

## **Cardiomyocytes Modulating Effects of *Annona Muricata* (Soursop) Leaf Extract on Testosterone - Induced Benign Prostatic Hyperplasia In Male Wistar Rats**

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### **ABSTRACT**

This present study aimed to determined Cardiomyocytes modulating effects of *annona muricata* (soursop) leaf extract on testosterone – induced benign prostatic hyperplasia. 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, animals were sacrificed, Organs such as the heart were harvested, weighed and preserved in labelled sample bottles containing 10% formaldehyde solution for histopathological studies of the heart. Data obtained were suggested 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. For the measured value, P-values less than 0.05 were regarded as statistically significant differences between the test groups and the control group. From this present study, histopathological evaluations of the heart, consistently revealed preserved heart tissue architecture in treated animals with *Annona muricata* leaf extract highlighting the extract's tissue-protective properties in benign prostatic hyperplasia.

**Keywords:** *Annona Muricata (soursop), cardiomyocytes modulation, testosterone – induced benign prostatic hyperplasia and wistar rats*

## INTRODUCTION

One of the promising natural remedies *Annona muricata* (soursop) for BPH and reproductive health issues and other comorbidity like heart pathology. [3,6] *Annona muricata*, commonly known as soursop, is a tropical fruit-bearing tree belonging to the Annonaceae family. Native to the tropical regions of the Americas, including the Caribbean, Central America, and northern parts of South America, *Annona muricata* is now widely cultivated in various tropical regions around the world.[5] The tree can grow up to 8-10 meters in height, featuring large, glossy, dark green leaves and heart-shaped fruits covered with soft spines. The fruit is known for its sweet, tangy flavor and is used in a variety of culinary applications.[8] *Annona muricata* leaves are rich in a variety of bioactive compounds, which contribute to their medicinal properties. Key phytochemicals identified in the leaves include acetogenins, alkaloids, and flavonoids. [10] Acetogenins exhibit a range of biological activities, including antitumor, antiparasitic, and antimicrobial effects. Notable acetogenins found in *Annona muricata* include annonacin, annonacinone, and squamocin [2,13]. The leaves contain several alkaloids, which are known for their therapeutic properties. Some of the alkaloids identified include asimilobine, stepharine, and reticuline. According to Fofana et al. (2012), these substances have shown analgesic, anti-inflammatory, anti-hypertensive, and antispasmodic properties. Flavonoids such as quercetin, kaempferol, and rutin have been found in the leaves.[4] They contribute to the anti-inflammatory and

antioxidant activities of *Annona muricata*[7,9]

## MATERIALS AND METHODS

### Materials

Equipment's 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

The study's several sections were conducted using 78 adult male rats that were acquired from the Department of Physiology's laboratory animal production facility at Abia State University in Uturu. While the remaining sixty (60) rats were employed for the testosterone-induced hyperplasia investigation, the remaining eighteen rats were used for the evaluation of the extract's acute toxicity and lethal dose. 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. The sixty rats utilized in this study section were divided into six groups of ten and given the following treatment:

### **Preparation of Plant Extracts**

Plant extracts were prepared in accordance with the method used by Orieki *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  $-4^{\circ}\text{C}$  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, heart were harvested, weighed and preserved in labelled sample bottles containing 10% formaldehyde solution for histopathological studies of the heart.

### **Histopathological Evaluation of the Prostate, Testes, Liver, Kidney and Heart Tissues**

The above-mentioned organs were identified and harvested into 10% formal saline for histopathological studies. This was done according to the method of Clayden, (1967) and John and Alan, (1977). Slices of the organs were fixed in 10% formalin for 48 hours and subsequently processed by placing them in ascending grades of alcohol in the following order, 50% alcohol for 1 hour, 70% alcohol for 1h hour, first 95% alcohol for 1 hour and second 95% alcohol for 1hour 15 minutes, first absolute alcohol for one and half hours and second absolute alcohol for 2 hours to ensure proper dehydration of the tissues. The dehydrated

tissues were then transferred to mixture of equal volumes of alcohol and xylene where they were left overnight and later cleared with two changes of xylene for 1 hour each. They were thereafter be infiltrated twice for 1 hour each with molten paraffin wax in the oven at 60°C before being embedded in paraffin wax, trimmed and mounted on wooden chuck, and then taken to the microtome for sectioning at 5µm thickness. . The slides were put on a staining dish, and excess wax was removed with two xylene changes. They were then hydrated for two minutes each with absolute, 95%, and 70% alcohol in decreasing order. Ehrlich haematoxylin was used to stain the slides for fifteen minutes. They were then rinsed with water for five minutes, differentiated in ten percent acid alcohol, then blued in a flowing faucet for ten minutes. For two minutes, they were counter-stained with filtered eosine. Excess eosine was eliminated using increasing alcohol grades

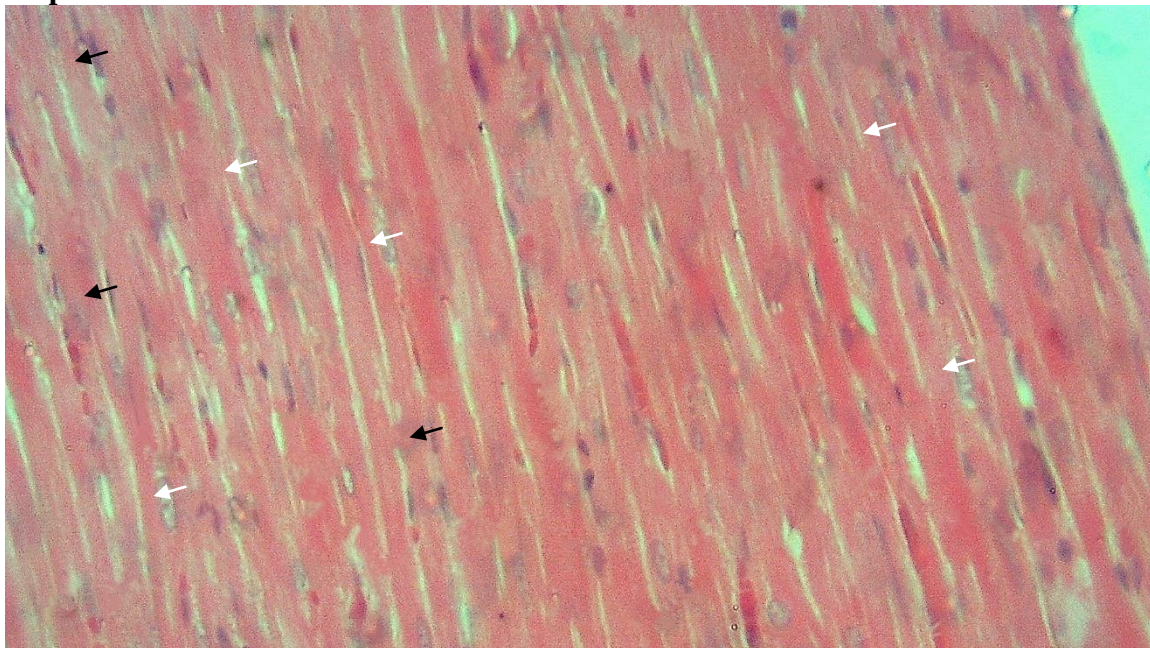
(75%, 95%, and 100%) for two minutes each. This was followed by two xylene changes and a depex mountant slip for each cover. The slides were examined under a light microscope, and a computer-connected Moticam 2.0 digital camera was used to take a few chosen pictures.

### Data Analysis

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc., Chicago, IL). 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

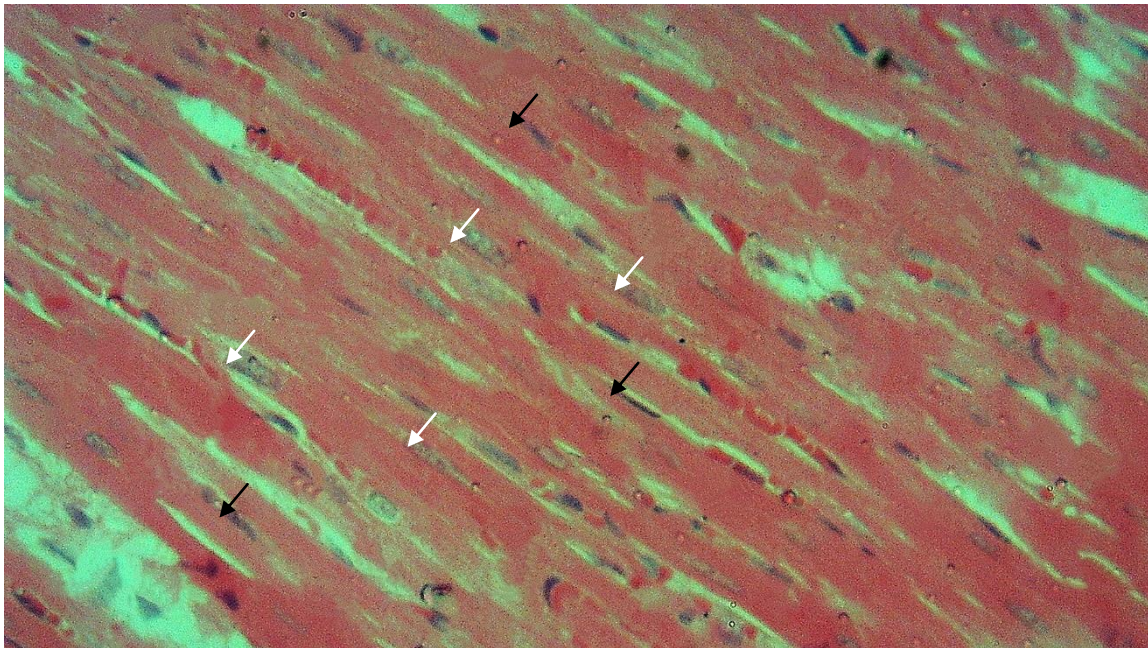
### Group 1



The sections of the heart presented in this group showed the normal myocardial histomorphology. Normal cardiomyocytes with oval to elongated, euchromatic nuclei (white arrow) were observed. Pericytes (black arrow).

HE x 400

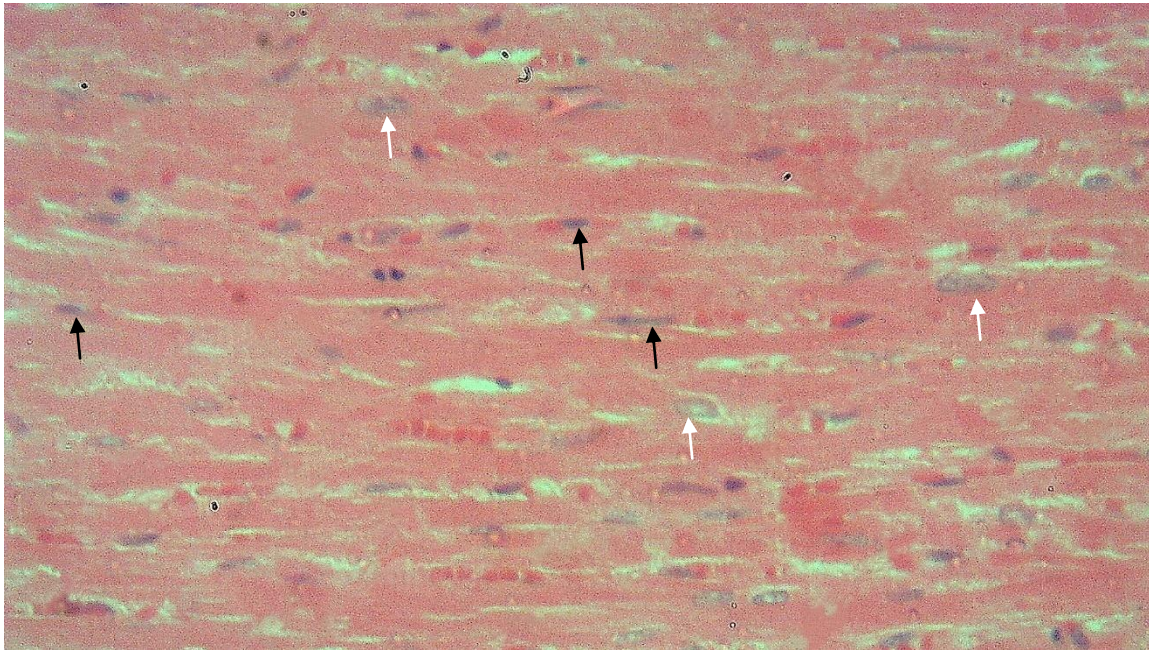
## Group 2



The sections of the heart presented in this group showed the normal myocardial histomorphology. Normal cardiomyocytes with oval to elongated, euchromatic nuclei (white arrow) were observed. Pericytes (black arrow).

HE x 400

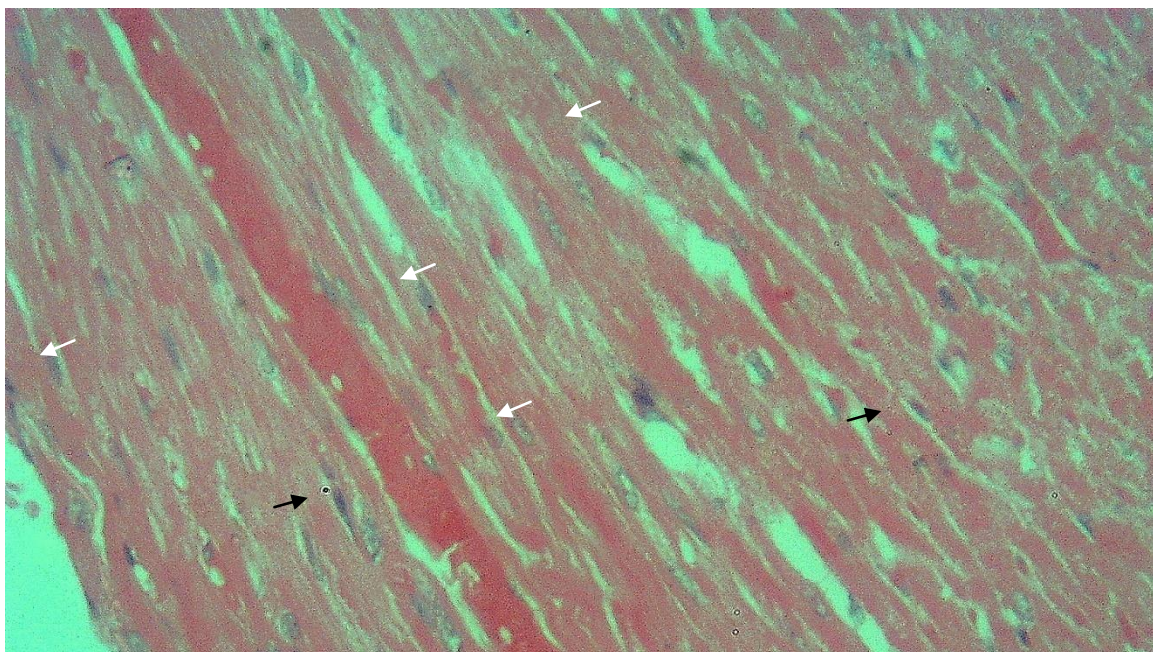
## Group 3



The sections of the heart presented in this group showed the normal myocardial histomorphology. Normal cardiomyocytes with oval to elongated, euchromatic nuclei (white arrow) were observed. Pericytes (black arrow).

HE x 400

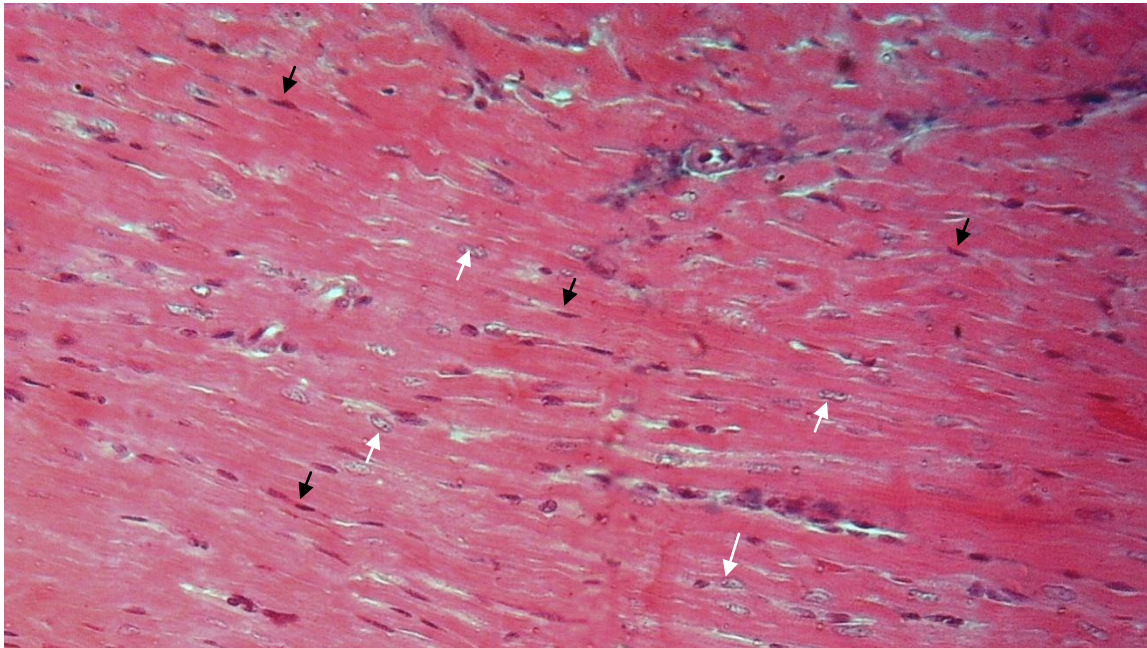
#### **Group 4**



The sections of the heart presented in this group showed the normal myocardial histomorphology. Normal cardiomyocytes with oval to elongated, euchromatic nuclei (white arrow) were observed. Pericytes (black arrow).

HE x 400

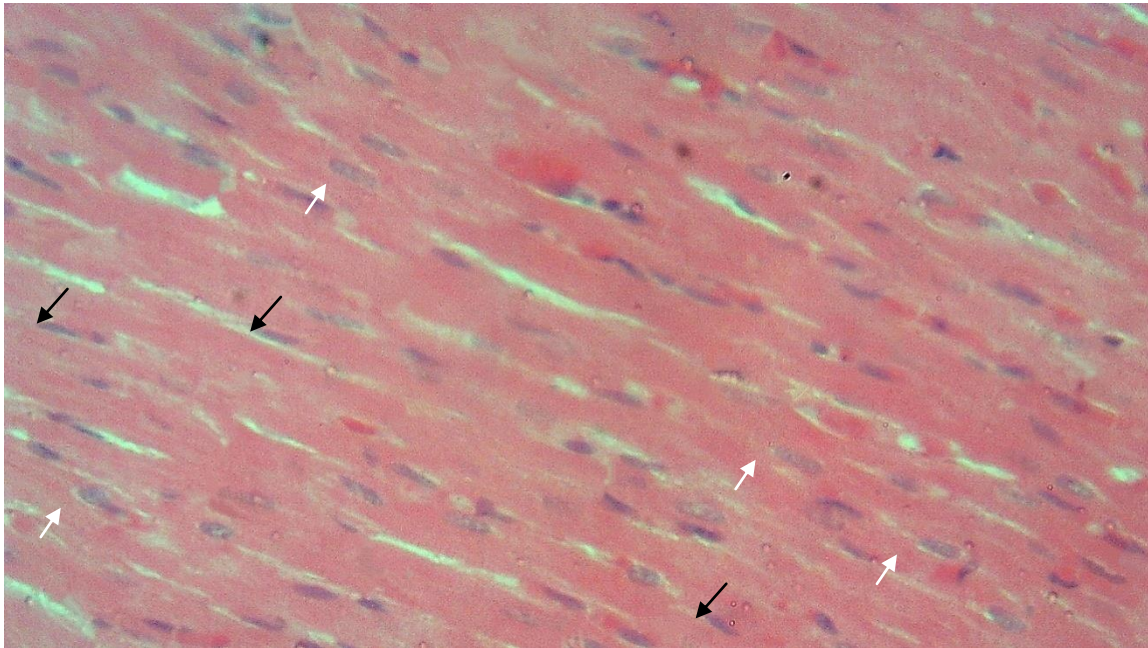
#### **Group 5**



The sections of the heart presented in this group showed the normal myocardial histomorphology. Normal cardiomyocytes with oval to elongated, euchromatic nuclei (white arrow) were observed. Pericytes (black arrow).

HE x 400

#### **Group 6**



The sections of the heart presented in this group showed the normal myocardial histomorphology. Normal cardiomyocytes with oval to elongated, euchromatic nuclei (white arrow) were observed. Pericytes (black arrow).

HE x 400

## DISCUSSION

The histopathological findings from this present study, provide insights into the impact of different treatments on the heart, across six distinct groups. In all groups (Groups 1 to 6), the sections of the heart consistently exhibited normal myocardial histomorphology. This includes normal cardiomyocytes with oval to elongated, euchromatic nuclei, which are characteristic of healthy cardiac tissue (HE x400). The presence of pericytes, which play roles in microvascular function and repair, suggests normal vascular support within the myocardium (black arrow). The maintenance of normal cardiac histology across all groups indicates that the treatments applied did not induce any observable pathological changes in the heart tissue. This aligns with the understanding that treatments like *Annona muricata* extract and Finasteride, as discussed in the literature, generally do not

exert adverse effects on cardiac morphology or function. [1,11] 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 heart, across these histopathological findings. [5,12]

## CONCLUSION

From this present study, histopathological evaluations of the heart, consistently revealed preserved heart tissue architecture in treated animals with *Annona muricata* leaf extract highlighting the extract's tissue-protective properties in benign prostatic hyperplasia.

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