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Some Male Reproductive Organs and Sperm Profile of Animal Models Treated with Aqueous Extract of *Musa Acuminata* (Banana)

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ABSTRACT

This research was conducted to assess some male reproductive organs and sperm profile of animal models treated with an aqueous extract of *musa acuminata*. Thirty male wistar rats were randomly redistributed into three groups of control (animals received water and food only), low dose (animals received water food and aqueous extract of musa acuminate at a low dose of 200mg/kgbw) and high dose (animals received food water and aqueous extract of *musa acuminata* at a dose of 400mg/kgBw) respectively. At the end of 21 days of administration period of the extract, animals were sacrificed and the testes and prostate removed for histological tissue processing. The caudal part of the epididymis was harvested for collection of semen analysis. Result of the study shows a significant ($p<0.05$) increase in final body weight of all the animals compared with their initial body weight. Histological observations of the testes of the control group shows seminiferous tubules filled with germ cells (Arrow) with adjoining interstitial tissue (IT). General parenchyma appears normal, low dose revealed seminiferous tubules filled with germ cells (Arrow) with adjoining interstitial tissue (IT). General parenchyma appears normal, while the high dose showed seminiferous tubules filled with germ cells (Arrow) with adjoining interstitial tissue (IT). General parenchyma shows mild necrotic interstitial tissue (IT). The histological results from the prostate gland reveal a crystal and dilated prostatic glands in the control group while the treated groups showed mildly hyperplastic glands and eosinophilic secretions. From Semen analysis the results show a significant increase ($p<0.05$) in percentage sperm motility in Low dose and High dose group, the highest percentage of sperm morphology was observed in High dose group, the highest percentage of sperm vitality was observed in the Low dose group while the sperm agglutination shows a significant decrease ($p>0.05$) in the High dose. In conclusion, extract of *Musa Acuminata* should be consume at a moderate quantity in other to achieve it health related benefits.

Keywords: *Musa Paradisiaca*; Sperm; Testes; Prostate; Histo-Architecture; Semen

Introduction

Herbal phytotherapeutic agents are currently used worldwide. When consumed for a long period, there may be a risk of toxic effects on body organs (Jenča, et al. [1,2]). Toxicity may manifest in substructures such as cells (cytotoxicity), sperm (spermatotoxicity) or organs such as the testes and the prostate gland (Wong, et al. [3]). The major evaluation points for toxicological assessments are the basic structural, functional, and biochemical parameters of injury; the dose-response relationships of the agent of toxicity and toxicological parameters; the mechanisms of toxicity (the fundamental biochemical alterations responsible for the induction and maintenance of the toxic response); reversibility of the toxic effect; and possible factors influencing response modification, such as route of exposure, species, and sex (Pagar, et al. [4]). Reports have indicated severe toxicity resulting from the use of herbal medicines or products (Shaito, et al. [5]). These

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toxicities arise from inherent poisonous phytochemicals, adulteration of medicines, contamination with various chemicals and heavy metals, herb-drug interactions, and poor-quality control of herbal products (Fokunang, et al. [6]).

The use of animals to predict the risks of various treatments, botanicals, and chemical products (Van Wyk, et al. [7]). According to Meng, et al. (2017), *Musa acuminata* is not a tree but rather an evergreen perennial. The inflorescence grows either horizontally or obliquely from the trunk, which is also known as the pseudostem. The trunk is made up of densely packed layers of leaf sheaths that arise from corms that are entirely or partially covered. The individual flowers range in color from white to yellowish-white and are negatively geotropic, meaning they grow up and away from the ground (Sangeetha [8]). The female flower is located toward the base (and develops into fruit), whereas a solitary inflorescence is positioned at the tip of the top-shaped bud between leathery bracts (Sangeetha [8]). The male reproductive gland, or gonad is known as the testicle or testis (plural testis) in all animals, including human. It resembles the female ovary in structure.

The testis is an organ that produce sperm as well as androgens, primarily testosterone. The anterior pituitary luteinizing hormones regulates the release of testosterone, whereas the anterior pituitary follicle-stimulating hormone and gonadal testosterone work together to regulate sperm production. (Corona, et al. [9]). Or gamete is called the sperm, red algae and some fungi generate is called the spermatia which are immobile sperm cells, whereas animals create spermatozoa, which are flagellated, motile sperm cells. While certain more basal plant, such as ferns and some gymaosperm, have hostile sperm (Yamatoto, et al. [10]). Poor penile erection, aberrant sperm, quality and volume, and abnormal ejaculation are few of the factors that might contribute to male infertility (Naz, et al. [11]). The prostate is a small, walnut-sized gland found in the male reproductive system. It is located just below the bladder and surrounds the urethra, the tube through which urine and semen exit the body (Prins, et al. 2010). The primary function of the prostate is to produce prostatic fluid, which forms a part of semen (Prins, et al. 2010). This fluid nourishes and protects the sperm, enabling their successful transportation and survival. Some common issues affecting the prostate include: Prostatitis: Inflammation of the prostate gland, often caused by bacterial infections, Benign prostatic hyperplasia (BPH): A non-cancerous enlargement of the prostate, which can cause urinary issues as it presses on the urethra. Prostate cancer: Malignant growth in the prostate gland that may spread to other parts of the body if not detected early (Prins, et al. 2010).

Semen analysis is a diagnostic test that evaluates the quality and characteristics of a male's semen and sperm. It is often used to assess male fertility or identify potential issues affecting reproductive health (Franken, et al. 2012). The test examines several parameters, including: Sperm count: The total number of sperm present in the ejaculate, Sperm morphology: The shape and appearance of sperm cells, as abnormalities can affect their ability to fertilize an egg, Sperm motility: The percentage of sperm that move forward progressively, which is crucial for reaching and fertilizing the egg, Volume: The amount of semen ejaculated, which can indicate issues with the accessory glands, Liquefaction time: The time it takes for the semen to become liquid after ejaculation (Oehninger, et al. 2021).

Materials and Methods

Plant Materials

The Banana (*Musa acuminata*) were gotten from the local market of Okuku in Yala Local Government Area of Cross River State of Nigeria. The unripe banana was properly washed, peeled, sliced and dried in an air-dried room temperature of about 27 °C for three weeks. It was blended and kept in an air tight container.

Extract Preparation

The banana powder was dissolved in 900ml of bottled table water in a plastic container. The mixture was properly stirred with a stick and allowed to stay for twenty-four (24) hours before it was filtered with a cloth-sieve. The filtrate was evaporated at about 45 °C with water bath to obtain the crude solid extract for three weeks and the extract obtained was stored in a refrigerator until the commencement of the administration.

Experimental Animals

Thirty (30) adult male Wistar rats were purchased from the animal house of the Department of Human Anatomy and Forensic Anthropology, University of Cross River State (UNICROSS), Okuku campus and were used for this study. The animals were randomly distributed into three group: Control, low dose and high dose, ten (10) animals for each group. The animals were acclimatized for two (2) weeks, they were housed in plastic cages under controlled light (12 hours' daylight cycle and 12 hours' dark cycle) and were fed with standard grower's vita feed and water before the start of the administration.

Experimental Design

The thirty (30) animals were allocated into three (3) groups consisting of ten animals in each group.

- Group A (control) received food and water only.
- Group B (Low dose) animals received food, water and extract of *Musa acuminata* at a dose of about 0.2mL/kg.
- Group C (High dose) animals received food, water and extract of *Musa acuminata* at a dose of 0.4mL/kg.

Termination of Experiment

At the end of administration which lasted for twenty-one days (21) the animals in all the groups were sacrificed a day after the final administration using cervical dislocation. The testes and the prostate glands of each animal were removed and the caudal epididymis separated from the testes and processed immediately for epididymal sperm parameters

Morphological Studies

All the animals were weighed using sensitive weighing balance before the commencement and at the end of the administration of the extract of *Musa acuminata* to ascertain the morphological changes.

Semen Analysis

Semen was obtained from the caudal epididymis by scraping the lumen into a lumen tube pre warmed with water bath at 50-60 C. A drop of this specimen was placed in a chamber and covered with a water glass. The following parameters; sperm motility, vitality, morphology and agglutination were determined using the makler counting chamber with Olympus microscope.

Tissue Processing

The testes were removed and preserved in a container with 10% neutral buffer formalin. This was done for seventy-two (72) hours to achieve good tissue penetration and effective fixation. After this they were placed in ascending grades of ethanol for dehydration. First they were treated with two (2) changes of 70% ethanol each lasting for one hour followed by 95% ethanol and then absolute ethanol for the same duration. Following dehydration, the tissue was cleared in three changes of xylene each lasting for fifteen minutes (Raad, et al. 2022). The impregnation in molten paraffin was at 580c was carried out overnight and the following morning the tissue were embedded in wax to form blocks. This tissue blocks were trimmed and sectioned at 3 to 5μm thickness using a microtome. The sections were floated in warm water (28 0C) and then taken up on albuminized glass slides. They were air dried and stained using the Masson Trichromes (Harris, 2011) staining method.

Statistical Analysis

Statistical analysis was done using statistical package for social sciences (SPSS) version 16 chicago Inc. one-way Analysis of variance (ANOVA), followed by Bonferroni's multiple data comparison test was used to perform the analysis. Result of descriptive statistics of the experiment data was presented as mean standard error (Mean ±SEM). Paired sample T-test were considered statistically significant at $p<0.05$.

Results and Analysis

Effect of Aqueous Extract of Musa Acumiata on the Body Weight of Adult Wistar Rats

Morphological observation from the study shows an observable significant ($P<0.05$) increase in the final mean body weight when compared with the initial body weight observable in control vs low dose, control vs high dose and low dose vs control but not observable in low dose vs high dose and high dose vs control dose. The final body weight of the control animals (115.0 ± 3.310) was significantly ($P<0.05$) higher than its initial body weight (91.40 ± 5.467). however, the mean final body weight of the low dose group (152.4 ± 6.723) and high dose group (160.2 ± 6.018) were significantly ($P<0.05$) higher than their initial body weights (133.8 ± 3.094) and (135.1 ± 6.125) respectively.

Histological Examination

(Plates 1-6).

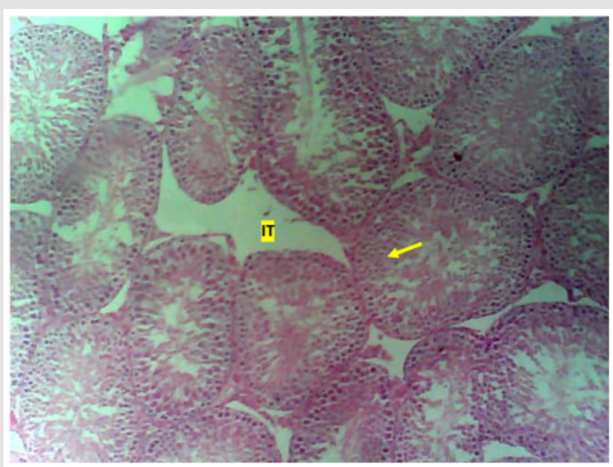

Plate 1: Photomicrograph of the testis in the control group showing seminiferous tubules filled with germ cells (arrow) with adjoining interstitial tissue (IT). General parenchyma appears normal. (H & E. X200).

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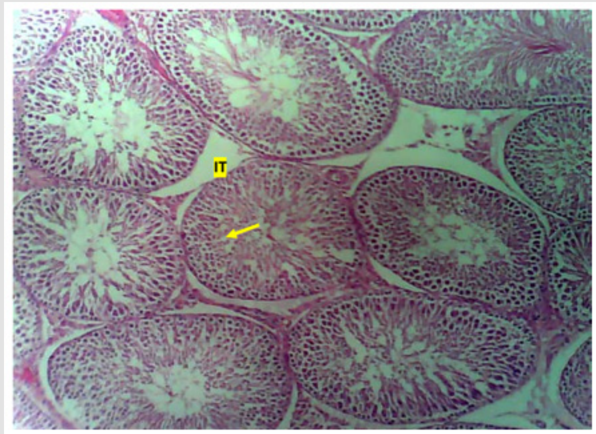

Plate 2: Photomicrograph of the testis in the *musa acuminata* low dose showing seminiferous tubules filled with germ cells (arrow) with adjoining interstitial tissue (it). general parenchyma appears normal. (H & E. X200).

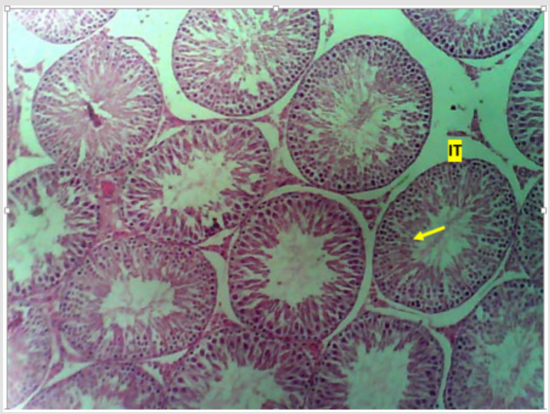

Plate 3: Photomicrograph of the testis in *musa acuminata* high dose group showing seminiferous tubules filled with germ cells (arrow) with adjoining interstitial tissue (IT). general parenchyma shows mild necrotic interstitial tissue. (H & E. X200).

Plate 4: Micrograph of the prostate gland in the control group shows crystally dilated prostatic glands (arrow). The prostate appears normal. (H &E .X100).

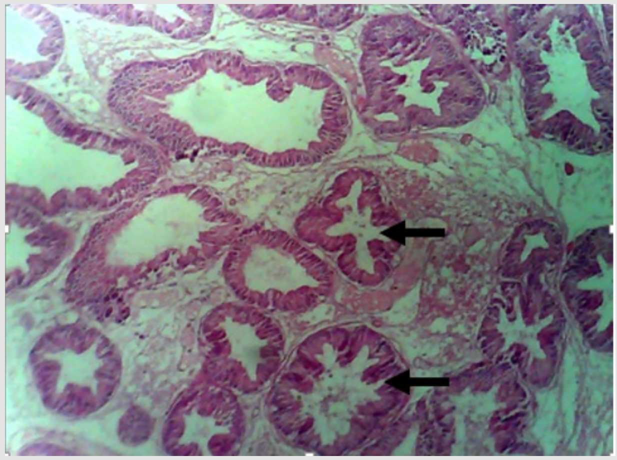

Plate 5: Micrograph of the prostate gland at *musa acuminate* low dose showing Mildly Hyperplastic glands with Eosinophilic secretion (arrow). (H & E X100).

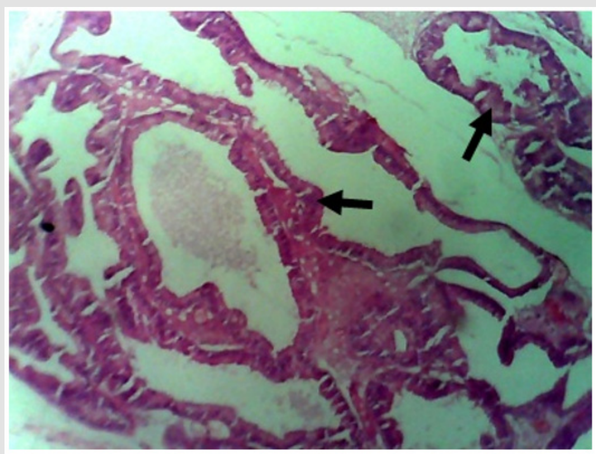

Plate 6: Micrograph of the prostate gland in *musa acuminate* high dose group showing Mildly Hyperplastic glands with Eosinophilic secretion (arrow). (H & E X 100).

Semen Analysis:

Sperm Motility: The result from this study shows a significant increase in (P < 0.05) percentage sperm motility in the low dose (63.33 ± 1.667) animals and high dose (63.33 ± 1.667) animals when compared with the control (60.00 ± 2.887) animals (Figure 1).

Sperm Vitality: There was a significant increase (P < 0.05) in sperm vitality of low dose (68.33 ± 1.667) and decrease in high dose (61.67 ± 1.667) when compared to the control group (61.67 ± 1.667) (Figure 2).

Sperm Morphology: Sperm Morphology is expressed as mean values in percentage. The highest percentage of sperm morphology (68.33 ± 1.667) was observed in high dose group which was significantly (P < 0.05) higher compared to the low dose and control group at (61.67 ± 1.667) and (61.67 ± 1.667) respectively (Figure 3).

Agglutination: There was a significant decrease (P < 0.05) in sperm Agglutination of High dose (4.667 ± 2.333) when compared to the low dose (7.333 ± 0.333) and control group (7.333 ± 0.6667) respectively (Figure 4).

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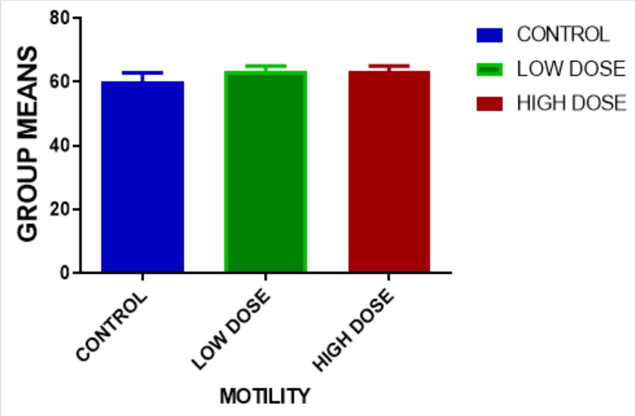

Figure 1: Showing the effects of *Musa acuminata* extract on sperm motility in wistar rts. Bars are presented as Mean ± SEM, n = 5 followed by Newman mean – Keuls Multiple Comparison Test.

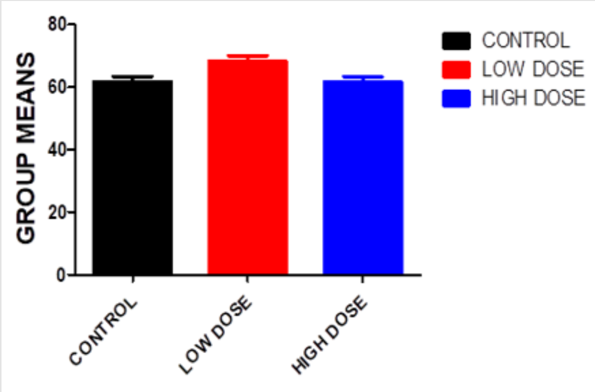

Figure 2: Showing the effects of *Musa acuminata* extract on sperm vitality in wistar rats. Bars are presented as Mean ± SEM, n = 5 followed by Newman – Keuls Multiple Comparison Test.

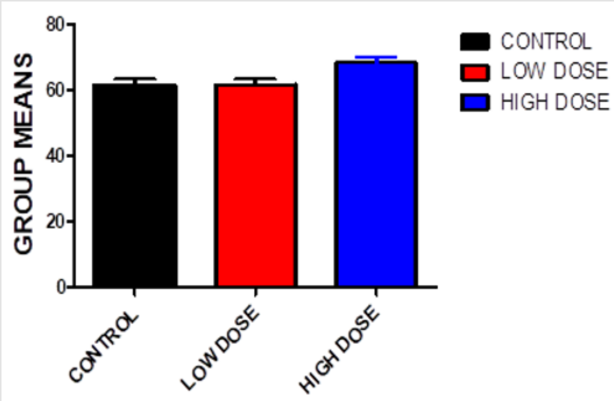

Figure 3: Showing the effects of *Musa acuminata* extract on sperm morphology in wistar rats. Bars are presented as Mean ± SEM, n = 5 followed by Newman - Keuls Multiple Comparison Test.

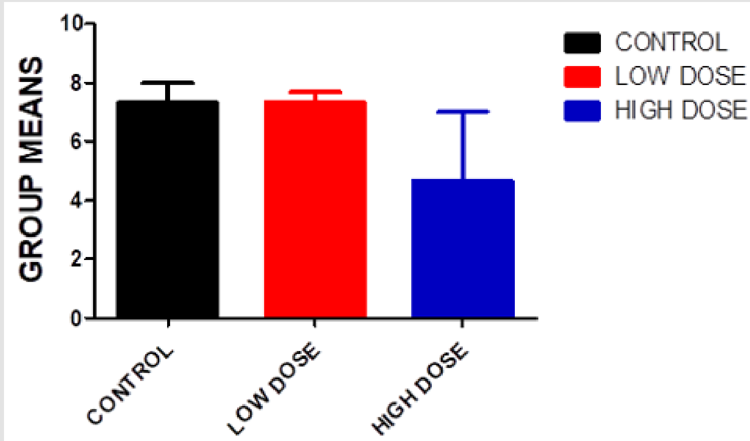

Figure 4: Showing the effects of *Musa acuminata* extract on sperm Agglutination in wistar rats. Bars are presented as Mean ± SEM, n = 5 followed by Newman – Keuls Multiple Comparison Test.

Discussion

The male reproductive system is highly sensitive to various environmental, dietary, and pharmacological interventions (Krzastek, et al. [12]). Recent research has emphasized the significance of plant-based supplements in enhancing reproductive health, with particular focus on their antioxidant and anti-inflammatory properties (Ayaz, et al. [13]). *Musa acuminata*, commonly known as banana, has been a subject of interest due to its rich content of bioactive compounds, including flavonoids, alkaloids, and phenolic compounds, which have been proposed to influence male reproductive health positively (Hashim, et al. [14,15]). In this study, we explored the effects of aqueous extract of *Musa acuminata* on the reproductive organs (Testes and the prostate gland) and sperm integrity in animal models. The results of this study show a significant increase in the final mean body weight of animals treated with *Musa acuminata* extract when compared with their initial body weight. This suggests that *Musa acuminata* extract may have general health benefits that extend beyond reproductive organs, potentially improving metabolic or systemic function. The observed increase could be attributed to the extract's potential to reduce oxidative stress, promote nutrient absorption, or influence endocrine pathways regulating growth (Ahmed, et al. [16]).

Histological analysis of the testes from control animals showed normal architecture, with intact seminiferous tubules and well-organized germ cell layers. This is consistent with previous studies that demonstrate the baseline integrity of testicular tissue in untreated animals (Chakraborty, et al. [17]). In contrast, the administration of *Musa acuminata* extract at both low and high doses caused noticeable alterations in the germ cell layers. At the low dose, mild regeneration of germ cell layers was observed, while at the high dose, there was evidence of mild to moderate degeneration of these layers. These findings suggest that *Musa acuminata* may exert dose-dependent effects on testicular function, with higher doses potentially leading to cellular damage or stress, which could compromise sperm production. While the low dose seems to stimulate germ cell regeneration, the high dose may overwhelm the testicular architecture, causing degenerative changes. These results are supported by findings from other plant-based extracts, such as those from Piper nigrum, which have been shown to exhibit dose-dependent effects on testicular histology (Tiwari, et al. [18]).

In the control group, the prostate gland exhibited normal histological features, with clear prostatic glands, consistent with the usual morphology observed in healthy animals. Following treatment with *Musa acuminata*, both the low and high doses induced mild hyperplasia of the prostatic glands, characterized by eosinophilic secretion. While this hyperplasia was not severe, it indicates that *Musa acuminata* extract may exert a stimulatory effect on the prostate, which allows with the findings of Lopes, et al. (2020). The mildly hyperplastic nature observed in both doses suggests that the extract might act as an endocrine modulator, possibly influencing testosterone or other hormones involved in prostate growth. This is in line with studies reporting that plant extracts, including those from *Musa acuminata*, can influence prostate gland morphology and function (Zhang, et al. [19]). Sperm analysis revealed several interesting trends. First, there was a mild increase in sperm motility in both the low- and high-dose *Musa acuminata* groups. This could reflect the antioxidant properties of the extract, which might improve sperm function by reducing oxidative stress, a known contributor to sperm dysfunction (Ali, et al., 2021). Interestingly, the low-dose group showed a significant increase in sperm vitality, which aligns with previous findings where moderate doses of plant extracts led to enhanced sperm quality (Sundararajan, et al. [20]).

In contrast, the high-dose group exhibited a significant reduction in sperm vitality. This suggests a potential toxic effect of high doses of *Musa acuminata* on sperm function, leading to cellular damage or disruption of the mechanisms required for maintaining sperm membrane integrity (Anand, et al. [21]). High doses of plant extracts have been reported to induce negative effects on sperm vitality due to excessive oxidative stress or hormonal imbalances, similar to what we observe here with *Musa acuminata*. Notably, the highest percentage of normal sperm morphology was observed in the high-dose group, which was significantly higher than in the control and low-dose groups. This is a surprising finding, as the high dose was associated with sperm vitality reduction. This paradox could be explained by the potential impact of *Musa acuminata* on spermatogenesis, where certain stressors may lead to improved morphological integrity of sperm but negatively affect their functional viability (Hasan, et al. [22]). Additionally, the significant reduction in sperm agglutination in the high-dose group further supports the notion that the extract may have an impact on sperm motility and integrity, likely through mechanisms that alter sperm membrane properties or interactions between sperm and seminal plasma proteins (Jain, et al. [23,24]).

Conclusion

The results of this study suggest that *Musa acuminata* aqueous extract has a significant impact on male reproductive health, with both beneficial and potentially harmful effects depending on the dose. At lower doses, the extract appears to stimulate testicular regeneration and improve sperm vitality, whereas higher doses may lead to testicular degeneration and decreased sperm vitality, though they promote better sperm morphology and reduce sperm agglutination. The observed effects on prostate gland morphology also indicate potential endocrine-modulating activity. Given the dual nature of the observed outcomes, further studies are needed to better understand the dose-response relationship, the molecular mechanisms involved, and the long-term implications for male reproductive health.

Author's Declaration

The authors declare that the research work in this article is original, and any liability arising from this work shall be their responsibility.

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