

Protective Effects of *Annona Muricata* Leaf Extract on Semen Quality in Male Wistar Rats

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ABSTRACT

The aim of this study is to determine the protective effects of *Annona Muricata* leaf extract on semen quality in male wistar rats. A total of 78 adult male rats obtained from the laboratory animal production unit of the Department of Physiology, Abia State University, Uturu were used for the different segments of the study. Eighteen of the rats were used for acute toxicity/lethal dose evaluation of the extract while the remaining sixty (60) were used for the testosterone-induced hyperplasia study. Prostate hyperplasia was induced in the rats via daily sub-cutaneous administration of testosterone propionate (5 mg/kg body weight) for 28 days. Sixty rats used for this segment of the study were assigned to 6 groups of 10 rats and treated as follows: Group 1, Normal control, no treatment. Group 2 Testosterone propionate (5 mg/kg) only and was the BPH control. Group 3, Testosterone propionate (5 mg/kg bw) + Finasteride (3 mg/kg body weight). Group 4, Testosterone propionate (5 mg/kg bw) + *Annona Muricata* Leaf extract (300 mg/kg body weight). Group 5, Testosterone propionate (5 mg/kg bw) + *Annona Muricata* Leaf extract (600 mg/kg body weight). Group 6, *Annona Muricata* Leaf extract (600 mg/kg bw) only. At the end of treatment with *Annona Muricata* Leaf extract, animals were sacrificed and Semen samples were collected from the caudal epidymis of each rat for semen analysis (Sperm mass motility, Sperm cell viability, Sperm concentration and Sperm morphology) was evaluated macroscopically. Data obtained were subjected to Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented with Student's *t* test for post-hoc test for comparisons of the means of the various doses and fractions. P- values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value. Conclusively, treatment with *Annona muricata* extract showed enhancement of sperm quality.

Keywords: Testosterone-induced hyperplasia, *Annona muricata* leaf extract, sperm quality (sperm mass motility, sperm cell viability, sperm concentration, sperm morphology) and wistar rats

INTRODUCTION

Histopathological studies focusing on testicular tissue provide insights into the effects of *Annona muricata* on male reproductive health, particularly in conditions of oxidative stress, inflammation, and toxicity.[1,4] Research indicates that *Annona muricata* exhibits protective effects on testicular tissues, especially under conditions that induce reproductive toxicity. For instance, a study by Abou-El-magd *et al.* (2024) investigated the impact of *Annona muricata* leaf extract on the testicular histology of rats exposed to high doses of Doxorubicin (DOX). The results showed that treatment with the extract significantly alleviated Doxorubicin -induced testicular damage, including reduction in seminiferous tubule degeneration and restoration of normal spermatogenesis. These protective effects are attributed to the antioxidant properties of *Annona muricata*, which mitigate oxidative stress and preserve the integrity of testicular tissue. Another study explored the ameliorative effects of *Annona muricata* on testicular damage induced by cadmium, a common environmental toxin known to disrupt endocrine function and impair reproductive health. Histopathological analysis revealed that rats treated with *Annona muricata* extract exhibited significant improvements in testicular architecture, with reduced histopathological alterations such as seminiferous tubule atrophy, interstitial edema, and Leydig cell hyperplasia. The study concluded that *Annona muricata* helps in preserving testicular function and structure, likely due to its anti-inflammatory and antioxidant effects.[3] The potential of *Annona muricata* to enhance spermatogenesis and improve sperm parameters has also been documented. Olawuyi (2023) evaluated the effects of *Annona muricata* on testicular

function in a rat model of Aluminium-induced testicular toxicity.[7] The results showed that Aluminium treatment induced histological changes in testicular tissue including degeneration of germ cells, lowering of sperm count and increased morphological abnormalities. Animals treated with aluminium along with *Annona muricata* fruit-extract showed recovery of normal testicular architecture and improved semen parameters. It is evident that Aluminum Chloride induced deleterious effect on the health of adult wistar rats as illustrated by the distortion of testis histology and semen parameters. These outcomes suggest that *Annona muricata* may support male fertility by counteracting the adverse effects of environmental toxins on testicular health. In addition to its protective role, *Annona muricata* has been studied for its potential to regulate hormonal imbalances that affect testicular function. Research by Eyeghre *et al.* (2023) found that treatment with *Annona muricata* extract normalized altered levels of reproductive hormones, including testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), in diabetic rats. Histopathological evaluation of the testes revealed reduced diabetic testicular damage and improved spermatogenesis, supporting the role of *Annona muricata* in modulating endocrine function and enhancing reproductive health.

MATERIALS AND METHODS

Materials

Equipments used include sensitive weighing balance, Electric weighing balance, Manual blender, Dissecting set, Dissecting board, 5ml syringes, 1ml syringes, test tubes, serum bottles, Sample bottles, EDTA bottles, whatman No 1 filter paper, Measuring cylinders, Pasteur pipette, Cotton wool, Micro pipette, Rat cages, Feeding and water trough, Oral

gauvage, Glass column, Microscope, Glass TLC plates, microscope slides, Glass wool, funnel, Spatula, Retort stand, Hand gloves, TLC tank, beaker, Oven, freezer, Refrigerator, Spectrophotometer(722N, Mindray, MC Quijing, China), Centrifuge, Rotary evaporator, Soxhlet extractor, GC-MS machine, Chemoilluminoscene machine and Haematology analyser (BC-2300, Mindray, MC Quijing, China).

ETHICAL APPROVAL

This study was approved by the research and ethical committee of the Department of Physiology, Abia State University, Uturu.

Animals

A total of 78 adult male rats obtained from the laboratory animal production unit of the Department of Biochemistry, Abia State University, Uturu were used for the different segments of the study. The extract's acute toxicity and lethal dose were assessed in 18 of the rats, and the testosterone-induced hyperplasia investigation was conducted in the remaining 60. The animals were housed in aluminum cages, under standard conditions 12/12h light-dark cycle at temperature of $25 \pm 2^\circ\text{C}$ and were fed with standard grower pellets (Grand Cereals Ltd, Abia State) and had access to clean drinking water for seven days of acclimatization before the initiation of experiment. All experiments were carried out in accordance with International Guideline for Biomedical Research (CIOMS/OMS, 1985) and as approved by the Department of Physiology, Abia State University, Uturu.

Experimental Design for the Hyperplasia Study

Prostate hyperplasia was induced in the rats via daily sub-cutaneous administration of testosterone propionate (5 mg/kg body weight) for 28 days. Sixty rats used for this

segment of the study were assigned to 6 groups of 10 rats and treated as follows:

- Group 1 - Normal control, no treatment
- Group 2 - Testosterone propionate (5 mg/kg) only and was the BPH control
- Group 3 - Testosterone propionate (5 mg/kg bw) + Finasteride (3 mg/kg bw)
- Group 4 - Testosterone propionate (5 mg/kg bw) + AML extract (300 mg/kg bw)
- Group 5 - Testosterone propionate (5 mg/kg bw) + AML extract (600 mg/kg bw)
- Group 6 - AML extract (600 mg/kg bw) only

At the end of treatment, animals were sacrificed and Semen samples were collected from the caudal epidymis of each rat for semen analysis.

Preparation of Plant Extracts

Plant extract were prepared in accordance with the method used by Oriek *et al.*, (2019). The freshly collected leaves of *Anona muricata* were air dried under shade for within 14 days and were thereafter pulverized into coarse powdered form using a locally fabricated grinding machine. Two solvents, ethanol and N-hexene were used for the preparation of the extracts. A known quantity (300g) of the powdered plant material was macerated in 1.5 litres of ethanol (AR) for 48 hours, been vigorously shaken every 2 hours within the period and was thereafter filtered with Whatman No. 1 filter paper to obtained a filtrate which was the extract in solution. The filtrate so obtained was concentrated at low temperature to obtain a solid dark-green extract which weighed 13.91 g and represented 4.64% extract yield. The extract was preserved in the refrigerator at -4°C until needed and is

hereafter referred to as *Anona muricata* leaf (AML) extract.

Semen Collection and Analysis

The epididymal reserve was used to harvest the sperm cells. The rats' epididymides were removed after they were anesthetized by inhaling chloroform. Each epididymis' caudal section was cut, and a smear was created on the heated glass slides for assessment.

Macroscopic examination

The color and consistency of the semen were assessed macroscopically and documented. Chibundu (2013)'s consistency scale (1-4) was applied.

Microscopic examination

Sperm mass motility:

The approach that El-Sherbiny (1987) outlined was used. Each treatment group's semen samples, obtained during epididymal washings, were examined for progressive motile sperm cells. This was completed very away upon collection. On a heated glass slide, a drop of semen was applied. It was then examined under a light microscope at lower magnifications of $\times 10$ and $\times 40$ and subjectively rated as a percentage.

Sperm cell viability (live proportion):

The proportion of sperm cells that are viable (alive) was determined by staining a drop of the collected semen (epididymal washings) with Eosin-Nigrosin stain. The stained glass slides were allowed to dry for 30 seconds before fixing with ethanol. A hand-held stopwatch manual counter was used to count the percentage of viable

sperm cells after the dyed slides were examined under a light microscope at $\times 100$ magnification (oil immersion). The percentage of viable sperm cells was determined by counting all 300 cells. The sperm cells that picked up the stain were dead, whereas the viable (living) sperm cells did not (El-Sherbiny, 1987).

Sperm concentration:

Using a technique outlined by Herbert (1992), the hemocytometer was used to measure the concentration of sperm cells in the semen samples. A red blood cell pipette was used to create a dilution of 1: 200. To immobilize the sperm cells, a 10% buffered formalin solution was utilized as the semen diluting fluid. The **haemocytometer** was charged with a drop of the sperm solution and allowed for 2 minutes on a wet paper (to allow the sperm cell to settle) before it was mounted on a light microscopic stage and viewed under $\times 40$ magnification.

The sperm concentration per ml = No. of cells counted $\times$ DF $\times 0.04 \times 10^6$
(**Note:** DF = Dilution factor) – Egbuka, 1995).

Abnormal sperm proportion (sperm morphology):

El-Sherbiny's (1987) approach was used to determine the aberrant sperm proportion. To check for primary and secondary abnormal sperm cells, as well as the percentage of differential abnormalities like head, tail, and mid-piece abnormalities, a drop of semen was stained with E/N stain, smeared on a glass slide, and examined at a lower magnification of $\times 40$ (El-Sherbiny, 1987).

Descriptive and Numerical Scales for Evaluation of Microscopic Pattern of Semen

Numerical scale	Descriptive scale	% sperm motility	Wave pattern
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0	V. Poor	0 – 20	Immotile, no wave
1	Poor	20 – 40	Stationary and bunting. Weak movements; no waves
2	Fair	40 – 50	Oscillatory or rotary movements. Few waves and eddies
3	Good	50 – 80	Progressive rapid movement. Waves and eddies seen.
4	V. good	80 – 90	Vigorous rapid movement. Eddies seen
5	Excellent	90 – 100	Very vigorous rapid movement. Rapid waves and eddies

Source: Peter, I. R (2002).

Individual motility score (1 – 4)

1. Progressive rectilinear motility – rapid straight forward movement
2. Circling motility – moves in circles because of mid and tail defects.
3. Reverse circling motility – moves in circles, in backward direction, no marked progress.
4. Pendulation motility – jerky, serpentine movement; no marked progress.

Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance (ANOVA). The Duncan Multiple Range Test was used to evaluate the data, and the Student's t test was used as a post-hoc test to compare the means of the different doses and fractions. For the measured value, P-values less than 0.05 were regarded as statistically significant differences between the test groups and the control group.

Data analysis

RESULTS

Table 4: Semen Quality.

Treatment s	Nor mal contr ol	BPH control (Testostero ne only)	Finesteride (3 mg/kg) + Testosterone	AML extract (200 mg/kg) + Testosterone	AML extract (400 mg/kg) + Testosterone	AML extract (800 mg/kg) + Testosterone
Semen colour (1-2)	2.00 ±0.00 ^b	1.00±0.00 ^a	2.00±0.00 ^b	2.00±0.00 ^b	2.00±0.00 ^b	2.00±0.00 ^b
Semen consistenc y (1-4)	4.00 ±0.00 ^b	2.33±0.58 ^a	3.67±0.58 ^b	3.67±0.58 ^b	4.00±0.00 ^b	4.00±0.00 ^b
Semen pH	7.05 ±0.03 ^b	6.66±0.13 ^a	7.03±0.02 ^b	6.95±0.10 ^b	7.01±0.03 ^b	7.02±0.05 ^b
Sperm mass motility (%)	94.77 ±2.16 ^c	68.73±1.19 ^a	85.73±1.31 ^b	87.10±1.97 ^b	94.13±2.84 ^c	94.83±1.21 ^c
Live proportion	95.47 ±0.9	75.93±4.52 ^a	92.60±1.70 ^{b,c}	90.50±2.07 ^b	94.03±1.00 ^{b,c,d}	97.07±0.25 ^d

(%)	3 ^{c,d}					
Sperm count (x10 ⁶ /CEp)	138.03±3.53 ^d	91.03±2.33 ^a	120.73±1.69 ^b	127.30±2.10 ^c	128.30±3.20 ^c	142.10±6.32 ^d
Normal sperm proportion (%)	96.17±1.19 ^{b,c}	85.77±5.55 ^a	91.93±1.32 ^{a,b}	94.30±1.41 ^{a,b}	94.33±0.83 ^{a,b}	97.07±0.76 ^c
Total abnormal sperm (%)	3.83±1.19 ^{a,b}	14.23±5.55 ^c	8.07±1.32 ^b	5.70±1.41 ^{a,b}	5.00±1.60 ^{a,b}	2.93±0.76 ^a

Values are presented as mean ± standard deviation (n = 3); and means with different letter superscripts are significantly (P < 0.05) different from paired mean across the row.

Table 1, presents the results of different treatments on semen quality parameters. Compared to the normal control group, the BPH control (Testosterone only) group shows significant reductions in semen quality indicators. Semen consistency (2.33±0.58), pH (6.66±0.13), sperm mass motility (68.73±1.19%), live proportion (75.93±4.52%), sperm count (91.03±2.33 x 10⁶/CEp), normal sperm proportion (85.77±5.55%), and total abnormal sperm (14.23±5.55%) are all adversely affected. Treatment with Finasteride (3 mg/kg) + Testosterone and AML extract at various doses (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone generally improves these parameters compared to the BPH control group. Specifically, these treatments increase sperm mass motility (85.73% to 94.83%), live proportion (90.50% to 97.07%), sperm count (120.73 x 10⁶/CEp to 142.10 x 10⁶/CEp), normal sperm proportion (91.93% to 97.07%), and reduce total abnormal sperm (2.93% to 14.23%). Semen colour and consistency remain consistently high across all treatment groups (2.00 and 4.00, respectively), indicating minimal variation in these parameters among the groups.

DISSCUSION

Semen quality parameters are critical indicators of male reproductive health, reflecting sperm viability, motility, and overall fertility potential. The BPH control group, induced by testosterone alone, exhibits significant impairments in various semen quality indicators compared to the normal control group, highlighting the adverse effects of hormonal imbalance on reproductive function. In the BPH control group, semen consistency and pH are notably altered, alongside reductions in sperm mass motility, live sperm proportion, sperm count, and normal sperm morphology. Conversely, total abnormal sperm proportion is increased, indicative of compromised sperm quality under testosterone-induced BPH conditions. Treatment with Finasteride + Testosterone and *Annona muricata* leaf extract at various doses + Testosterone demonstrates beneficial effects on semen quality parameters. These treatments consistently improve sperm mass motility, live sperm proportion, sperm count and normal sperm morphology. They also reduce total abnormal sperm count, indicating enhanced sperm viability and morphology compared to the BPH control group. Studies on *Annona muricata*'s effects on reproductive health underscore its potential in improving semen quality parameters. The extract's bioactive

compounds, including flavonoids, alkaloids, and acetogenins, likely contribute to its protective effects on sperm function and fertility. For instance, research has shown that *Annona muricata* extracts possess antioxidant properties that can mitigate oxidative stress-induced damage to sperm cells, thereby preserving sperm motility and viability.[5,6] Furthermore, the modulation of hormonal pathways and reduction of oxidative stress by *Annona muricata* extracts may contribute to improvements in sperm quality parameters observed in experimental studies.[4] These mechanisms highlight the potential of *Annona muricata* as a therapeutic agent for addressing reproductive disorders associated with BPH-induced hormonal imbalance.[2,8]

CONCLUSION

Conclusively, treatment with *Annona muricata* extract showed enhancement of sperm quality.

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