**Exploring the Therapeutic Potential of *Psidium guajava* (Guava) Leaf Extract in Lead Acetate-Induced Male Reproductive Dysfunction in Albino Rats**

 **ABSTRACT**

This study investigated the therapeutic potential of Psidium guajava (guava) leaf extract against lead acetate-induced male reproductive dysfunction in albino rats. Twenty-eight male albino rats were randomly divided into 4 groups (n=7 per group): Group A (negative control) received only food and water; Group B (positive control) received 30 mg/kg of lead acetate once daily for 14 days; groups C, and D, received 30 mg/kg of lead acetate once daily for 14 days followed by 250and 500 mg/kg of guava leaf extract respectively, once daily for another 14 days. After the treatment period, rats were anesthetized, sacrificed, and blood samples were collected into plain bottles through cardiac puncture for the assay of rat-specific luteinizing hormone (rLH), follicle stimulating hormone (rFSH), testosterone, malondialdehyde (MDA), and superoxide dismutase (SOD) using an enzyme-linked immunosorbent assay (ELISA). Epididymis samples were collected for semen analysis, and testes were processed for histological examination. Statistical analysis was performed using GraphPad Prism, with significance set at p<0.05. The results indicated that flavonoids are the most abundant phytochemicals present in guava leaves, with a concentration of 8.01 mg/mL. Sperm motility and count showed considerable improvement in rats treated with 250 and 500 mg/kg of extract following exposure to lead acetate. The seminal pH was restored to near-neutral values in the guava-treated rats following lead acetate administration for 14 days. Lead-induced higher values of LH and FSH were considerably reduced in 250 and 500 mg/kg treated rats. However, the induced significant fall in the testosterone and testosterone-LH ratio values was observed to be tremendously elevated in the 250 and 500 mg/kg treated rats. Finally, considerable fall and rise in the values of MDA and SOD respectively were observed in rats treated with 250 and 500 mg/kg of guava extract after exposure to lead acetate. Dose-dependent responses were observed in FSH and testosterone values. Conclusively, the 250 and 500 mg/kg doses of *Psidium guajava* leaf extract in the form of post-treatment intervention, considerably improved the sperm count, motility, and also restored the near-neutrality of seminal pH. In addition, testosterone, LH, and FSH concentrations were improved towards their optimal concentration.

**Keywords:** *Psidium guajava*, Guava Leaf Extract, Testosterone, Luteinizing Hormone, Follicle Stimulating Hormone, FSH-LH ratios, Testosterone-LH ratio, Lead, Endocrine disruption, Male

1. **INTRODUCTION**

In recent years, environmental pollution has become a significant global concern, particularly regarding the accumulation of heavy metals in various ecosystems (Ben-Chioma et al. 2023). Heavy metals are natural components of the earth's crust; however, anthropogenic activities such as industrial processes, gas and oil activities, mining, and agricultural practices have significantly contributed to their release into the environment, leading to widespread contamination (Elekima et al. 2024). Among these heavy metals, lead (Pb) stands out as one of the most toxic and pervasive pollutants, posing serious threats to human and animal health due to its persistent nature and ability to accumulate in living organisms (Elekima et al. 2020).

Guava (*Psidium guajava* L.) is a tropical fruit widely cultivated and consumed for its nutritional and medicinal properties. Guava leaves, in particular, have gained attention for their rich phytochemical composition, including flavonoids, tannins, terpenoids, and polyphenols, which exhibit various therapeutic effects such as antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic properties (Kumar et al. 2021; Chinatu et al. 2023). Additionally, guava leaves have been traditionally used in the treatment of various ailments, including gastrointestinal disorders, wounds, and skin infections (Kumar et al., 2021). Moreover, recent research has shown promising therapeutic properties of guava leaf extract against heavy metal-induced toxicity (Kumar et al*.* 2021). Studies have demonstrated its ability to mitigate oxidative stress, inflammation, and cellular damage induced by lead (Boskabady et al. 2018; Ruksiriwanich et al. 2022). Therefore, the essence of this work is to investigate the therapeutic (post-treatment) potential of guava leaf extract in lead acetate-induced male reproductive dysfunctions in albino rats.

Male reproductive dysfunction refers to any condition that affects a man’s ability to fertilize a viable ovum (Zegers-Hochschild et al. 2017; Aworu et al. 2022). It encompasses a range of conditions and can result from various factors, including exposure to hormonal imbalances, environmental pollutants, lifestyle choices, and underlying medical conditions (Aworu et al., 2022; Oni et al. 2023). Reproductive dysfunction impacts male reproductive health, potentially leading to decreased sperm quality, production, and motility, and reduced testosterone levels, among others. Toxin-induced infertility could affect both men and women and may stem from various biological, environmental, and lifestyle factors (Zegers-Hochschild et al. 2017; Aworu et al. 2022). In men, toxin-induced infertility often leads to abnormalities in sperm production, motility, and morphology as well as hormonal disruptions (Carlo et al. 2023; Ruksiriwanichet al. 2022). Heavy metals, such as lead, cadmium, and mercury have been linked with impaired sperm quality and reduced fertility in men through the disruption of the antioxidant-oxidation balance (Kumar 2018; Ruksiriwanich et al. 2022). Antioxidants are vital components for cell survival and viability by combating of oxidative stress (Li et al. 2022). While antioxidants are known to combat oxidative stress, their impact on cellular function can be complex, as excessive levels may lead to unintended physiological disruptions (Chaudhary et al*.* 2023).

Therefore, this research is aimed at evaluating the effect of guava leaf extract on testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH), and their ratios as well as oxidative markers such as malondialdehyde (MDA) and superoxide dismutase (SOD) in male rats exposed to lead acetate over a period of 14 days.

**2. MATERIALS AND METHODS**

**2.1 Materials**

Materials used in this study include guava leaf from Yeghe, Khana, Rivers State, Nigeria, lead acetate (Molychem, India), pH meter, Olympus microscope, centrifuge, electronic weighing balance, 10% formal saline, Microplate Reader, Shandon AS 325 Rotary Microtome, Leica tissue processor, Haematoxylin and Eosin stain, rLH, rFSH, and testosterone ELISA Kits purchased from Calbiotech, USA. MDA and SOD ELISA kits were purchased from Elabscience, India.

**2.2 Experimental Animal**

Male rats were obtained from the Department of Anatomy at the College of Medical Sciences, Rivers State University, Port Harcourt, and transported in well-ventilated, wired cages to the animal house located in the Department of Animal and Environmental Sciences at Rivers State University, Port Harcourt. They were kept in a controlled environment with a 12-hour light/dark cycle. Additionally, the rats had unrestricted access to solid poultry chow as their food and water *ad libitum*. Prior to the commencement of the study, a 14-day acclimatization period was observed.

**2.3 Collection and Preparation of Guava Leaf**

The guava leaves grown in Yeghe, Rivers State, were obtained and identified at the Department of Plant Science and Biotechnology, Rivers State University by Dr. M. G. Ajuru, a plant specialist. The guava leaves were carefully sorted to remove any dead matter or unwanted particles. The leaves were left to air-dry for 14 days at room temperature, after which they were finely ground using a blender. And 600g were measured into 1000mL of 80% v/v ethanol for 72 hours. The extraction was carried out using a Soxhlet extractor at a temperature of 600C. The yielded crude weighed 15.62g and was transferred into a sample bottle and refrigerated at 40C before the commencement of the experiment.

**2.4 Experimental Design**

A total of 28 male albino rats weighing 200 grams were randomly divided into 4 groups, 7 rats per group.

**Group A** = Negative control, given food and water only

**Group B** = Positive Control, treated with 30mg/kg of lead acetate daily for 14 days

**Group C** = Treated with 30mg/kg of lead acetate daily for 14 days, followed by 250mg/kg treatment of guava leaf extracts daily for another 14 days

**Group D** = Treated with 30mg/kg of lead Acetate daily for 14 days, followed by 500mg/kg treatment of guava leaf extracts daily for another 14 days.

The LD50 of lead acetate was established following the Kerber-Breham method as described by Isrea et al. (2021), while the dose selection of guava leaf extract was established following Lork’s method as described by Enegide et al. (2013)

**2.5 Specimen Collection, Preparation, and Analysis**

At the end of the experimental study, the rats were allowed to fast overnight and anaesthetized using chloroform followed by collection of 5mL of whole blood specimen through cardiac puncture. The collected blood specimens in plain bottles were allowed to clot at room temperature and then spun for 10 minutes at 4500 rpm to obtain the serum. The serum samples were transferred into another plain bottle and stored at -200C pending the time of analysis. LH, FSH, testosterone, MDA, and SOD were assayed using ELISA as described by Engvall & Perlmann (1971). The epididymis was immediately excised to obtain semen used for semen analysis as described by Lars & Jackson (2022). The testes were harvested and fixed in 10% formal saline for histological examination.

**2.6 Statistical Analysis**

Raw data were analysed using GraphPad Prism version 8.02and presented as **Mean±Standard Deviation.** O**ne-way ANOVA** was used to compare the groups alongside the use of Tukey’s multiple test. Statistical significance was set at p<0.05.

**3 RESULTS**

**3.1 Results of the Quantitative Analysis of the Phytochemicals of Guava Leaves**

This result indicates that the most abundant phytochemicals present in guava leaves are flavonoids with a concentration of 8.01mg/ml, relative to saponins, tannins, anthraquinones, alkaloids, and phenols with concentrations of 6.07, 5.03, 0.01, 0.50, and 2.87mg/ml, respectively (Table 1).

**Table 1: Results of the Quantitative Analysis of Phytochemical Components of Guava**

**Leaves**

|  |  |
| --- | --- |
| **Phytochemical** | **Concentration (mg/ml)** |
| Flavonoid | 8.01 |
| Saponins | 6.07 |
| Tannins | 5.03 |
| Anthraquinones | 0.01 |
| Alkaloids | 0.50 |
| Phenol | 2.87 |

**3.2 Results of Measurable Sperm Parameters**

Sperm active motility was significantly higher in Group D (500mg/kg treated group) compared to Group C (250mg/kg treated group) and the positive control group treated with lead acetate (Group D). However, the negative control group (Group A) had a significantly higher value of active motility compared to Groups D, C, and B. In addition, Groups C and D had significantly lower sperm counts compared to Group B but higher values compared to Group A (Table 2). Finally, the pH of the seminal fluid had significantly higher values in Group B compared to the treated groups and the negative control. However, there were no significant differences in the pH between the treated groups and the negative control at p<0.05 (Table 2).

**3.3 Results of Testosterone, Luteinizing Hormone, Follicle Stimulating Hormone, and**

 **their Ratios**

The luteinizing hormone showed significantly lower values in the 250mg/kg (Group C) and 500mg/kg (Group D) treated rats than the positive control rats (Lead acetate-induced rats). However, these groups indicated significantly higher values of LH compared to the negative control. In addition, FSH also indicated a significantly lower value in the 500mg/kg treated rats compared to the 250mg/kg treated group and the positive control group treated with lead acetate. However, their values were significantly higher compared to the negative control group. Moreover, the testosterone levels showed significantly lower values in the 250 and 500mg/kg treated groups compared to the negative control, but their values were significantly higher compared to the positive control rats treated with lead acetate. However, the testosterone values of Group D (500mg/kg treated group) were significantly higher than the 250mg/kg treated group (Group C) (Table 3).

The LH/FSH ratio showed significantly higher values in Group D than Groups C and B, while Group D had a significantly lower value of FSH/LH ratio, than Groups C, A, and B. Finally, the T-LH ratio showed significantly higher values in Group D compared to Groups C, A, and B. All comparisons were set at p<0.05 (Table 3).

**3.3 Results of the Oxidative Stress Markers**

The oxidative stress markers considered were Malondialdehyde (MDA) and superoxide dismutase (SOD). The results of MDA in the 500mg/kg treated group (Group D) showed significantly lower values compared to the 250mg/kg treated group (Group C) and the positive control group (Group B). Likewise, Group C indicated significantly lower values compared to Group B. However, Group A had significantly lower values than Groups B, C, and D at p<0.05 (Table 4).

Regarding SOD, the results indicated significantly higher values of SOD in Groups C and D compared to Group B. However, Group A had significantly higher values compared to Groups B, C, and D at p<0.05 (Table 4).

**Table 2:** **Results (Mean±SD) of Measurable Sperm Parameters in Different Groups of Lead-Induced Testicular Toxicity in**

 **Rats Post-Treated with Guava Leaf Extract**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameters | GROUPA | GROUPB | GROUPC | GROUPD | F value | P value | Remark |
| Active Motile (%) | 81.67±12.1a | 3.75±2.7b | 26.25±8.5c | 48.3±7.64d | 48.69 | <0.0001 | S |
| Dead (%) |  7.0±6.48a | 92.5±9.6b | 65.0±7.07c | 40.0±10.0d | 121.5 | <0.0001 | S |
| Count (cells/ml)x107 | 7.04±0.7c | 0.88±0.8b | 2.0±0.11a | 2.28±1.25a | 3.147 | 0.0221 | S |
| pH | 7.41±0.37a | 8.50±0.6b | 7.75±0.29a | 7.83±0.29a | 4.875 | 0.0026 | S |

**Post Hoc (Tukey’s):** Values in the row with different superscripts differ significantly at p<0.05

**Table 3: Results (Mean±SD) of Different Groups of Lead-Induced Testicular Toxicity in Rats Post-Treated with Guava Leaf**

 **Extract**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameters | GROUPA | GROUPB | GROUPC | GROUPD | F value | P Value | Remark |
| rLH (miu/ml) | 2.40±1.08a | 5.07±0.21b | 3.63±0.37c | 3.22±0.13c | 14.98 | <0.0001 | S |
| rFSH (miu/ml) | 1.72±0.44a | 4.40±0.38b | 3.62±0.31c | 2.50±0.23d | 70.80 | <0.0001 | S |
| TESTO (ng/ml) | 4.57±0.81a | 0.20±0.09b | 1.28±0.08c | 2.10±0.66d | 43.60 | <0.0001 | S |
| rLH/rFSH | 1.49±0.75a | 1.16±0.15b | 1.01±0.16b | 1.29±0.14a | 4.059 | 0.0034 | S |
| rFSH/rLH | 0.86±0.45a | 0.87±0.11a | 1.01±0.14b | 0.78±0.08c | 7.531 | <0.0001 | S |
| T/rLH | 2.14±0.65a | 0.04±0.02b | 0.36±0.04c | 0.66±0.23c | 32.76 | <0.0001 | S |

**Keys:** rLH = Rat Specific-Luteinizing Hormone, rFSH = Rat Specific-Follicle Stimulating Hormone, TESTO = Rat Specific-Testosterone, T/LH = Testosterone-Luteinizing Hormone. **Post Hoc (Tukey’s):** Values in the row with different superscripts differ significantly at p<0.05. Group A = Negative control group, Group B = Positive control group, Group C = Received 30mg/kg of lead acetate for the first two weeks and treated with 250mg/kg of guava leaf extract for another two weeks, Group D = Received 30mg/kg of lead acetate for the first two weeks and treated with 500mg/kg of guava leaf extract for another two weeks.

**Table 4: Results (Mean±SD) of Oxidative Stress Parameter of Different Groups of Lead-Induced Testicular Toxicity in**

 **RatsPost-Treated with Guava Leaf Extract**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameters | GROUPA | GROUPB | GROUPC | GROUPD |  F value | P Value | Remark |
| MDA (ng/ml) | 193.2±26.38a | 397.3±38.21b | 289.8±22.44c | 261.8±21.64d | 40.98 | <0.0001 | S |
| SOD(ng/ml) | 36.0±6.36a | 12.67±2.42b | 25.67±1.37c | 27.67±1.86c | 23.84 | <0.0001 | S |

**Keys:** MDA = Malondialdehyde, SOD = Superoxide dismutase. **Post Hoc (Tukey’s):** Values in the same row with different superscripts differ significantly at p<0.05

**3.4 Histological Examination of Testicular Tissues**

The results of the histological examination of the testes in the negative control and treated groups are shown in plates A to D. The H&E staining technique was adopted. The negative control group (Plate A) showed a well-defined basement membrane (BM) lined with Sertoli cells and spermatogonia alongside distinct Leydig cell areas without clusters. In addition, the photomicrograph showed spermatocytes with defined flagellation in the lumen of the seminiferous tubules migrating toward the basement membrane (BM). Also indicated were well-stained Sertoli cells (arrow) lining the basement membrane. These features are indicative of normal testicular histology.

In the 30mg/kg lead acetate treated group, (Plate B), for 14 days, the histology indicated distorted basement membrane (circled area), distorted and poorly differentiated Leydig cells (LC), gross loss of sertoli cells, spermatogonia (sp), and spermatids in their respective layers alongside vacuolations (V). There was an absence or severe loss of flagellation with aggregated nuclear materials; pycnosis (arrows) in the spermatogonia and spermatids layers of the lumen (F) of the seminiferous tubules, indicative of a degenerated testicular tissue.

Regarding the group C treated group (that is, 30mg/kg of Pb for 14 days + 250mg/kg of Guava Leaf Extract for 14 days) indicated as Plate C, the histology showed an intact basement membrane (BM) with Sertoli cells. The Leydig cells (LC) were well-defined, but there were indications of loss at some junctions. Scanty spermatids were observed in the lumen, and the seminiferous tubules (F) indicated the loss and distortion of flagellated materials of mature spermatocytes migrating toward the basement membrane.

Finally, in Group D-treated with 30mg/kg of Pb for 14 days + 500mg/kg of Guava Leaf Extract for 14 days (Plate D), the basement membrane (BM) was intact with Sertoli cells. There were also clustered and poorly differentiated Leydig cells (LC) as well as a poor number of spermatogonia (SP), spermatids,and flagellation in the lumen (F) of the seminiferous tubules. In addition, there were also aggregations of nuclear materials (pycnosis) in the spermatids and spermatogonia layers of the lumen (rectangular area), as well as loss of testicular parenchymal materials with vacuolation (V). However, the lumen of the seminiferous tubules (F) indicated the improved flagellation in some areas (left lobule) with mature spermatocytes migrating toward the basement membrane, indicative of gross testicular injuries with recovery tendencies.

****

**Plate A:** H&E Stain, Mag., x200, Group A. Treatment: NIL. Well-defined basement membrane (BM) lined with Sertoli cells and spermatogonia. Distinct Leydig cells (LC) areas without clusters. A photomicrograph showed spermatocytes with defined flagellation in the lumen (F) of the seminiferous tubules migrating towards the basement membrane (BM). Also indicated were well-stained Sertoli cells (arrow) lining the basement membrane. Inference: Normal testicular tissue. **Plate B:**H&E Stain, Mag., x200, Group B. Treatment: 30mg/kg of Pb for 2 weeks. Distorted basement membrane (circled area). Distorted and poorly differentiated leydig cells (LC). Gross loss of Sertoli cells, spermatogonia (sp), and spermatids in their respective layers alongside vacuolations (V). Absence/severe loss of flagellation with aggregated nuclear pycnosis (arrows) in the spermatogonia and spermatids layers of the lumen (F) of the seminiferous tubules. Inference: Histology of a degenerated testicular tissue.**Plate** **C:** H&E Stain, Mag., x200, Group C. Treatment: 30mg/kg of Pb for 2 weeks + 250mg/kg of Guava Leaf Extract for 2 weeks. The basement membrane (BM) is intact with Sertoli cells. The Leydig cells (LC) are well-defined but indicated loss at some junctions. The spermatogonia appear close to the Sertoli cells and the basement membrane. Scanty spermatids were also observed in the lumen. The lumen of the seminiferous tubules (F) indicated the loss/distortion of flagellated materials of mature spermatocytes migrating toward the basement membrane. Inference: Severe loss of Leydig cells and flagellated materials. P**late D:**H&E Stain, Mag., x200, Group D. Treatment: 30mg/kg of Pb for 2 weeks + 500mg/kg of Guava Leaf Extract for 2 weeks. The basement membrane (BM) is intact with sertoli cells. Clustered and poorly differentiated Leydig cells (LC). Poor number of spermatogonia (SP) and spermatids, as well as poor flagellation in the lumen (F) of the seminiferous tubules. However, there are aggregations of nuclear materials (pycnosis) in the spermatids and spermatogonia layers of the lumen (rectangular area). There are losses of testicular parenchymal materials with vacuolation (V) as well as a distorted basement membrane (circled area). The lumen of the seminiferous tubules (F) indicated the improved flagellation in some areas (left lobule) with mature spermatocytes migrating toward the basement membrane. Inference: Gross testicular distortion with loss of parenchymal materials indicating recovery tendencies.

**4. DISCUSSION**

The highest concentration of flavonoids observed in our findings amongst other phytochemicals compared to saponins, tannins, anthraquinones, alkaloids, and phenol, is in line with the reports of Arima & Danno (2002) and Chinatu et al. (2023), who reported that guava leaves are rich in flavonoids. The high flavonoid content suggests that guava leaves may serve as a potent natural antioxidant source for medicinal applications. Francis et al. (2002), Hochma et al. (2019), and Dubale et al. (2023) reported that saponins and tannins contributed to the disruption of bacterial membranes and therefore exert antimicrobial effects. Anthraquinones, alkaloids, and phenols are also phytochemicals with antioxidant properties with medicinal importance. Adeyemi et al. (2006) documented that alkaloids such as guavacine present in guava leaf extract exhibit antibacterial properties against various pathogens. Antioxidants are very useful in the removal of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are responsible for oxidative stress. In this study, the presence of phytochemicals, rich with antioxidant properties, particularly flavonoids, could offer protective attributes to free radical cum oxidative stress induced by the lead acetate.

The significantly higher values of LH and FSH in the 250mg/kg and 500mg/kg treated groups compared to the negative control group is an indication of a therapeutic or ameliorative potential of guava leaf extract in the mitigation of testicular toxicity and injury on the testicles following the administration 30mg/kg of lead acetate for 14 days. The induction may have disrupted the hypothalamic-pituitary-gonadal (HPG) axis due to poor response of the testosterone production in the negative feedback loop in regulating hormonal balance. LH and FSH are gonadotrophic hormones responsible for the stimulation of the Leydig and Sertoli cells of the testes necessary for the synthesis for the synthesis and production of testosterone viz-a-viz sperm cells and their maturation. The higher values of LH and FSH observed in the lactate acetate treated rats could be associated with the destruction of the receptors of the Leydig and Sertoli cells of the testes, failing to produce testosterone in response to their stimuli. The non-responsive Leydig and sertoli cells and low testosterone in the plasma could have triggered the release of more gonadotropins (LH and FSH). The testicular damages as seen in the histology examination, such as distorted basement membrane, distorted and loss of Leydig cells (LC) and Sertoli cells, spermatogonia (sp), and spermatids, would have accounted for the significant fall in the testosterone concentration. The poor production of testosterone and response of the testicular cells, in turn, stimulated an increase in the production of gonadotrophins like the LH and FSH through the negative feedback mechanism. This could have accounted for the significantly higher values of LH and FSH in the lead acetate-induced rats. Our findings are also in agreement with the reports of Chen et al. (2019) and Zhou et al. (2020) who documented that the increase in LH is suggestive of lead acetate disruption of the hypothalamic-pituitary-gonadal (HPG) axis causing impairment of the testicular function, potentially as a compensatory response to reduced testosterone levels. Our findings further suggest a dose-dependent ameliorative or therapeutic role of guava leaf extract regarding testosterone production as seen in 500mg/kg treated rats.

The ratios of LH/FSH and FSH/LH did not exhibit significant variations among groups. , However, the significantly higher values in LH/FSH ratio as observed in the 500mg/kg treated group suggests a more favorable endocrine environment for testosterone synthesis compared to the lower dose group C. In addition, Kim & Koo (2023) documented T/LH ratio as a key indicator of Leydig cell responsiveness to LH stimulation and testosterone production. Therefore, an increase in the T/LH ratio is suggestive of better Leydig cells function, androgenesis, and spermatogenesis in males and vice versa. The drastic significant reduction in lead acetate-treated group (Group B) compared to the negative control group (Group A) further supports the hypothesis of lead-induced testicular dysfunction. The significantly higher values of T/LH ratio observed in the negative control group indicate optimal Leydig cell functioning as noted by Zirkin & Papadopoulos (2018). Our observations agree with the work of Ajibade et al*.* (2022) who demonstrated that exposure to toxicants such as lead could impair testosterone synthesis by disrupting Leydig cell activity and hormonal balance. Therefore, the considerably improved values of T/LH ratio in the guava-treated rats is an indication of the ameliorative capabilities of the guava extract in resolving lead acetate induced-testicular or endocrine disruptions.

In addition, following the loss of testicular parenchymal materials as a result of lead-induced testicular damage, resulting in low production of testosterone, spermatogenesis was also affected, resulting in a significant fall in the total sperm cell concentration, active motility, and increased dead cells. Our findings align with the findings of Zirkin & Papadopoulos (2018) and Rabiu et al. (2019) who also observed that lead exposure induces oxidative stress, leading to impaired sperm motility and viability. The considerable fall in total sperm count, active motility, and increased cell death could be linked to the non-responsive Leydig and Sertoli cells of the testes responsible for the production of testosterone, that is needed for sperm production and maturation. The non-responsiveness of the Leydig and Sertoli cells of the testes, also accounted for the higher values of LH and FSH in the lead acetate-treated rats. The higher values of dead sperm cells in the treated groups also suggest that lead exposure rendered sperm cells completely non-motile or dead, rather than just sluggish. This could be associated with the loss of energy production necessary for movement and sustenance. Our observation concurs with the reports of Archibong et al. (2018) and Kaltsas (2023), who observed that lead exposure, induced oxidative stress, which damages the sperm structures and functions necessary for motility. The significantly improved motile cells seen in the 250 and 500mg/kg treated groups suggesting improved antioxidant activities. Ekaluo et al. (2016) demonstrated that guava leaf extract enhances spermatogenesis by modulating oxidative stress and supporting Sertoli and Leydig cell functions.

The elevated pH can negatively affect sperm motility and viability. However, the 250 and 500mg/kg treated groups showed moderated pH levels, suggesting guava leaf extract might aid in restoring normal seminal fluid balance. Lead is known to induce seminal fluid acidity, disrupting the acid-base balance that is crucial for sperm cells survival, maturation, and motility. Our observation concurs with the documentation of Akinola et al*.* (2007) who observed improved seminal parameters in rats treated with guava leaf extract in gossypol-associated sperm toxicity in Wistar rats.

However, with the administration of guava leaf extract for 14 days, the testicular tissues had recovery tendencies on the testicular tissues particularly in the 500mg/kg treated group as indicated by intact basement membranes (BM) with Sertoli cells, Leydig cells, improved flagellation in some areas (left lobule) with mature spermatocytes migrating toward the basement membrane amidst poor number of spermatogonia and spermatids alongside pycnosis. These histological changes via the therapeutic intervention could be associated with improved total sperm count, motility, and reduced sperm cell death. Regarding LH and FSH, significantly lower values with an improved testosterone concentration were seen. These observations imply that guava leaf extract had ameliorative or therapeutic effects on the endocrine disruption induced by lead acetate toxicity. The ameliorative or therapeutic potential is associated with a high concentration of phytochemicals like flavonoids, with a concentration of 8.01mg/ml compared to saponins, tannins, anthraquinones, alkaloids, and phenols with concentrations of 6.07, 5.03, 0.01, 0.50, and 2.87mg/ml, respectively. Phytochemicals are natural antioxidants that scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) in a biological system. Our findings align with Abwage et al. (2021) and Ibrahim et al*.*(2021) works. They observed that bioactive compounds such as flavonoids mitigated oxidative stress and improved Leydig cell function.

Our findings are further supported by a considerable reduction in the MDA value in the 250mg/kg and 500mg/kg doses. The MDA is an indicator of lipid peroxidation due to oxidative stress. Therefore, the higher the MDA values, the more likely the oxidative stress in the system, lipid membrane damage, and cell death. The significantly increased SOD values further align with the results of MDA. SODs are antioxidant enzymes in mammals. Its response to oxidative stress is inversely proportional, that is, as oxidative stress builds up, there is a depletion of SOD, due to the overwhelming activities of ROS and RNS and vice versa. Therefore, the higher values of SOD in the treated groups compared to the positive control group indicate the ameliorative or therapeutic potential of guava leaf extract in mitigating lead acetate-induced testicular damage and endocrine disruption. The mitigating potential of guava leaf extract in restoring oxidative balance as indicated by the plasma levels of SOD and MDA in the treated rats could also be due to the rich presence of phytochemicals in the guava leaf extract. These phytochemicals, particularly flavonoids have shown to exhibit antioxidant characteristics by removing free radicals. Our findings concur with Vigeh et al. (2011), who documented the disruption of endocrine balance and steroidogenesis through lead exposure by increasing reactive oxygen species (ROS). In addition, our findings were in line with Chinatu et al. (2023) work, which showed a significant reduction in MDA values and increase in SOD values rats treated with a high-fat diet and feud adjuvant for 2 weeks. Again, our work also aligns with the observation of Freire et al. (2014), who documented that elevated MDA levels in lead-exposed rats were significantly reduced following treatment with guava leaf extract. Finally, our observations also concur with the reports of Sobral–Souza et al. (2019) who documented the chelating, antioxidant, and cytoprotective effects of guava leaf extract against mercury and aluminum toxicity.

**5. CONCLUSION**

This study revealed that guava leaf extract has ameliorative and therapeutic potential against lead-induced endocrine disruption, resulting in restoration of damaged testicular tissues, hormonal balances, sperm motility, viability, and concentration. Moreover, testosterone revealed a dose-dependent response in the 250 and 500 mg/kg doses.

Disclaimer:

We, the author(s) of this manuscript, take full responsibility for any "False Positive Similarity Score" generated by plagiarism detection software or tools. We believe that such a similarity score may be due to technical errors related to the software’s internal data storage system, repository, or other system-related issues. We hereby affirm and guarantee the originality and authenticity of our manuscript.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that No generative AI technology and text-to-image generators have been used during the writing and editing of this manuscript.

**ETHICAL APPROVAL**

We hereby declare that the Principles of laboratory animal care (NIH publication No. 85 23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Rivers State University research/ethics committee with file No: RSU/CV/APU/74/VOL.VIII/104.

**REFERENCES**

.Abwage, S. A., Agu, S. T., Saganuwan S. A, & Abu, A. H. (2021) Evaluation of potentials of aqueous ethanolic extract of Psidium guajava on reproductive functions of male Albino rats, Sokoto Journal of Veterinary Sciences, 19(3): 174 – 181.

Adeyemi, O. O., Okpo, S. O., &Ogunti, O. O. (2006). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of Psidium guajava in rats and mice. African Journal of Biomedical Research, 9(2), 89-93.

Akinola, O. B., Oladosu, O. S. & Dosumu, O. O. (2007). Spermatoprotective activity of the leaf extract of Psidium guajava Linn. Nigerian Postgraduate Medical Journal, 14(4), 273-276.

Archibong A. E, Rideout M. L, Harris K. J, Ramesh A. (2018). Oxidative Stress In Reproductive Toxicology. Current Opinion in Toxicology, 7,95-101. Doi: 10.1016/J.Cotox.2017.10.004.

Arima, H., &Danno, G. (2002). Antibacterial action of several tannins against Staphylococcus aureus. Journal of Natural Products, 65(3), 345-347.

Aworu, A. M., Ben-Chioma, A. E., Elekima, I., Holy B. & Bartimaeus, E. S. (2022). Pattern of Some Sperm Proteins, Anti-oxidants, Testosterone, and Prostate-Specific Antigen of Infertile Males with Sperm Cell Deformities in Port Harcourt, Rivers State. Asian Journal of Research in Medical and Pharmaceutical Sciences 11(2): 36-47

Ben-Chioma, A.E., Ijoma, V., Sheudeen, A., Ibioku, E. &Ollor, A.O. (2023). Determining Heavy Metals in Soil and Water in a Bioremediating Area in Nkeleoken-Alode Eleme Community Rivers State. Sokoto Journal of Medical Laboratory Science, 8(3): 82 - 105

Boskabady, M., Marefati, N., Farkhondeh, T., Shakeri, F., Farshbaf, A. &Boskabady, M. H. (2018). The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review. EnvironmentInternational, 120(1), 404-420.

Carlo G, Valentina M, Virgilio D, Giordano P, Jeremy Y. T, Giulio M., Andrea B. G., Daniele C. (2023). The influence of lead exposure on male semen parameters: A systematic review and meta-analysis. Reproductive Toxicology, 118, 108387; ,https://doi.org/10.1016/j.reprotox.2023.108387.

Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., AbdullRazis, A. F., Modu, B., Calina, D. & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Frontiers in Chemistry, 11 (1), 1-24.

Chen, H., Ge, R. S., &Zirkin, B. R. (2019). Leydig Cells: from stem cells to aging. Molecular and Cellular Endocrinology, 306(1-2), 9-16.

Chinatu, N. P, Elekima, I., Ben-Chioma, A. E., Brown H. (2023). Anti-Arthritis, Antioxidant and Anti - Inflammatory Potential of Ethanolic Extract of Guava Leaves on Rats Exposed to High Fat Diet and Freud Adjuvan. Journal of Complementary and Alternative Medical Research, 24 (3), 46-53,

Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. (2023). Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. Journal of Experimental Pharmacology, 8 (15), 51-62. doi: 10.2147/JEP.S379805.

Ekaluo, U.B., Ikpeme, E.V., Uno, U. U., Umeh, S.O. and Erem, F. A., (2016). Protective Role of Aqueous Guava Leaf Extract Against Caffeine Induced Spermatotoxicity in Albino Rats. Research Journal of Medicinal Plants, 10: 98-105.

Elekima, I, Chukwukere A. P., Nwojo, A. M., George-Opuda, I.M (2024). Bonny Light Crude Oil Toxicity: Histopathological and Biochemical Upshots on Cardiac and Hepatocellular Tissues. Asian Journal of Research in Biochemistry, 10(1), 1, 1-10

Elekima, I., Edookue, B. R., Pepple N. F., Aworu, A. M, & Ben-Chioma, A. E. (2020). Evaluation of Some Heavy Metals in Selected Sea Foods Directly from the Creeks in Rivers State, Nigeria. Journal of Advances in Medical and Pharmaceutical Sciences, 22(10): 29-39

Enegide, C., David, A. & Solomon, A. (2013). A new method for determining acute toxicity in animal models. Toxicology International, 20(3), 224-226.

Engvall, E., &Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. Immunochemistry, 8(9), 871–874.

Francis, G., Kerem, Z., Makkar, H. P., & Becker, K. (2002). The biological action of saponins in animal systems: A review. British Journal of Nutrition, 88(6), 587-605.

Freire, J. M., de Abreu, C. M. P., Duarte, S. M. S., de Paula, F. B. A. & Lima, A. R. (2014). Evaluation of the protective effect of guava fruits and leaves on oxidative stress. Acta Scientiarum. Biological Sciences, 36(1), 35-40.

Ibrahim, I. A., Shalaby, A. A., Abd Elaziz, R. T. & Bahr, H. I. (2021). Chlorella vulgaris or Spirulina platensis mitigate lead acetate-induced testicular oxidative stress and apoptosis with regard to androgen receptor expression in rats. Environmental Science and Pollution Research, 28(39), 39126-39138.

Kaltsas, A. (2023). Oxidative Stress and Male Infertility: The Protective Role of Antioxidants. Medicina, 59(10), 1769. https://doi.org/10.3390/medicina59101769

Kim, T. J., & Koo, K. C. (2023). Testosterone to Luteinizing Hormone Ratio as a Potential Predictor of Sperm Retrieval in Non-Obstructive Azoospermia Patients. Yonsei Medical Journal, 64(7), 433-439. doi: 10.3349/ymj.2023.0054

Kumar, M., Tomar, M., Amarowicz, R., Saurabh, V., Nair, M. S., Maheshwari, C., Sasi, M., Prajapati, U., Hasan, M., Singh, S., Changan, S., Prajapat, R. K., Berwal, M. K. &Satankar, V. (2021). Guava (Psidium guajava L.) leaves: nutritional composition, phytochemical profile, and health-promoting bioactivities. Foods, 10(4), 1-20.

Kumar, S. (2018). Occupational and environmental exposure to lead and reproductive health impairment: An overview. Indian Journal of Occupational and Environmental Medicine, 22(3), 128-137.

Lars, B. & Jackson K. B. (2022). The sixth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen: ensuring quality and standardization in basic examination of human ejaculates. Fertility and Sterility, 117 (2), 246-251

Li, S., Fasipe, B. &Laher, I. (2022). Potential harms of supplementation with high doses of antioxidants in athletes. Journal of Exercise Science and Fitness, 20(4), 269-275.

Oni E. S., Bartimaeus, E. S., Nwachuku, E. O., Elekima I., Ohaka, T. P., &Wojuade S. K. (2023). Characterization of sperm cells in infertile male subjects in southwest Nigeria World Journal of Biology Pharmacy and Health Sciences, 2023, 16(01), 182–185

Rabiu, S., Abubakar, M. G., Sahabi, D. M., & Makusidi, M. A. (2019). Effect of lead on the activity of antioxidant enzymes and male reproductive hormones. Journal of Toxicology and Environmental Health Sciences, 11(7), 84-89.

Ruksiriwanich, W., Khantham, C., Muangsanguan, A., Phimolsiripol, Y., Barba, F. J., Sringarm, K., Rachtanapun, P., Jantanasakulwong, K., Jantrawut, P., Chittasupho, C., Chutoprapat, R., Boonpisuttinant, K. & Sommano, S. R. (2022). Guava (*Psidium guajava* L.) Leaf extract as bioactive substances for anti-androgen and antioxidant activities. Plants, 11(24), 1-14.

Sobral – Souza C E., Ana R.P. S, Nadghia F. L, Janaina E. R, Amanda K. S, José G.M. C, Irwin R.A. M, Francisco A.B. C, Larissa A. R, Henrique D.M. C. (2019). *Psidium guajava* bioactive product chemical analysis and heavy metal toxicity reduction. Chemosphere, 216,785-793,

Vigeh M, Smith DR, Hsu PC. (2011). How does lead induce male infertility? Iran Jourmal of Reproductive Medicine, 9(1), 1-8.

Zegers-Hochschild, F., Adamson, G. D., Dyer, S., Racowsky, C., de Mouzon, J., Sokol, R., Rienzi, L., Sunde, A., Schmidt, L., Cooke, I. D., Simpson, J. L. &Van der Poel, S. (2017). The international glossary on infertility and fertility care, 2017. HumanReproduction, 32(9), 1786-1801.

Zhang, T., Ru, Y. F., Wu, B., Dong, H., Chen, L., Zheng, J., Li, J., Wang, X., Wang, Z., Wang, X., Shen, X., Wu, J., Qian, J., Miao, M., Gu, Y. & Shi, H. (2021). Effects of low lead exposure on sperm quality and sperm DNA methylation in adult men. Cell & Bioscience, 11(1), 1-11.

Zhou, W., Zhang, L., & Chen, Y. (2020). Role of follicle-stimulating hormone in spermatogenesis. Frontiers in Endocrinology, 11, 598.

Zirkin, B. R. & Papadopoulos, V. (2018). Leydig cells: formation, function, and regulation. Biology of Reproduction, 99(1), 101-111.