragmentation in germ cells, perhaps due to an overproduction of reactive oxygen species (ROS). Lead exposure stimulates the generation of intracellular reactive oxygen species (ROS), which can cause morphological and genetic damage to the testes. Reactive oxygen species (ROS) are known to damage the polyunsaturated fatty acids in phospholipids inside cell membranes, leading to impaired cellular function and contributing to gene alterations. Reactive oxygen species may generate genetic modifications, including point mutations, since oxidative DNA damage, signified by 8-OHdG, can lead to DNA base mutations such as G>T/C>A transversions [39]. Oxidative stress constitutes merely one of several mechanisms responsible for DNA fragmentation; others encompass germ cell apoptosis during spermatogenesis and defects in chromatin remodelling and compaction during spermiogenesis. Lead exposure can compromise the DNA integrity of germline cells, potentially resulting in cellular death. Under normal settings, apoptosis is a natural mechanism that regulates the cell population in testicular tissue and removes damaged cells. Excessive apoptosis may disrupt male reproductive function. Moreover, it has been observed that germinal DNA fragmentation is typically more significant in spermatids, corroborating the assertion that PbAc mostly impacts spermiogenesis. This may be elucidated by a chemical mechanism, since lead may disrupt the DNA of germ cells and hinder the normal progression of nuclear condensation, a vital phase in spermiogenesis [32].

In the groups administered IG extracts, the testicular concentration of O8HdG was significantly decreased compared to the PbAc group, indicating a reduction in DNA damage. The potential of IG to counteract the reduction in the activity of antioxidant enzymes like SOD is likely due to its bioactive compounds that promote antioxidant defense mechanisms. IG may enhance the expression or activity of SOD by neutralizing reactive oxygen species (ROS), thereby reducing oxidative stress. This antioxidant action helps restore the balance of oxidative and antioxidative processes in cells, preventing further damage to tissues and supporting the proper function of enzymes like SOD, which are crucial for protecting cells from oxidative damage. The reduction in lipid peroxidation products and the enhancement of cellular activity in testicular tissue, following IG treatment in this study, further indicate increased free radical scavenging and improved detoxification of hydrogen peroxide and lipid hydroperoxides, which are potent agents that induce DNA fragmentation.

Elevated testicular levels of MDA have been implicated in cases of sperm cells exhibiting a higher percentage of aberrant morphology and greater mortality. Cells have defensive mechanisms against the detrimental effects of reactive oxygen species (ROS). Superoxide dismutase (SOD) neutralizes superoxide radicals, transforming them into hydrogen peroxide (H_2O_2), which is then rapidly converted to water by catalase (CAT) or glutathione peroxidase (GPx) [40]. Additionally, glutathione peroxidase converts lipid hydroperoxides into alcohols. The inhibition of any antioxidant enzymes may result in harmful consequences owing to the buildup of superoxide radicals and hydrogen peroxide [29]. IG extracts markedly reduced the

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MDA levels in rats treated with lead acetate. The activities of the antioxidant enzyme superoxide dismutase in the testis were normalized after IG treatment compared to rats treated with lead acetate. This observation, together with the decreased malondialdehyde (MDA) levels in the IG-treated groups, indicates the reactive oxygen species (ROS) scavenging ability of IG such as flavonoids.

Treatment with IG improved the balance of oxidative stress by reducing oxidants and enhancing antioxidant enzyme activity. Specifically, IG reduced the levels of oxidative markers such as MDA and 8OHdG, which were measured in this study, while boosting the activity of antioxidant enzymes like SOD. This combination helped mitigate the effects of oxidative damage, improving the overall oxidative status in the treated rats. This plant is abundant in flavonoids and ascorbic acid, which have demonstrated significant antioxidant properties. These antioxidants function by donating electrons to oxidants, resulting in the formation of molecules that are incapable of oxidizing other substances. Lead may attract electrons from lipid molecules, resulting in the formation of lipid peroxide. The antioxidants in IG can donate electrons to lead ions and inhibit fatty acid oxidation, resulting in a substantial reduction of MDA levels in all groups treated with IG extract. The antioxidants from IG may have inhibited the binding of Pb2+ to sulfhydryl groups in proteins, including cellular antioxidants, as Pb2+ can obstruct antioxidant inactivation while concurrently binding with oxidants, thereby averting cellular damage from oxidants.

The phytochemical analysis of ethanol and ethyl acetate fractions of *Irvingia gabonensis* seed extracts (EEIG and EAF) demonstrated a significant polyphenol content [3], which aids in alleviating oxidative stress caused by lead (Pb^{2+}) exposure by improving redox homeostasis and safeguarding cells from reactive oxygen species (ROS). This corresponds with earlier research suggesting that IG can mitigate xenobiotic-induced organ damage by enhancing antioxidant levels. The research indicated that IG fractions had substantial ferric ion reducing antioxidant activity (FRAP), associated with phenolic chemicals recognized for their strong free radical scavenging abilities. Assays such as DPPH and total antioxidant capacity (TAC) demonstrated significant antioxidant capabilities, indicating the critical function of phenols in neutralizing free radicals [3].

Subsequent investigation revealed that IG fractions comprise phytochemicals like flavonoids, alkaloids, glycosides, saponins, sterols, and tannins, each offering various medicinal advantages [3]. Tannins facilitate inflammation reduction, evidenced by decreased TNF-α in testicular tissues, whereas saponins and sterols have antioxidant, immunostimulatory, and wound-healing properties.

5. Conclusions

This study found that lead acetate (PbAc) disrupts the pituitary-gonadal axis, leading to hormonal imbalances, oxidative stress, apoptosis, and inflammation in testicular tissue. PbAc exposure reduced reproductive hormones and increased markers of apoptosis and inflammation, impairing testicular function and DNA integrity. Treatment with Irvingia gabonensis extracts (EEIG and EAF) effectively counteracted these effects by restoring hormone levels, reducing oxidative stress, and enhancing anti-apoptotic Bcl-2 expression. IG extracts also protected testicular cells from Pb-induced damage, suggesting their potential as a natural remedy against Pb-induced reproductive toxicity.

Ethical statement

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

Authors' contributions

Conceptualized, experimented and wrote the first draft of the manuscript, O.P.A.; Designed and supervised the experiment, O.S.A.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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