

The Protective Effects of *Annona Muricata* (Soursop) Leaf Extract Against Testosterone – Induced Prostate Hyperplasia and Reproductive Dysfunction in a Male Rat Model

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ABSTRACT

*In a male rat model, the purpose of this study was to examine the preventive effects of annona muricata leaf extract against testosterone-induced prostate enlargement and reproductive failure. A total of 78 adult male rats was 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. Rats were given testosterone propionate (5 mg/kg body weight) subcutaneously every day for 28 days in order to cause prostate hyperplasia. 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, animals were sacrificed, Organs including testes, prostate, liver, kidney and heart were harvested, weighed and preserved in labelled sample bottles containing 10% formaldehyde solution for histopathological studies of the testes and prostate. 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. From this present study, Histopathological evaluations of the testes and prostate consistently revealed preserved tissue architecture in treated animals with Annona muricata leaf extract highlighting the extract's tissue-protective properties in benign prostatic hyperplasia and related male reproductive disorders.*

Keywords: *Annona muricata* (soursop), protective effects, prostate, testes, testosterone – induced benign prostatic hyperplasia and wistar rats

INTRODUCTION

Histopathological studies focus on examining tissue changes at the microscopic level to understand the effects of *Annona muricata* on prostate health, especially in conditions such as benign prostatic hyperplasia (BPH) and prostate cancer [4,5]. Histopathological analysis of prostate tissue involves examining the architecture of the prostate gland, the presence of inflammatory cells, and any signs of hyperplasia or malignancy[9,14]. Studies have shown that *Annona muricata* exhibits significant effects on prostate tissue, which is explained by its abundant phytochemical makeup, which includes tannins, alkaloids, flavonoids, acetogenins, and saponins. [4].

One study investigated the effects of *Annona muricata* extract on rats induced with testosterone-induced prostatic hyperplasia. The results demonstrated that treatment with *Annona muricata* extract led to a significant reduction in prostate size and weight compared to the control group. Histopathological examination revealed decreased epithelial thickness, reduced glandular hyperplasia, and lowered inflammatory cell infiltration in the prostate tissues of treated rats[2]. These findings suggest that *Annona muricata* possesses anti-proliferative and anti-inflammatory properties, which are beneficial in managing conditions like BPH. In another study, the anticancer potential of *Annona muricata* was assessed in prostate cancer models. Histopathological analysis of prostate tissues from treated animals showed a marked reduction in tumor size and number. The treatment with *Annona muricata* extract led to the induction of apoptosis, as evidenced by increased apoptotic bodies and reduced mitotic figures in the prostate tissues.[15] The apoptotic effect is primarily attributed to the acetogenins present in *Annona*

muricata, which have been shown to inhibit mitochondrial complex I, leading to the activation of the intrinsic apoptotic pathway [10].

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 gavage, 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, Chemo illuminoscene 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 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. 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:

Preparation of plant extracts

Plant extracts were prepared in accordance with the method used by Orieko *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-hexane 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 obtain 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.

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 body weight) + Finasteride (3 mg/kg body weight)
Group 4 - Testosterone propionate (5 mg/kg body weight) + *Annona Muricata*

Leaf extract (300 mg/kg body weight)

Group 5 - Testosterone propionate (5 mg/kg body weight) + *Annona Muricata*

Leaf extract (600 mg/kg body weight)

Group 6 - *Annona Muricata* Leaf extract (600 mg/kg body weight) only
At the end of treatment, animals were sacrificed, Organs including testes, prostate, liver, kidney and heart were harvested, weighed and preserved in labelled sample bottles containing 10% formaldehyde solution for histopathological studies.

Histopathological evaluation of the prostate, testes, liver, kidney and heart tissues

For histological analysis, the aforementioned organs were identified and extracted into 10% formal saline. This was carried out using Clayden's (1967) and John and Alan's (1977) methodology. To guarantee adequate dehydration of the tissues, organ slices were first preserved in 10% formalin for 48 hours. They were then processed by arranging them in increasing alcohol grades in the following order: 50% alcohol for one hour, 70% alcohol for one hour, first 95% alcohol for one hour and second 95% alcohol for one hour and fifteen minutes, first absolute alcohol for one and a half hours, and second absolute alcohol for two hours. Following their transfer to a combination of equal parts alcohol and xylene, the dehydrated tissues were allowed overnight before being cleaned with two changes of xylene for an hour each. A microtome was used to segment them at a thickness of 5µm after they had been infiltrated twice

for an hour each with molten paraffin wax in an oven set to 600C. After that, they were imbedded in paraffin wax, cut, and placed on a wooden chuck. Clean albuminized slides were used to choose the sections, which were then floated in a floating-out bath. After placing the slides in a staining dish, excess wax was removed using two xylene changes. The slides were then hydrated for two minutes each using descending alcohol grades: 100% alcohol, 95% alcohol, and 70% alcohol. The slides were infiltrated with Ehrlich haematoxylin for 15 minutes, followed by a 5-minute water wash, a 10-minute differentiation in 10% acid alcohol, and a 10-minute blueing under running water. For two minutes, they were counter-stained with filtered eosine. Excess eosine was eliminated using increasing alcohol grades (75%, 95%, and 100%) for two minutes each. This was followed by two

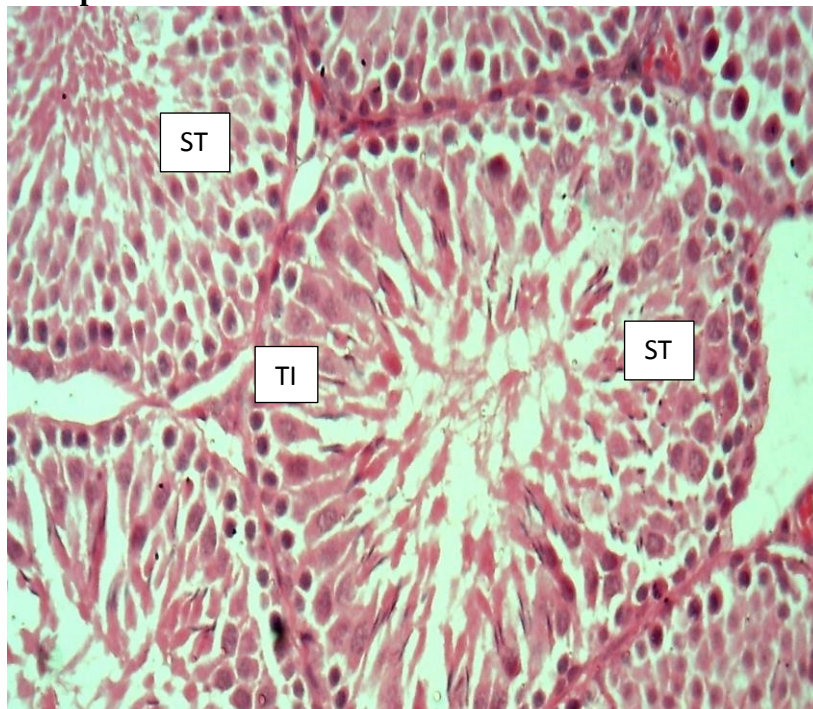
xylene changes and a depex mountant slip for each cover. The slides were examined under a light microscope, and a computer-connected Moticam 2.0 digital camera was used to take a few chosen pictures.

Data Analysis

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.

RESULTS

Group 1



Sections of the **testes** presented in this group showed the normal testicular histo-architecture. Normal seminiferous tubules (ST) lined by stratified epithelium of germ cells and normal testicular interstitium (TI) were observed. HE x200

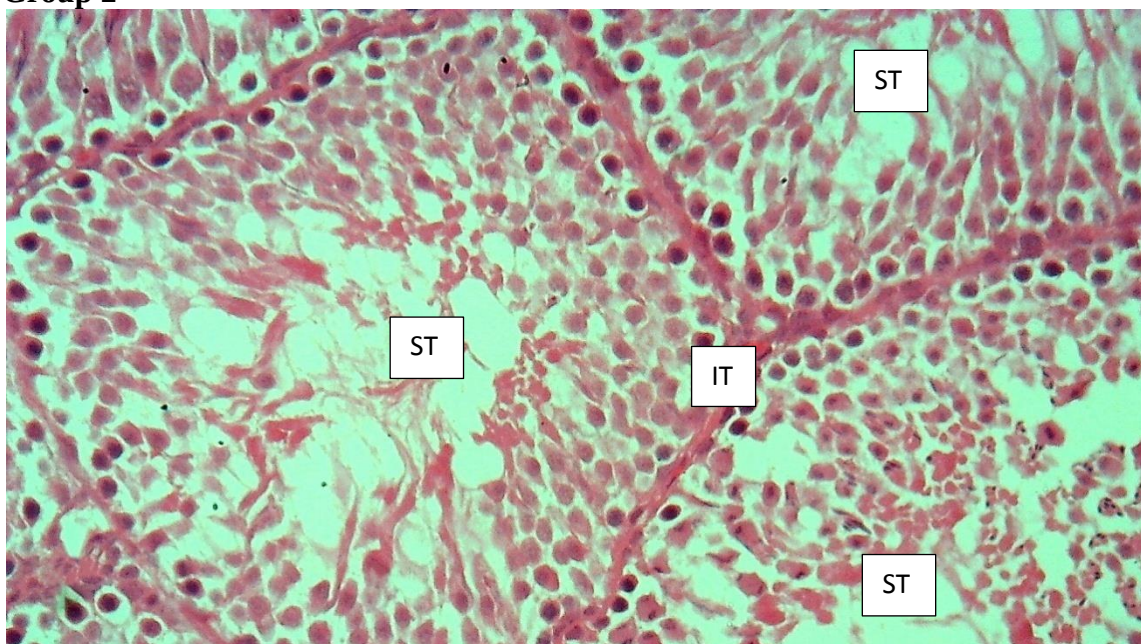


Sections of the **Prostate** presented in this group showed the normal histomorphology of the different lobes of the prostate. The acinar (A) were lined with cuboidal to low columnar epithelium with occasional papillary infoldings.

Interstitium (IT)

HE x200

Group 2

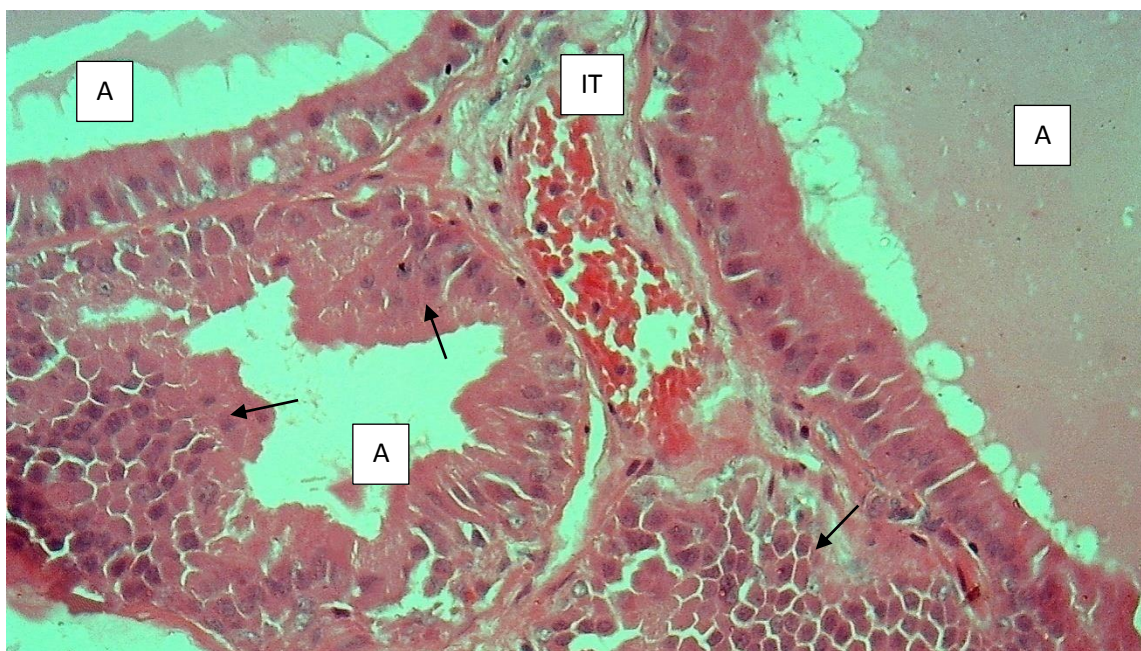


Sections of the **Prostate** presented in this group showed multifocally widespread areas of epithelial hyperplasia (arrow).

Acini (A)

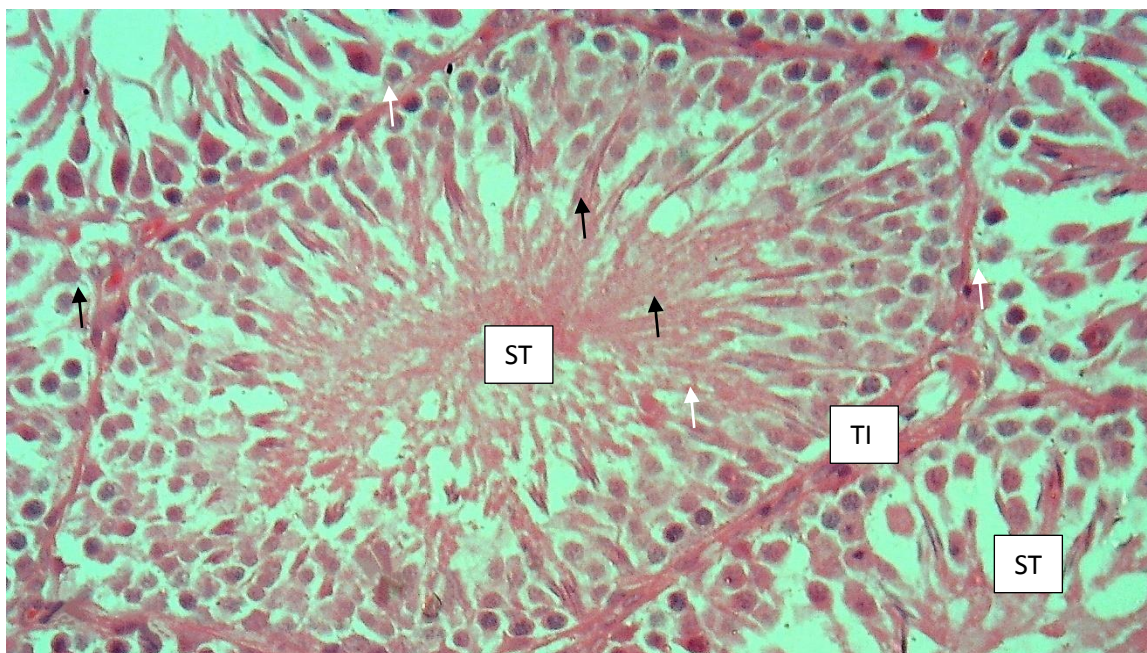
Interstitium (IT)

HE x200

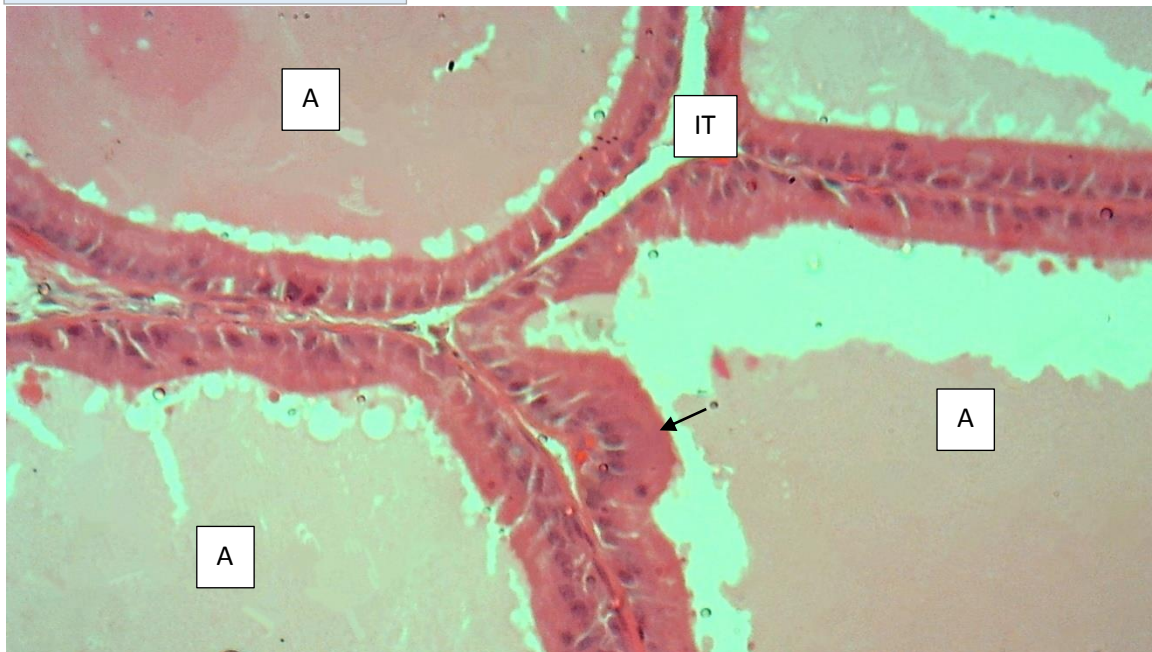


Sections of the **testes** presented in this group showed the normal testicular histo-architecture. Normal seminiferous tubules (ST) lined by stratified epithelium of germ cells and normal testicular interstitium (TI) were observed. HE x200.

Group 3



Sections of the **testes** presented in this group showed the normal testicular histo-architecture. Normal seminiferous tubules (ST) lined by stratified epithelium of germ cells and normal testicular interstitium (TI) were observed. HE x200

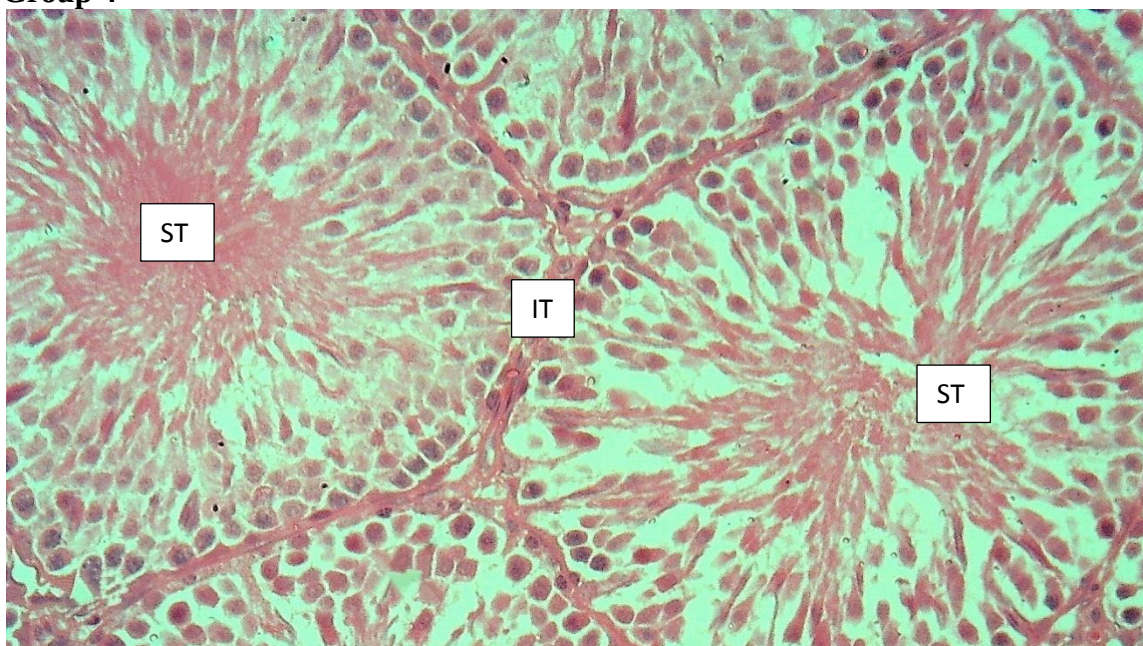


Sections of the **Prostate** presented in this group showed the normal histomorphology of the different lobes of the prostate. The acinar (A) were lined with cuboidal to low columnar epithelium with occasional papillary infoldings (arrow).

Interstitium (IT)

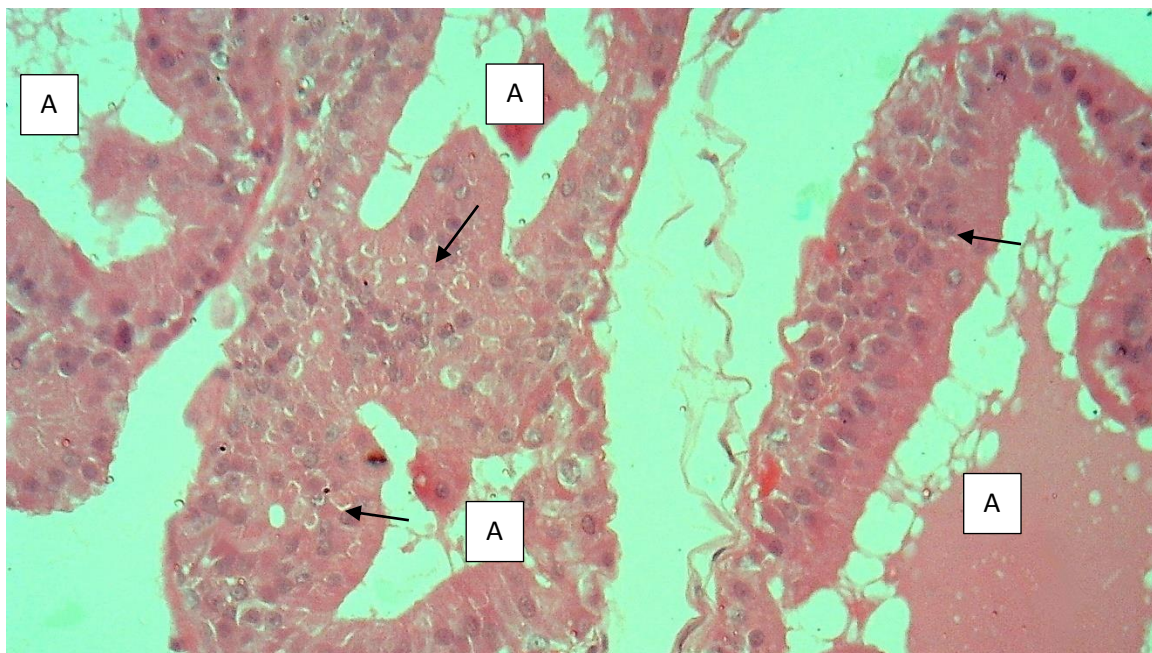
HE x200

Group 4



Sections of the **testes** presented in this group showed the normal testicular histo-architecture. Normal seminiferous tubules (ST) lined by stratified epithelium of germ cells and normal testicular interstitium (TI) were observed.

HE x200.



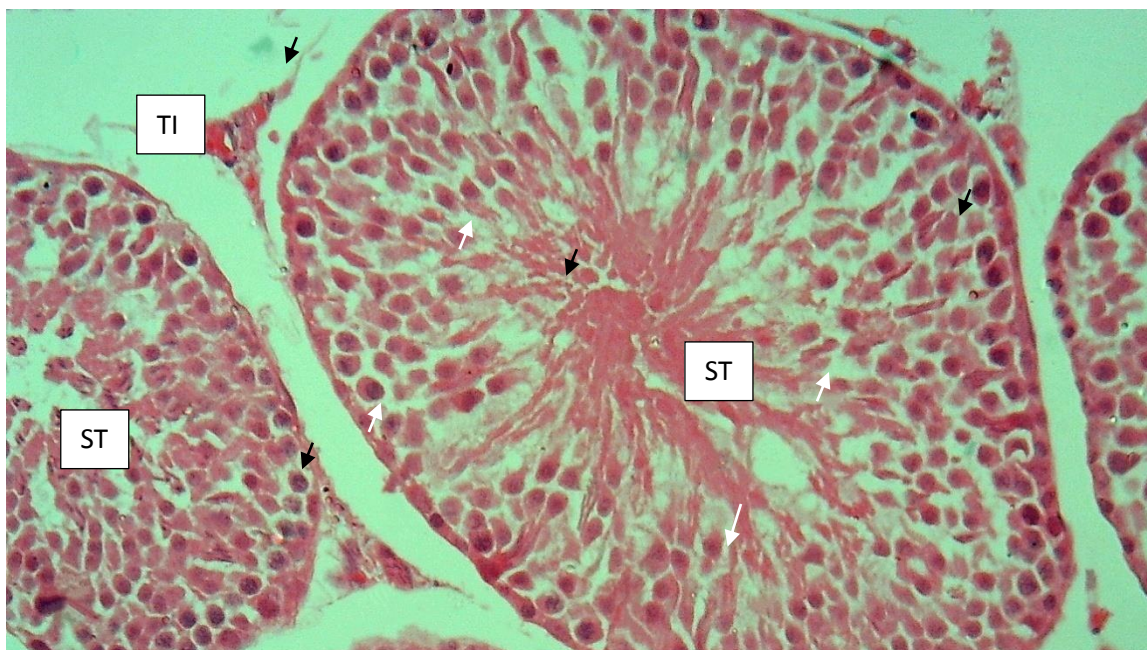
Sections of the **Prostate** presented in this group showed multifocally widespread areas of epithelial hyperplasia (arrow).

Acini (A)

Interstitium (IT)

HE x200

Group 5



Sections of the **testes** presented in this group showed the normal testicular histo-architecture. Normal seminiferous tubules (ST) lined by stratified epithelium of germ cells and normal testicular interstitium (TI) were observed.

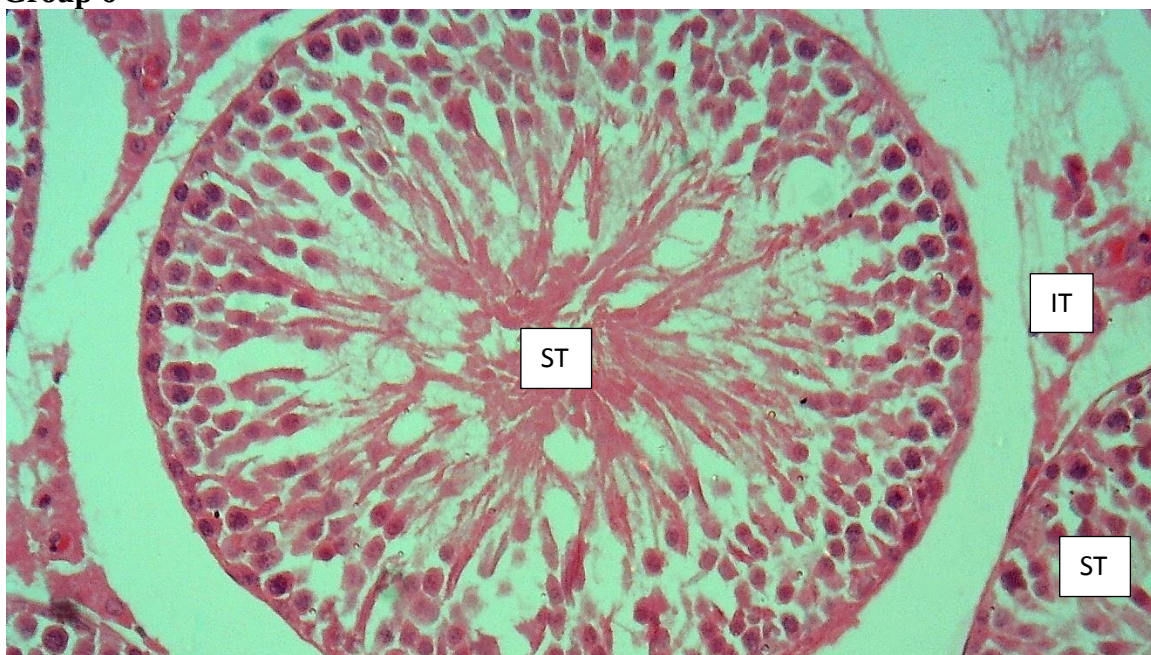
HE x200



Sections of the **Prostate** presented in this group showed the normal histomorphology of the different lobes of the prostate. The acinar (A) were lined with cuboidal to low columnar epithelium with occasional papillary infoldings.

Interstitium (IT)

Group 6



Sections of the **testes** presented in this group showed the normal testicular histo-architecture. Normal seminiferous tubules (ST) lined by stratified epithelium of germ cells and normal testicular interstitium (TI) were observed.

HE x200

DISCUSSION

The histopathological findings from the study provide insights into the impact of different treatments on the testes, and prostate across six distinct groups. In all groups (Groups 1 to 6),. Similarly, the testicular histopathology in all groups (Groups 1 to 6) revealed normal testicular architecture. This includes normal seminiferous tubules lined by stratified epithelium of germ cells and a normal testicular interstitium (HE x200).

These findings indicate that neither the induction of BPH with testosterone nor the subsequent treatments significantly disrupted testicular morphology. The preservation of normal testicular histoarchitecture is crucial for maintaining male reproductive function, including spermatogenesis and hormone production. Studies on *Annona muricata* suggest potential protective effects on testicular tissues, preserving normal architecture and function against oxidative stress and other insults [1,3,7]. In Groups 2, 4, and 6, sections of the prostate showed multifocally widespread areas of epithelial hyperplasia (arrow), while Groups 1, 3, and 5 displayed normal histo-morphology.

The acini were generally lined with cuboidal to low columnar epithelium with occasional papillary infoldings (HE x200), typical of normal prostate tissue.

The observed epithelial hyperplasia in some treatment groups (Groups 2, 4, and 6) may reflect responses to BPH induction and subsequent treatments. Hyperplastic changes in the prostate gland are commonly associated with benign prostatic enlargement, a condition exacerbated by testosterone and mitigated by treatments like Finasteride and potentially *Annona muricata* extract.[6]

It is evident that *Annona muricata* possesses compounds with antioxidant, anti-inflammatory, and tissue-protective properties, which may explain its potential in preserving normal tissue architecture observed in the testes, and prostate across these histopathological findings [8,13]. The reduction or prevention of epithelial hyperplasia in the prostate by these treatments underscores their therapeutic potential in managing conditions like BPH [4,11,12]

CONCLUSION

Histopathological evaluations of the testes, and prostate consistently revealed preserved tissue architecture in treated animals with *Annona muricata* leaf extract highlighting the extract's tissue-protective properties in benign prostatic hyperplasia and related male reproductive disorders.

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