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# *Annona muricata* L. Fruit Pulp Improves Hormone Profile and Semen Quality in Testosterone-Induced Benign Prostatic Hyperplasia Male Rats

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ABSTRACT

Fruit pulp of *Annona muricata* is utilized in traditional medicine to treat pain, urethritis, inflammation and to promote breast milk production after childbirth. In this study, the effect of the aqueous *Annona muricata* fruit pulp extract (AAMFPE) on the hormonal profile and semen quality in testosterone-induced benign prostatic hyperplasia (BPH) in rats was investigated. In the acute toxicity study, single dose of up to 5 g/kg AAMFPE was orally administered. Healthy adult male rats were divided into 6 groups (n=5) and groups 2-6 were induced with a daily dose of 5 mg/kg testosterone propionate for 28 days by intraperitoneal injections, while group 1 served as a normal control. After induction, group 2 served as a negative control, group 3 received 5 mg/kg finasteride orally, while groups 4-6 received different oral doses of AAMFPE: 500 mg/kg, 1000 mg/kg and 1500 mg/kg respectively for 14 consecutive days via the oral route. The results showed a significant decrease ($p<0.05$) in testicular weight, prostate weight, prostate-specific antigen and testosterone as well as an increase in luteinizing hormone and follicle stimulating hormone in rats treated with AAMFPE, finasteride and normal control animals compared to the negative control group. In addition, treatment with AAMFPE improved semen quality, sperm morphology and histomorphology of both testis and prostate in male rats compared to the untreated BPH-induced group. In conclusion, AAMFPE is not toxic but reduces testicular and prostate weight, and improves hormone profile and sperm quality in BPH-induced male rats.

***Keywords*:** Benign prostatic hyperplasia, *Annona muricata*, Prostate-specific antigen, Hormone profile, Sperm quality.

## Introduction

Infertility refers to a woman's inability to conceive after engaging in regular, unprotected sexual activity for a year. In many developing countries, including Nigeria, infertility is predominantly perceived as a problem that only affects women.[1] However, scientific studies show that men are responsible for 20-30% of all cases of infertility.[2] The prostate is a pea-sized, rubbery organ located in the pelvis between the base of the penis and the rectum. It plays an essential role in male reproduction as it is partly the source of seminal fluid. It can enlarge in a pathological condition (benign prostatic hyperplasia (BPH) or benign prostatic enlargement (BPE)), which often occurs in men over the age of 50.[3,4] According to the American Neurology Association, the incidence of BPH increases in direct proportion to age, occurring in 8-50% of men aged 40-60 years and in 90% of men over the age of 85.[5]

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Uncontrolled enlargement of the prostate leads to several clinical signs and symptoms such as urethral stricture, frequency of urination, haematuria, hesitancy, urinary dribbling, recurrent urinary tract infections, obstructive uropathy and pyelonephritis.[6,7] Although the pathophysiology of BPH is not clearly understood, androgens and products of their metabolism have been linked to the etiology of prostate enlargement and the associated restructuring and proliferation of cellular elements in the prostate.[7] Besides androgen stimulation, oxidative stress, inflammation, and aging are risk factors for BPH. Dihydrotestosterone from the reduction of testosterone in a reaction catalyzed by 5-reductase causes increased growth of the prostate and the development of the disease.[4,6] Treatment options for BPH include drug treatment and surgical removal of the enlarged prostate. Surgical treatment of BPH is associated with various complications including bleeding and multiple blood transfusions, erectile dysfunction, decreased libido and postoperative sepsis. Drug treatment with 5-reductase inhibitors such as finasteride and dutasteride and 1-adrenoceptor antagonists such as tamsulosin, doxazocin and terazocin can cause impotence, gynaecomastia, erectile dysfunction and impaired libido as well as retrograde ejaculation.[4,8] Changes in sperm DNA, sperm count and sperm motility have been observed in people taking BPH medications.[9] Most BPH patients therefore refuse both drug and surgical treatments, making ethnomedicine an alternative treatment option. The fruit pulp of *A. muricata* is one of the edible medicinal plants used in ethnomedicine for the treatment of prostate enlargement. *Annona muricata* is a tropical medicinal plant known for its edible fruit pulp. The fruit pulp is utilized in traditional medicine to treat pain, cancer, urethritis, inflammation, and to boost breast milk after childbirth.[10-12] Previous studies have shown that *A. muricata* have anticancer, [13] anti-proliferative effect against BPH-1 cells as well as antioxidant activities.[14] Despite the use of *A. muricata* in ethnomedicine, there is still little or no literature on the ameliorative

effect on hormonal profile and semen quality in testosterone-induced BPH in male rats. This study evaluated the effect of AAMFPE on the hormonal profile and semen quality in testosterone-induced BPH in rats.

## Materials and Methods

*Plant collection and identification*
The *A. muricata* fruits were purchased at Nkwo market in Abayi, Osisioma Ngwa LGA, Abia State, Nigeria. Identification of *A. muricata* fruit was carried out by a taxonomist from Michael Okpara University of Agriculture, Umudike. The plant name was confirmed on http://www.worldfloraonline.org, accessed 22 January 2024. A herbarium number (MOUAU/ZEB/HERB/23/003) was assigned and the *A. muricata* fruit was kept in the herbarium.

*Preparation of A. muricata fruit pulp extract*
The ripe fruits of *A. muricata* were thoroughly washed, then peeled and the seeds were removed from the pulp. To prepare AAMFPE, 500 g of the fresh fruit pulp was blended with 200 mL of distilled water using an electric blender for 5 min. The resulting mixture was then filtered and concentrated at 60 $^{O}$C using hot air-oven. The final product obtained was a creamy-white paste-like extract weighing 86.32 g, which corresponds to an extract yield of 17.26%.
The % yield was calculated as

$$\% \text{ yield of AAMFPE} = \frac{\text{Weight of dried AAMFPE (86.32)}}{\text{Weight of the fresh fruit pulp of } A. \textit{ muricata} \text{ (500)}} \times \frac{100}{1} \quad \text{....I}$$

*Animal study*
Healthy male rats (unmated, weighing between 175-220 g) were used. These experimental rats were housed in adequately ventilated cages in the animal house. The rats were provided with both food and water. The environmental temperature was controlled at 25 ± 2 °C, and a 12-hour cycle of light and darkness was provided. Humidity was between 60 and 90 during the acclimatization and experimental period. International guidelines for the use of laboratory animals were strictly followed. Ethical approval for this study was granted by the Ethics Committee of Abia State University on 20 September 2022 (approval number: ABSU/REC/BMR/080).

*BPH induction*
The method outlined by Gong et al.[15] was utilized. Healthy, sexually mature male rats were randomly divided into six (6) groups (n=5). Group I (normal control) received 1 mL/kg olive oil (vehicle), while groups 2-6 received a subcutaneous injection of testosterone propionate (TP) diluted with olive oil at the dose 5 mg/kg body weight once daily for 28 days. After BPH induction, the experimental rats were treated daily for 14 days as follows: Group I served as the normal control group and did not receive TP and was not treated with either the standard drug or AAMFPE. Group II served as a negative control and received TP but no treatment with the standard drug or AAMFPE. Group III received TP and orally 5 mg/kg body weight finasteride (standard drug). Group IV received TP and orally 500 mg/kg AAMFPE. Group V received TP and orally 1000 mg/kg AAMFPE and group VI received TP and orally 1500 mg/kg AAMFPE. The dose select was based on the acute study of AAMFPE. After the 14-day treatment period, the experimental rats were euthanized via cervical dislocation on the 15th day after an overnight fasting. Blood samples were collected via cardiac puncture with a sterile needle and syringe.

*Hormone assay*
The quantitative determination of prostate-specific antigen (PSA), testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were carried out using Chemiluminescence immunoassay (CLIA) with kits manufactured by Autobio Diagnostics Co., Ltd., Zhengzhou, China. The procedures outlined in the kits were strictly followed throughout the determination.

*Semen collection and analysis*
The sperm cells were obtained from the epididymal reserve. Rats were anesthetized using chloroform through inhalation, and their epididymides were extracted. The distal segment of each epididymis was meticulously dissected, and a smear was prepared on pre-warmed glass slides for subsequent analysis.[1]

*Sperm samples colour and consistency*
The macroscopic examination and recording of sperm involved assessing its consistency and colour. The consistency scale, as established by Chibundu[16], was utilized, while the colour scale consisted of three categories: 1 (white), 2 (milky white), and 3 (creamy white). To ensure precision, the consistency scale ranged from 1 (watery) to 4 (extremely thick), with 2 representing slightly thick and 3 indicating thick consistency.

*Sperm mass motility*
In this study, we adopted the technique described by El-Sherbiny.[17] The semen samples collected from each treatment group were analyzed to assess the presence of motile spermatozoa. This analysis was performed promptly following the collection. A small aliquot of semen was applied to a heated glass slide and examined under a light microscope at magnifications of ×10 and ×40. Only sperm cells that exhibited linear forward movement were considered for the motility assessment. Sperm cells that showed circular motion, backward movement, or pendular activity were not considered. The assessment of sperm motility was conducted using a defined scale: 1= vigorous progressive movement, 2= progressive movement, 3= cycle movement and 4= stationary movement.

*Sperm cell viability (Live Proportion)*
A single drop of harvested sperm (epididymal washings) underwent staining with Eosin-Nigrosin in order to assess the proportion of viable sperm cells. Following staining, the glass slides were air-dried for half a minute before being fixed with ethanol. The assessment of viable sperm cells on the stained slides was performed utilizing a handheld stopwatch and a manual counter, while observing under a light microscope at a magnification below 100 (oil immersion). A cumulative count of 300 cells was recorded to ascertain the percentage of viable sperm cells. Specifically, 300 sperm cells were analyzed, with the viable sperm cells represented as a percentage of the total count. It was noted that viable sperm cells did not take up the stain, in contrast to the non-viable cells, which absorbed the stain.[17]

*Sperm concentration*
Haemocytometer was employed for quantifying the sperm cell concentration in semen samples as described by Herbert[18]. A 1:200 dilution was prepared using a pipette crafted from red blood cells. To immobilize the spermatozoa, a 10% buffered formalin solution was used as the diluent for the semen. A drop of the sperm suspension was applied to the haemocytometer and allowed to remain undisturbed for 2 minutes on a damp paper surface, to facilitate the settling of the sperm cells. Following this, the haemocytometer was positioned on the stage of a light microscope and observed at a magnification of ×40.

The sperm concentration per ml = No. of cells counted × Dilution factor × $0.04 \times 10^{6}$.[19]……II

*Abnormal sperm proportion (Sperm Morphology)*
The technique outlined by El-Sherbiny[17] was used to evaluate the percentage of abnormal spermatozoa. A minimal volume of semen was combined with Eosin-Nigrosin stain, and the mixture was uniformly distributed on a glass slide. The slide was then analyzed under a magnification of ×40 to identify both essential and non-essential abnormal sperm cells. This analysis sought to ascertain the prevalence

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of different types of irregularities, including abnormalities in the head, tail and midpiece regions.

*Statistical analysis*

Statistical analysis was conducted using Microsoft Excel and Graphpad Prism version 10.0.2. A significance level of $p \leq 0.05$ was determined by employing a one-way analysis of variance (ANOVA) in combination with the Tukey post-hoc test.

## Results and Discussion

Benign prostatic hyperplasia is still the most common age-related non-malignant tumour in men.[5,20] It is the most common urological disease in men over the age of 50.[21] This condition poses a significant threat to life and diminishes the overall quality of life and life expectancy in men. This study evaluated the effect of AAMFPE on hormone levels and semen quality of testosterone-induced BPH male rats. Administration of AAMFPE at the dose up to 5 g/kg did not result in any toxic symptoms or death. Therefore, AAMFPE is not toxic but safe for therapeutic purposes. All experimental rats gained weight throughout the study (Figure1). However, administration of testosterone to the rats resulted in a significant increase ($p<0.05$) in testicular weight, prostate weight and PSA levels compared to the normal control group. AAMFPE consumption also significantly reduced the weight of the prostate and testes compared to the negative control group. In addition, the administration of the extract led to a decrease in PSA levels in the test rats compared to the negative control group. The reduction in testicular and prostate weight and the decrease in PSA levels observed in the rats treated with 500 mg/kg, 1000 mg/kg and 1500 mg/kg AAMFPE are similar to the effects caused by treatment with the standard drug (finasteride) (Figure 2). Previous studies have shown that testosterone administration increases testicular and prostate weight and PSA levels.[21-24] Increase in prostate weight is a common indicator of benign prostatic hyperplasia. Studies by Arayombo et al.[21] and Veeresh et al.[25] have shown that the increase in prostate weight is an important tool for assessing the progression of BPH, which is characterized by the proliferation of prostatic muscle cells, epithelial cells and fibroblasts.

Enlargement of the prostate can be characterized by narrowing of the urethra, leading to either partial or complete obstruction of urine flow or the occurrence of chronic or acute urinary retention. The decrease in prostate weight observed in this study following the administration of AAMFPE is consistent with the findings of Sasidharan et al.[4] and Eyeghre et al.[12] in which administration of 400 mg/kg *C. bonuc* and 500 mg/kg *A. muricata* resulted in a significant reduction in the weight of the prostate and testes in experimental animals. This reduction in prostate weight was also supported by the results of Oluwatosin et al.[26] who reported that the administration of the hexane fraction of *A. muricata* seeds resulted in a remarkable reduction in prostate weight in male rats compared to the control group. Prostate-specific antigen, a known biomarker for BPH, is a glycoprotein produced in the secretory epithelium of the prostate.[4] Serum levels of PSA increase in both BPH and prostate cancer, with a more marked increase in the latter.[27,28] The increase in PSA levels in the testosterone-induced group indicates progression of BPH. In contrast, the decrease in PSA levels in the rats treated with finasteride and AAMFPE demonstrates the potential of the extract in the treatment of BPH and leads to promising results comparable to those of the standard drug. The suppression of the isoenzymes of 5α-reductase (types 1 and 2), which facilitate the transformation of testosterone into the biologically active dihydrotestosterone, is considered a plausible mechanism by which finasteride and AAMFPE exert their effect against BPH.[5,29] Dihydrotestosterone is recognized for its ability to enhance the transcription of various peptide growth factors, including insulin-like, fibroblast and epidermal growth factors. In addition, it activates androgen receptors, all of which play a role in the pathogenesis of BPH.[30]

In addition, both FSH and LH levels were significantly increased after administration of AAMFPE and standard medication finasteride compared to the negative control group. The testosterone, FSH and LH levels in the test groups did not differ from that of the normal control group. Gonadotropins, also known as adenohypophyseal hormones, are peptide hormones released by the anterior pituitary gland. They play a vital role in the regulation of ovarian and testicular functions and thus influence gametogenesis.[31] They are essential for male fertility as they are involved in spermatogenesis.[32] The observed increase in FSH and LH levels in the extract-treated group compared to the negative control group indicates a potential improvement in the reproductive capacity of the rats (Table 1). This is particularly significant considering that FSH and especially LH contribute to male reproductive function by stimulating testosterone production within the normal physiological range.[33] Treatment with the standard drug finasteride and AAMFPE in experimental rats resulted in a restoration of testosterone levels similar to that of the normal control group. Our findings are similar to the report of Onyegeme-Okerenta et al.[7] who demonstrated that treatment with the aqueous leaf extract of *A. muricata* resulted in the normalization of testosterone and FSH levels in animals subjected to high doses of testosterone propionate.

Medicinal plants such as *M. pudica* and *C. dactylon* have been shown to improve sperm quality in rats as they are rich in flavonoids and phytoconstituents such as sterols, tannins, glycosides and quercetin.[34] This study showed that AAMFPE improves both sperm morphology and sperm quality in male rats. Sperm motility, sperm count, normal and abnormal sperm, head and tail abnormalities, midline abnormalities and total abnormal sperm are well-established parameters used in urology to assess sperm quality and male fertility status. Factors such as varicocele, orchitis, high testicular temperatures, smoking, alcohol consumption, diabetes, pelvic surgery and environmental factors such as exposure to heavy metals are all known to influence the above parameters.[35] The increase in THAb, TTAb, TCD, MPAb and TAS levels observed in the testosterone-induced animals was reversed when the male rats received the standard drug or different doses of AAMFPE (Table 2). This reversal is similar to the level observed in the normal control group. The sperm colour, consistency, pH, progressive motile sperm, live sperm percentage, sperm count, normal sperm percentage and total abnormal sperm in the AAMFPE-treated group showed no significant difference ($p>0.05$) compared to the positive control group (Table 3). Our results leave no doubt that AAMFPE improves sperm quality better than finasteride.

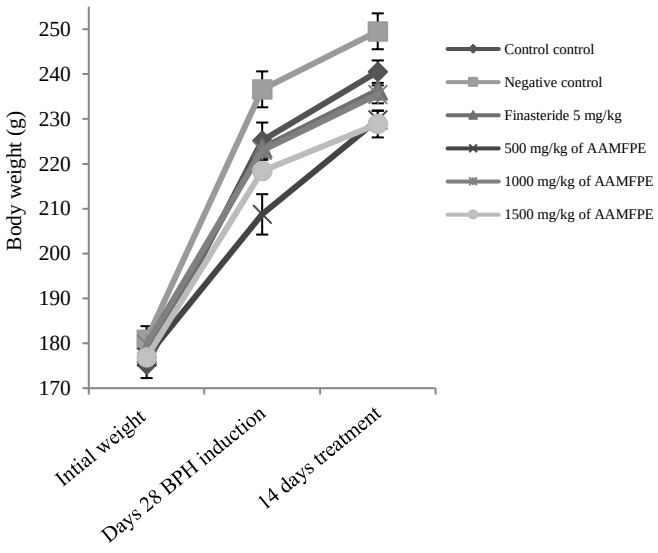


**Figure1:** Body weight of testosterone-induced benign prostatic hyperplasia in adult male rats

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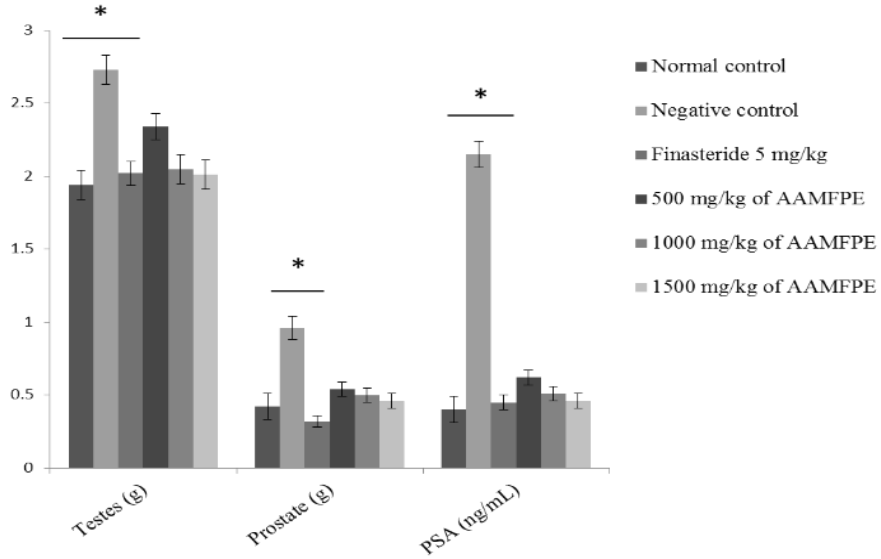


**Figure 2:** Weight of testes, prostate and value of PSA of testosterone-induced benign prostatic hyperplasia in adult male rats

**Table 1:** The effect of *A. muricata* fruit pulp on profiles of selected hormones on testosterone-induced benign prostatic hyperplasia in adult male rats

| Parameter/Treatments | Normal control | Negative control | Positive control (5 mg/kg Finasteride ) | AAMFPE (mg/kg) | | |
|---|---|---|---|---|---|---|
| | | | | 500 | 1000 | 1500 |
| Testosterone (ng/mL) | 11.50 ± 0.89 | 17.50 ± 1.46* | 12.89 ± 0.78 | 12.98 ± 1.00 | 11.84 ± 0.43 | 11.75 ± 0.24 |
| FSH (miu/mL) | 7.90 ± 0.78 | 5.74 ± 0.82* | 7.30 ± 0.57 | 6.30 ± 0.72 | 6.70 ± 0.88 | 7.32 ± 0.93 |
| LH (miu/mL) | 5.97 ± 0.62 | 4.10 ± 0.31* | 5.39 ± 0.67 | 5.28 ± 0.71 | 5.45 ± 0.94 | 5.64 ± 0.67 |

Values in the row bearing (*) are not statistically significant ($P$ >0.05). Values represent the mean ± SD for n=3. FSH, Follicle stimulating hormone; LH, Luteinizing hormone.

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**Table 2:** The effect of aqueous *A. muricata* fruit pulp on sperm morphology of testosterone-induced benign prostatic hyperplasia in adult male rats

| Parameters | Normal control | Negative control | Positive control (5 mg/kg Finasteride) | AAMFPE (mg/kg) 500 | 1000 | 1500 |
|---|---|---|---|---|---|---|
| THAb (%) | 1.26 ± 0.12 | 2.99 ± 0.83* | 3.46 ± 0.22* | 2.21 ± 0.31 | 2.03 ± 0.02 | 1.93 ± 0.03 |
| TTAb (%) | 0.75 ± 0.05 | 1.13 ± 0.18* | 2.12 ± 0.03* | 0.77 ± 0.18 | 0.90 ± 0.03 | 0.86 ± 0.02 |
| TCD (%) | 1.70 ± 0.37 | 3.26± 0.80* | 3.21 ± 0.09* | 2.16 ± 0.09 | 2.87 ± 0.11 | 2.03 ± 0.02 |
| M-PAb (%) | 0.37 ± 0.29 | 3.52 ± 0.88* | 0.68 ± 0.13 | 0.80 ± 0.74 | 0.67 ± 0.16 | 0.43 ± 0.19 |
| TAS (%) | 4.08 ± 0.77 | 10.90 ± 0.96* | 9.48 ± 0.08* | 5.94 ± 1.22 | 6.47 ± 0.21 | 5.26 ± 0.18 |

Values in the row bearing (*) are not statistically significant ($P$ >0.05). Values represent the mean ± SD for n=3. THAb, total head abnormality; TTAb, total tail abnormality; TCD, total cytoplasmic droplets, MPAb, mid piece abnormality; TAS, total abnormal spermatozoa.

**Table 3:** The effect of *A. muricata* fruit pulp on the semen quality of testosterone-induced benign prostatic hyperplasia in adult male rats

| Parameter/Treatments | Normal control | Negative control | Positive control 5 mg/kg Finasteride) | AAMFPE (mg/kg) 500 | 1000 | 1500 |
|---|---|---|---|---|---|---|
| Semen colour (1-2) | 2.00 ± 0.00 | 2.00 ± 0.00 | 2.00 ± 0.00 | 2.00 ± 0.00 | 2.00 ± 0.00 | 2.00 ± 0.00 |
| Semen consistency (1-4) | 4.00 ± 0.00 | 2.00 ± 0.00* | 3.30 ± 0.00 | 4.00 ± 0.00 | 3.67 ± 3.67 | 4.00 ± 0.00 |
| Semen pH | 7.04 ± 0.01 | 7.03 ± 0.08 | 7.10 ± 0.01 | 7.02 ± 0.06 | 7.11 ± 0.04 | 7.15 ± 0.02 |
| % progressive motile sperm cells | 81.09 ± 1.40[a] | 65.27 ± 0.60* | 72.10 ± 2.19** | 71.21 ± 0.99** | 77.03 ± 1.05 | 84.48 ± 3.32 |
| Live proportion (%) | 91.28 ± 0.78 | 81.71 ± 2.05* | 79.27 ± 1.07* | 94.88 ±0.33 | 93.52 ± 1.45 | 93.09 ± 0.47 |
| Sperm count (x$10^6$/Cep) | 131.47 ± 2.76 | 113.64 ± 3.19* | 109.38 ± 3.33* | 141.70 ± 2.09 | 131.40 ± 4.90 | 142.78 ± 2.14 |
| Normal sperm proportion (%) | 95.92 ± 0.77 | 89.10 ± 0.96* | 90.52 ± 0.08* | 94.06 ± 1.22 | 93.53 ± 0.21 | 94.74 ± 0.18 |
| Total abnormal sperm (%) | 4.08 ± 0.77 | 10.90 ± 0.96* | 9.48 ± 0.08* | 5.94 ± 1.22 | 6.47 ± 0.21 | 5.26 ± 0.18 |

Values in the row bearing (*) are not statistically significant ($P$ >0.05).Values represent the mean ± SD for n=3

## Conclusion

In conclusion, the acute toxicity evaluation showed that AMFPE did not exhibit toxic effects at doses up to 5 mg/kg body weight, suggesting its safety for therapeutic applications. The result also showed that administration of AAMFPE over a 14-day period reduced testicular and prostate weights and improved PSA levels in male rats whose BPH was induced with testosterone propionate. In addition, male rats treated with AAMFPE showed improved hormonal profile and sperm morphology, such as abnormalities of total head, total tail, total cytoplasmic droplets, midpiece, and total abnormal sperm. The parameters of sperm consistency, pH, percentage of progressively motile sperm, percentage of live sperm, sperm count, percentage of normal sperm and total abnormal sperm were better in AAMFPE-treated rats. The pulp of *A. muricata* could, therefore, be useful in the treatment of male infertility.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

## References

1. Orieke D, Ohaeri OC, Irene I, Ijioma SN. Semen quality, hormone profile and histological changes in male albino rats treated with *Corchorus olitorius* leaf extract. Avicenna J Phytomed. 2019; 9(6): 551-562. doi: 10.22038/AJP.2019.13426.
2. Vander BM, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem. 2018; 62:2-10. doi:10.1016/j.clinbiochem.2018.03.012.
3. Anosike CA, Ugwu CC, Ogara AL, Ozioko CS, Ogugofor MO. Effect of methanol leaf extract of *Duranta erecta* in rats induced with benign prostatic hyperplasia. Trop J Nat Prod Res. 2020; 4(10): 790-795. doi.org/10.26538/tjnpr/v4i10.21.
4. Sasidharan S, Srinivasakumar KP, Bhaumik A, Kanti S, Nair JH.. Administration of *Caesalpinia bonduc* seed extracts ameliorates testosterone-induced benign prostatic hyperplasia (BPH) in male wistar rats. Res Reports Urol. 2022; 14: 225-239.
5. Abdel-Aziz AM, Gamal, NF, Salah A, Mohammed MM, Ali AI, Ibrahim YF. Amelioration of testosterone-induced benign prostatic hyperplasia using febuxostat in rats: The role of VEGF/TGFβ and iNOS/COX-2. Eur J Pharmacol. 2020; 889: 173631.
6. Ogbu PN, Ugota EO, Onwuka RU, Ogbu IM, Aloke C. Effect of acetogenin fraction of *Annona muricata* leaves on antioxidant status and some indices of benign prostatic hyperplasia in rats. Redox Report. 2020; 25(1): 80-86. doi: 10.1080/13510002.2020.1804711.
7. Onyegeme-Okerenta B, Anacletus F, Agene K, Eteng M. Ameliorating potential of *Annona muricata* on testosterone propionate-induced benign prostatic hyperplasia in male wistar rats. Int. J. Biochem. 2022; 5: 28-36.
8. Irwig MS, Kolukula S. Persistent sexual side effects of finasteride for male pattern hair loss. J Sexual Med. 2011; 8: 1747–1753.
9. Tu HY, Zini A. Finasteride-induced secondary infertility associated with sperm DNA damage. Fertil Steril. 2011; 95(6). https://doi.org/10.1016/j.fertnstert.2010.12.061.
10. Coria-Tellez AV, Montalvo-Go E, Yahia EM, Obledo-Vázquez E. 2018. *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arabian J Chem. 2018; 11: 662–691. doi: 10.1016/j.arabjc.2016.01.004.
11. Chinyere IA, Emmanuel PI, Chidinma OV, Sylvester NC. Evaluation of the antidiarrhoeal activity of the methanol pulp extract and fractions of *Annona muricata* Linn (Annonaceae). Trop J Nat Prod Res. 2022; 6(6): 974-979.doi.org/10.26538/tjnpr/v6i6.26.
12. Eyeghre O, Chijioke D, Ezeokafor E, Oparaji C, Sixtus A, Chukwuma C, Martins O, Igbokwe V. The impact of *Annona muricata* and metformin on semen quality and hormonal profile in Arsenic trioxide-induced testicular dysfunction in male Wistar rats. Magna Scientia Adv Res Rev. 2023; 08(01): 001–018.
13. Agu KC, Okolie NP, Falodun A, Engel-Lutz N. In vitro anticancer assessments of *Annona muricata* fractions and in vitro antioxidant profile of fractions and isolated acetogenin (15-acetyl guanacone). J. Cancer Res. Pract. 2018; 5 (2):53-66.
14. Roduan MR, Hamid RA, Cheah YK, Mohtarrudin N. Cytotoxicity, antitumor-promoting and antioxidant activities of *Annona muricata in vitro*. J Herbal Med. 2019; 15: 100219. doi: 10.1016/j.hermed.2018.04.004.
15. Gong GY, Xi SY, Li CC, Tang WL, Fu XM, Huang YP. *Bushen tongluo* formula ameliorated testosterone propionate-induced benign prostatic hyperplasia in rats. Phytomed. 2023;120: 155048.
16. Chibundu UC. Response of Pre-Pubertal Bucks to Administration of Estradiol B. Project. Federal University of Technology Owerri, Nigeria. 2013; pp. 30.
17. El-Sherbiny A.M. Seasonal Variation in Seminal Characteristics of Rabbits. M.Sc. Dissertation. Fac. of Agric, Ain-Shams University. 1987.
18. Herbert U. Growth and Reproductive Characteristics of Rabbits Fed on Leucaena (*Leucaena leucocephala*) and (*Gliricidia sepium*) foliage. Ph.D. thesis. 1992.
19. Egbuka CG. New Approach in Estimating Sperm Concentration in Farm Animals. Third Edition, Niger Press Plc. 1995; pp 16 -24.
20. Yu ZJ, Yan HL, Xu FH, Chao HC, Deng LH, Xu XD, Huang JB, Zeng T. Efficacy and side effects of drugs commonly used for the treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia. Frontiers Pharmacol. 2020; 11: 658. doi: 10.3389/fphar.2020.00658.
21. Arayombo B, Adewole O, Ojo S, Adelodun S, Adefule A, Bejide R, Salako K, Olabanji I, Ojewole A. Effects of the aqueous extract of *Mimosa pudica* on experimentally-induced prostatic hyperplasia. Int J Contemporary Res Rev. 2018; 9(02): 20439–20444. doi: 10.15520/ijcrr/2018/9/02/433.
22. Akanni OO, Owumi SE, Olowofela OG, Adeyanju AA, Abiola OJ, Adaramoye OA. Protocatechuic acid ameliorates testosterone-induced benign prostatic hyperplasia through the regulation of inflammation and oxidative stress in castrated rats. J Biochem Mol Toxicol. 2020; 34(8): e22502. doi:10.1002/jbt.22502.

23. Huang JJ, Cai Y, Yi YZ, Huang MY, Zhu L, He F, Liu XW, Huang BY, Yuan M. Pharmaceutical evaluation of naftopidil enantiomers: Rat functional assays in vitro and estrogen/androgen induced rat benign prostatic hyperplasia model *in vivo*. Eur J Pharmacol. 2016; 791:473-481. doi:10.1016/j.ejphar.2016.09.009.-
24. Park E, Lee MY, Jeon WY, Lee N, Seo CS, Shin HK. Inhibitory effect of Yongdamsagan-Tang water extract, a traditional herbal formula, on testosterone-induced benign prostatic hyperplasia in rats. Evid Based Complement Alternat Med. 2016; 2016:1428923. doi:10.1155/2016/1428923.
25. Veeresh BSV, Veeresh B, Patil AA, Warke A. 2010. Lauric acid and myristic prevent testosterone induced prostatic hyperplasiain rats. Eur. J. Pharmacol. 2010; 625: 262–265. doi:10.1016/j.ejphar.2009.09.037.
26. Oluwatosin A, Taiwo D, Olubukola O, Olusoji J. Hexane fraction of *Annona muricata* (Soursop) seed ameliorates testosterone-induced benign prostatic hyperplasia in rats. Biomed Pharmacother. 2019; 111: 403-413. https://doi.org/10.1016/j.biopha.2018.12.038.
27. Andriole GL, Crawford ED, Grubb RL, Buys SS, Chia D, Church TR, Fouad MN, Gelmann EP, Kvale PA, Reding DJ, Weissfeld JL, Yokochi LA, O'Brien B, Clapp JD, Rathmell JM, Riley TL, Hayes RB, Kramer BS, Izmirlian G, Miller AB, Pinsky PF, Prorok PC, Gohagan JK, Berg CD, PLCO Project Team. Mortality results from a randomized prostate-cancer screening trial. The New Engl. J. Med. 2009; 360(13): 1310-1319. doi: 10.1056/NEJMoa0810696.
28. Gharaee-Kermani M, Macoska JA. Promising molecular targets and biomarkers for male BPH and LUTS. Curr. Urol. Reports 2013; 14: 628–637.
29. Hu X, Wang YH, Yang ZQ, Shao YX, Yang WX, Li X. Association of 5-alpha-reductase inhibitor and prostate cancer incidence and mortality: a meta-analysis. Transl Androl Urol. 2020; 9(6): 2519–2532.
30. Jarvis TR, Chughtai B, Kaplan SA. Testosterone and benign prostatic hyperplasia. Asian J Androl. 2015; 17(2):212-216. doi: 10.4103/1008-682X.140966.
31. Ramaswamy S, Weinbauer GF. Endocrine control of spermatogenesis: Role of FSH and LH/testosterone. Spermatogenesis. 2015; 4(2): e996025. doi: 10.1080/21565562.2014.996025.
32. Hollander-Cohen L, Golan M, Levavi-Sivan B. Differential regulation of gonadotropins as revealed by transcriptomes of distinct LH and FSH cells of fish pituitary. Int J Mol Sci. 2021; 22(12): 6478. doi: 10.3390/ijms22126478.
33. Crosnoe LE, Grober E, Ohl D, Kim ED. Exogenous testosterone: a preventable cause of male infertility. Transl Androl Urol. 2013; 2(2): 106-113. doi: 10.3978/j.issn.2223-4683.2013.06.01.
34. Khalid M, Shah J, Suresh D, Singh R, Reddy I, Kumar, S. Evaluation of anti-diarrhoeal potential of ethanolic extract of *Mimosa pudica* leaves. Int J Green Pharm. 2011; 5: 75. 10.4103/0973-8258.82096.
35. Kobayashi H, Nagao K, Nakajima K. Focus issue on male infertility. Adv. Urol. 2012; 2012:823582. doi:10.1155/2012/823582.