**Exploring the Therapeutic Potential of *Psidium guajava* (Guava) Leaf Extract on Testosterone, Luteinizing Hormone, and Follicle Stimulating Hormone in Lead Acetate-Induced Endocrine Disrupted Male 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 250 and 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 assay of luteinizing hormone (rLH), follicle stimulating hormone (rFSH), testosterone, malondialdehyde (MDA) and superoxide dismutase (SOD) using 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 result indicated that flavonoids are the most abundant phytochemicals present in guava leaves, with a concentration of 8.01mg/ml, relatively to saponins, tannins, anthraquinones, alkaloids, and phenols with concentrations of 6.07, 5.03, 0.01, 0.50, and 2.87mg/ml, respectively. Measurable sperm parameters indicated significantly higher active motility in Group D compared to Groups C and B. However, group A had a significantly higher value of active motility compared to groups D, C, and B. The total sperm concentration count showed that Groups C and D had significantly higher values compared to Group B, but significantly lower values compared to Group A. Finally, the pH of the seminal fluid had significantly higher values in Group B compared to Groups A, C, and D. LH indicated significantly lower values in Group C and Group D compared to Group B. In addition, FSH also indicated a significantly lower value in Group D-treated rats compared to Groups C and B. More so, testosterone results indicated significantly lower values in Group C and D compared to Group A, but their values were significantly higher compared to Group B. However, the testosterone values of Group D were significantly higher than Group C. The LH/FSH ratio indicated significantly higher values in Group D compared to Groups C and B while in the FSH/LH ratio, Group D had a significantly lower value than Groups C, A, and B. Finally, the testosterone-LH ratio showed significantly higher values in Group D compared to Groups C, A, and B. The results of MDA in Group D showed significantly lower values compared to Groups C and B. Likewise, Group C indicated significantly lower values compared to the positive control group. However, group A had significantly lower values compared to Groups B, C, and D. SOD indicated significantly higher values in Groups C and D compared to Group B. However, group A had significantly higher values compared to Groups B, C, and D. Conclusively, lead acetate impairs male reproductive function in rats, but *Psidium guajava* leaf extract offers some therapeutic (post-treatment) benefits, especially at the 500mg/kg dose.

**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 activities (Kumar et al., 2021; Chinatu et al., 2023). Additionally, guava leaves have been traditionally used in folk medicine for 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 heavy metals such as 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, 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 (Akingbemi, 2020; Ruksiriwanich et al., 2022). Heavy metals like lead, cadmium, and mercury have been associated with impaired sperm quality and reduced fertility in men mostly through the disruption of the antioxidant-oxidation balance (Kumar 2018; Ruksiriwanichet al., 2022). Antioxidants are an important component for cell survival and vitality through the 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 might lead to unintended physiological disruptions (Chaudhary et al*.,* 2023).

Therefore, this research is aimed to evaluation of effect of guava leaf extract on testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH), and their ratios as well as oxidative markers such 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 (Hanna with model number: HI99001), 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, U.S.A. MDA and SOD ELISA kits purchased from Elabscience, India.

**2.2 Experimental Animal**

Male rats were procured from the Department of Anatomy at the College of Medical Sciences, Rivers State University, Port Harcourt, and were 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. The rats were kept in a controlled environment with a 12-hour light and 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 with an accession/collection Number of RSUPbH019. The guava leaves were carefully sorted to remove any dead matter or unwanted particles. The leaves were left to air-dry over a period of 14 days at room temperature, after which they were finely ground using a blender, and 600g were measured into 1000 mL of 80% v/v ethanol for 72 hours. The extraction was done using a Soxhlet machine at a temperature of 60 0C. The yielded crude weighed 15.62g and was transferred into a sample bottle and refrigerated at 4 0C before being used for the study.

**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 a collection of 5 mL 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 -20 0C 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 in order to obtain the semen and used immediately for semen analysis and sperm count as described by WHO (2021). 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 8.02 and 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**

The results of measurable sperm parameters indicated significantly higher active motility 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, when the total sperm concentration or count and the number of dead cells were considered, Groups C and D had significantly lower values compared to Group B but significantly 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 result obtained indicated significantly lower values of LH in the 250mg/kg (Group C) and 500mg/kg (Group D) treated rats compared to 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. More so, testosterone results indicated 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).

When the hormonal ratios were considered, in the LH/FSH ratio, significantly higher values were observed in Group D compared to Groups C and B, while in the FSH/LH ratio, Group D had a significantly lower value compared to the Group C, negative control as well as the positive control rats. Finally, the Testosterone-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 |
| rTESTO (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 |
| rT/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, rTESTO = 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**

 **Rats Post-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 aklongside distinct Leydig cell areas without clusters. In addition, the photomicrograph showed spermatocytes with defined flagellation in the lumen of the seminiferous tubules migrating towards 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 treaded 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.

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**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. Akinmoladun et al. (2010) and Francis et al. (2002) 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.

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. 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, Hernandez et al., (2018), documented T/LH ratio as a key indicator of Leydig cell responsiveness to LH stimulation. 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.

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 Mohammadi et al. (2018) who also observed that lead exposure induces oxidative stress, leading to impaired sperm motility and viability. 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 Nguyen et al. (2020) and Oseni et al. (2021), who observed that lead exposure induced oxidative stress, which damages the sperm flagella necessary for movement. The significantly improved motile cells seen in the 250 and 500mg/kg treated groups suggest improved antioxidant activities derived from the guava leaf. Aralepo & Olaleye (2021) also demonstrated that guava leaf extract may enhance spermatogenesis, potentially by modulating oxidative stress and supporting Sertoli cell function.

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. Our observation concurs with the documentation of Ezeonu et al*.* (2018) who observed a balanced pH of seminal fluid in rats treated with guava leaf extracts.

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 the improved total sperm count, motility, and reduced sperm cell death. More so, the LH and FSH values tend to fall significantly with an improved testosterone concentration. These observations imply that guava leaf extract had ameliorative or therapeutic effects on the endocrine disruption induced through lead acetate toxicity. These ameliorative or therapeutic potentials could be associated with the high concentration of phytochemicals such as 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. Phytochemicals are known natural antioxidants involved in the scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in a biological system. Our findings align with the reports of Ojulari et al. (2019) and Ibrahim et al*.* (2021), who observed that the bioactive compounds in guava, such as flavonoids and antioxidants, may have contributed to this protective effect by mitigating oxidative stress and improving Leydig cell function.

Our findings are further supported by the significant reduction in the MDA values of the 250mg/kg and 500mg/kg treated rats. 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. In addition, the significant increase in the SOD values further aligns with the results of the MDA. SODs are enzyme antioxidant 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 is an indication of ameliorative or therapeutic potential of guava leaf extract in the mitigation of lead acetate induced testicular damages and endocrine disruption. Our findings are in line with the report of Adhikari et al. (2019), who documented the disruption of endocrine balance and inhibited steroidogenesis through lead exposure by increasing reactive oxygen species (ROS). More so, our findings were in line with the reports of Chinatu et al. (2023), who observed significant reduction in the values of MDA and a corresponding increase in the values of SOD in oxidative stressed-rats treated with high fat diet and feud adjuvant for 1 week and 2 weeks. Again, our work also agrees with the observation of Freire et al. (2014) who also documented that elevated MDA levels in lead-exposed rats were significantly reduced following treatment with guava leaf extract. Finally, our observations also concurs with the reports of Sobral – Souza et al., (2019) who documented chelating, antioxidant and [cytoprotective](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/cytoprotective-agent) effects of guava leaf extract against mercury and aluminum toxicity.

**5. CONCLUSION**

This study showed that guava leaf extract has ameliorative and therapeutic potential in lead-induced endocrine disruption, particularly on the hypothalamus-pituitary-testicular axis, resulting restoration of damages to testicular tissues, hormonal balances, and measurable sperm parameters such as sperm motility, viability, and concentration. Testosterone indicated a dose-dependent response in the 250 and 500mg/kg treated rats.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

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

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

Animal ethic committee approval has been collected and preserved by the author (s).

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