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# A Natural Product Molecule of Artocarpus Heterophyllus Reverses Inflammation Due to the Effect of Acute Exposure of Prostate Tissue to Testosterone Enanthate in Wistar Rats

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## Abstract

Benign prostatic hyperplasia (BPH) is a prevalent non-cancerous enlargement of the prostate, mainly affecting aging men. Current treatments, including drugs and surgery, often cause adverse effects, prompting the search for alternative therapies. This study evaluated the impact of Aqueous *Artocarpus heterophyllus* seed extract (AAHS) on testosterone enanthate-induced BPH in adult Wistar rats. Thirty male Wistar rats (12 weeks old, 180-200g) were acclimated under standard laboratory conditions before being divided into six groups: standard control, negative control (BPH-induced), positive control (treated with finasteride), and three treatment groups receiving AAHS at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg.

The $LD_{50}$ and phytochemicals of AAHS were determined. BPH was induced by injecting testosterone enanthate (0.2 mL of 25mg/kg), and treatments were administered for 28 days. At the end of the study, the rats were sacrificed using halothane anesthesia, and their prostrate and blood samples were collected for analysis. Testosterone enanthate-induced BPH led to significant prostate enlargement, histopathological changes, and increased oxidative stress. However, AAHS treatment demonstrated dose-dependent improvements, including reduced prostate weight, restored normal tissue structure, lower malondialdehyde (MDA) levels, higher superoxide dismutase (SOD) activity, and decreased prostate-specific antigen (PSA) levels. These findings suggest that AAHS has anti-inflammatory and antioxidant properties, making it a promising natural alternative for BPH management. Further research is needed to understand its mechanisms of action and potential clinical applications.

## Keywords

Benign prostatic hyperplasia; *Artocarpus heterophyllus*; Testosterone enanthate; Oxidative stress; Wistar rats.

## Introduction

Benign Prostatic Hyperplasia (BPH) is a common urological condition characterized by non-cancerous enlargement of the prostate gland, predominantly affecting older males [1]. Its global prevalence increases with age, with more than 50% of men over 50 years and 90% of men over 80 years experiencing symptoms associated with BPH [2]. The pathophysiology involves hyperplasia of stromal and epithelial cells, primarily driven by hormonal imbalances, particularly involving testosterone and its potent metabolite, dihydrotestosterone (DHT) [3]. The resulting prostate enlargement can compress the urethra, leading to lower urinary tract symptoms (LUTS) such as urinary retention, frequency, urgency, and nocturia [4].

The current management strategies for BPH include pharmacological treatments like alpha-blockers, 5-alpha reductase inhibitors, and surgical interventions such as transurethral resection of the prostate (TURP) [5]. However, these options often present side effects, such as sexual dysfunction, hypotension, invasive complications, and are quite expensive for the local population. Consequently, attention has shifted toward exploring natural products such as herbal concoctions with potential therapeutic benefits in managing BPH [6], which are cheap, effective, and safe as an alternative to complement the currently used drugs with doubtful efficacy and safety. This study aims to assess the therapeutic potential of A. heterophyllus seed extract in a testosterone enanthate-induced BPH model in Wistar rats.

## Materials and Methods

### Plant collection and preparation

Fresh and mature fruits of *Artocarpus heterophyllus* were procured from Ishaka Market in Bushenyi District. The plant was taken to the Department of Science at Mbarara University of Science and Technology for identification by ***Dr. Eunice A. Olet***. The plant was assigned herbarium number **MA-2025-001**. The plant was then taken to the pharmacology laboratory, where the fruits were cut, and the seeds were extracted, washed, and shade-dried. The dried *Artocarpus heterophyllus* seeds were then ground into a fine powder, weighed, and stored in airtight containers awaiting extraction.

## Preparation of the extract of seeds of Artocarpus heterophyllus

The *Artocarpus heterophyllus* seed powder was extracted following a method described [7], where 1000 grams of the powdered sample were added to a container and 10 liters of distilled water were added. The mixture was allowed to stand for 24 hours with occasional stirring using a magnetic stirrer. Then, the mixture was strained using a double muslin cloth and filtered using Whatman filter paper No.1. The filtrate was poured on a tray and put into a hot air oven at $40^0$C °C - 60°C for four (4) days to obtain dry cake extracts of aqueous extracts of the *Artocarpus heterophyllus seed*.*Artocarpus heterophyllus seed cake* extract was scraped from the tray and placed in a universal container and preserved in a refrigerator at 4 – 6 $^0$C pending analysis.

### Determination of $LD_{50}$ of the aqueous *artocarpus heterophyllus* seed extract

The acute oral toxicity test of the aqueous *Artocarpus heterophyllus* seed extract was evaluated in adult Wistar rats according to the method of [8]. Briefly, twelve female nulliparous and non-pregnant adult Wistar rats (6 weeks old, weighing 150 –200 g) were used. Animals were allowed to acclimatized for at least 7 days before dosing. They were randomly allocated to the different phases and dose groups via stratified sampling. For phase I, three groups of three animals each were used, and another three groups with one animal each were used in phase II. In phase I, animals in the same group were marked differently for easy identification and housed together in clearly labelled cages, while in phase II, each animal was housed in a separate, clearly labeled cage.

Before administration of a single dose of the aqueous *Artocarpus heterophyllus* seed, the rats were made to fast for 18 hours but allowed access to drinking water *ad libitum*, while retained in clean cages to prevent coprophagy. The solutions were administered once as a bolus by oral gavage, using a curved, ball-tipped gastric gavage needle affixed to a glass syringe to the rats as follows;

**Phase I:**

- Group I (3 animals); Every single animal in the group received 100 mg/kg of the aqueous *Artocarpus heterophyllus* seed extract orally.
- Group II (3 animals); Every single animal in the group received 500 mg/kg of the aqueous *Artocarpus heterophyllus* seed extract orally.
- Group III (3 animals); Every single animal in the group received 1000 mg/kg of the aqueous *Artocarpus heterophyllus* seed extract orally.

The treated animals were fasted for 1 hour with continuous observations after administration of the test substances; intermittently for 4 h, throughout 24 h, and then frequently for 24 h for 14 days. And because no death was recorded at any of the dosage levels in phase I after 24 hours, three other animals were grouped and dosed as follows in phase II.

**Phase II:**

- Group I (1 animal); Animal received 1600 mg/kg of the aqueous *Artocarpus heterophyllus* seed extract orally.


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- Group II (1 animal); Animal received 2900 mg/kg of the aqueous *Artocarpus heterophyllus* seed extract orally.
- Group III (1 animal); The Animal received 5000 mg/kg of the aqueous *Artocarpus heterophyllus* seed extract orally.

After administration in phase II, the same observation procedure was repeated as demonstrated in phase I. Gross behavioral changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhea, mortality, and other signs of toxicity manifestation were checked for and recorded daily. Body weights of all animals were determined shortly before the test substance was administered and after days 4, 7, 10 and 14. The death of any of the animals that occurred, the geometric mean of the least dose that killed the rats, and the highest dose that did not kill rats were taken as the median lethal dose. The median lethal dose was taken to be the value above the experimentally tested doses. Where death was recorded at the end of the experiment, the $\mathbf{LD_{50}}$, was determined using the following formula;

$$\boldsymbol{LD_{50}} = \sqrt{(\boldsymbol{D_0} \times \boldsymbol{D_{100}})}$$

Where $\mathbf{D_0}$ = Highest dose that gave no mortality,
$\mathbf{D_{100}}$ = Lowest dose that produced mortality.

## Experimental animal

Thirty (30) adults male Wistar rats weighing between 150 and 200 grams each were obtained from the Department of Pharmacology's Animal House at the Kampala International University. They were left to acclimatize for 14 days in the laboratory conditions before the experiment while under close monitoring, and ensuring standard laboratory conditions of 12 hours of natural light and 12 hours of darkness, room temperature (20±2 ◦C), and relative humidity of 50±5%. The animals were randomly assigned to the different treatment groups, and each group was housed in different cages (Okolo *et al.,* 2023). The animals were fed on standard commercial dry rat pellets (Ngaano® feed company Ltd, Kampala, Uganda) throughout the experiment. Additionally, clean water was availed *ad libitum*. The experimental study and animal handling followed the "Guide for Care and Use of Laboratory Animals" (National Research Council, 2010) and the Animal Ethics Committee of Kampala International University.

## Study design

The study was an experimental design. Thirty (30) adult Wistar rats weighing 150-180g were divided into 5 groups (n=6). The five groups of rats were then subjected to the following oral treatments (using a graduated syringe and stainless intubation cannula).

- **Group 1 (Normal Control):** This group received only water and feed for 28 days without any treatment.
- **Group 2 (Negative Control):** This group was induced with benign prostatic hyperplasia (BPH) by administering a single dose of 0.2 mL of 25 mg/kg testosterone enanthate.

- **Group 3 (Positive Control):** This group was induced with BPH using 0.2 mL of 25 mg/kg testosterone enanthate and subsequently treated with 0.2 mL of 10 mg/kg body weight of finasteride.
- **Group 4 (Low-Dose Treatment):** This group was induced with BPH using 0.2 mL of 25 mg/kg testosterone enanthate and treated with 0.2 mL of 250 mg/kg aqueous Artocarpus heterophyllus seed extract.
- **Group 5 (Medium-Dose Treatment):** This group was induced with BPH using 0.2 mL of 25 mg/kg testosterone enanthate and treated with 0.2 mL of 500 mg/kg aqueous Artocarpus heterophyllus seed extract.
- **Group 6 (High-Dose Treatment):** This group was induced with BPH using 0.2 mL of 25 mg/kg testosterone enanthate and treated with 0.2 mL of 1000 mg/kg aqueous Artocarpus heterophyllus seed extract.

| GROUPS | GROUP CATEGORY | INDUCEMENT | TREATMENT ACCORDING TO KG/B. W |
|---|---|---|---|
| Group 1 | Normal group | - | Distilled water |
| Group 2 | Negative control | 25mg/kg of testosterone enanthate | BPH-induced and distilled water |
| Group 3 | Positive control | 25mg/kg of testosterone enanthate | Standard drug (10mg/kg finasteride gavage ) |
| Group 4 | Low-dose group | 25mg/kg of testosterone enanthate | 250 mg/kg of aqueous *Artocarpus heterophyllus* seed extract |
| Group 5 | middle dose group | 25mg/kg of testosterone enanthate | 500 mg/kg of aqueous *Artocarpus heterophyllus* seed extract |
| Group 6 | High dose group | 25mg/kg of testosterone enanthate | 1000 mg/kg of aqueous *Artocarpus heterophyllus* seed extract |

**Table 1:** Summary of the treatment regimen per experimental group.

## Termination of studies

On completion of the experiment, all the animals were sacrificed by Chloroform Inhalation. The organ weight of the prostrate of each rat was measured on day 28th for all groups using a Metler electronic weighing machine.

## Collection of samples and measurement of enzymatic parameters

5 mL of Blood samples were collected through cardiac puncture from the posterior vena cava, placed in a clean, dry container, and allowed to stand for 20 minutes to clot. The blood samples were retrieved from the walls of the container with the help of an applicator stick and centrifuged for 20 minutes at 2500g. Serum was carefully transferred into another dry blood specimen container using a sterile, dry Pasteur pipette. This was labelled and stored in a refrigerator at **2-8 $^{0}$C** pending PSA analysis.

The prostate gland was removed, weighed, and examined for gross morphological changes. The excised prostate glands from the rats were cleaned, and a portion was homogenized in 10% freshly prepared sodium phosphate-buffered saline and later used for for MDA and SOD. Furthermore, another portion of the prostate gland was fixed in 10% neutral formaldehyde buffer for 5 days pending histological

processing.

## Determination of prostate weight and prostatic index

Determination of prostate weight and prostatic index. The prostate gland of all the experimental animals was harvested and weighed at the end of the trial. The prostatic index was determined using the formula [9].

**Prostate index = Prostate weight / Body weight**

**Percentage of inhibition = 100 – [ Treatment group − Negative control / Disease group − Negative control × 100]**

## Measurement of malondialdehyde (MDA) activity

Malondialdehyde, a lipid peroxidation end product in tissue homogenate, was measured according to the method described by [10].

## Principle

The test is based on the reaction of MDA with Thiobarbituric acid (TBA); forming MDA-TBA2 product that absorbs strongly at 532nm to pink colored Compound [11]. MDA is a byproduct of lipid peroxidation that reacts with thiobarbituric acid (TBA) to form a pink complex with a peak u of 532 nm.

An aliquot of clear tissue homogenate (200 µl) was mixed with 2 ml of Trichloroacetic acid (TCA) and 0.2 ml HCl (10% and 0.2%, respectively). The tubes were centrifuged at 3000 rpm for 10 minutes to remove the precipitated proteins. Then 1 ml of 0.67% TBA was added to the mixture and incubated at 95% for 30 minutes in a water bath to allow TBA to react with MDA. A BSSUV-202 spectrophotometer was used to measure the absorbance of the pink-colored extract at 532 nm. The amount of MDA formed per gram weight of the tissue was calculated using a molar extinction coefficient of 1.56x105 M-1 cm-1 and expressed as nmol of MDA formed per gram of the tissue.

$$\text{Concentration of MDA} = \frac{\text{Absorbance of TBA} - \text{MDA complex at 532nm}}{\textit{Molar extinction coefficient of MDA X weight of tissue}}$$

## Measurement of superoxide dismutase (SOD) activity

SOD level was determined by the procedure described by [12]. Superoxide dismutase (SOD) catalyzes superoxide dismutation into oxygen and hydrogen peroxide. The assay is based on SOD's ability to inhibit epinephrine autoxidation (adrenaline). To equilibrate the spectrophotometer, two milliliters of tissue supernatant were added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2). The reaction was started by quickly mixing 0.3 ml of freshly prepared 0.3 mM adrenaline into the mixture. The reference tube was made up of 2.5 ml of 0.05 M carbonate buffer (pH 10.2), 0.3 ml of substrate (adrenaline), and 0.2 ml of water. The increase in absorbance (Abs) at 480 nm caused by the formation of adrenochrome (Adreno) was measured at 30 seconds and 150 seconds. The SOD activity was calculated as shown below:

1. **Increase in Abs per min** $= \frac{A1 - A0}{2.5}$

   $A_0$ = Abs after 30 seconds


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$A_1$ = Abs after 150 seconds

2. $\%\ \textbf{age inhibition} = \frac{(Increase\ in\ Abs/\min of\ reference - Increase\ in\ Abs/\min of\ adreno)\ X\ 100}{Increase\ in\ Abs/\min of\ reference}$

Where one unit of SOD activity was defined as the amount of SOD required to inhibit the auto-oxidation of adrenaline to adrenochrome by 50% for 1 minute.

### Serum PSA determination

Blood samples from the rats' hearts were taken after they were sedated to measure the amount of free prostate-specific antigen (PSA) in the blood using a PSA ELISA kit [13]. This study evaluated the effect of *Artocarpus heterophyllus* extract on these biomarkers to determine its therapeutic potential in BPH management.

### Histopathological examination

This was carried out as demonstrated by the method of [14]. The prostate was dissected out and fixed immediately in 10% formal saline for 24 hours. Having washed the specimens, they were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax (melting point of 50- 56 °C). Paraffin sections were cut at 5µm thickness using a rotary microtome (Model MR 60, Russian); finally, the sections were then stained with Harris Haematoxylin and Eosin and examined using a light microscope (Zeiss Axiophot, Germany), and photographs were taken with an automatic photomicrographic system [15].

### Ethical considerations

Before the commencement of the research, clearance was sought and approved by Kampala International University's Research Ethics Committee (KIU-REC), **KIU-2024-462**. After approval by the KIU REC, the study was registered with the Uganda National Council for Science and Technology. National and international ethics guidelines about maintaining and handling experimental animals during and after research were strictly adhered to during the study.

The 3Rs (replacement, refinement, and reduction) was observed through the study. Replacement was ensured by using statistical data already obtained in previously done studies. Refinement was ensured during the experimentation, as animals were kept in standard cages of a stocking density of seven animals per cage with a spacing of two cubic feet per rat.

### Data/statistical analysis

Data was analyzed using SPSS v 27. For the organ weight ratios and oxidative stress tests, data obtained from this study was analyzed by determining differences between groups by one-way ANOVA, followed by Turkey's Honest Post-hoc test. Results were presented as mean ± standard error of the mean (SEM), and values at $p \leq 0.05$ were considered statistically significant.

## Results

### Plant extract yield

The aqueous extraction of *Artocarpus heterophyllus* seeds yielded 106.4g of extract from 1021g of seed


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powder, representing an extraction efficiency of 10.42%. This yield provided sufficient material for subsequent analyses and treatment protocols. (Table 1.2).

| Solvent | Weight of seed powder (g) | Weight of extract (g) | Percentage Yield (%) |
|---|---|---|---|
| Distilled water | 1021 | 106.4 | 10.42 |

**Table 1.2:** Extraction yield of Artocarpus heterophyllus seed extract.

## Qualitative phytochemical profile of the aqueous seed extract of Artocarpus heterophyllus

Phytochemical analysis revealed a diverse profile of bioactive compounds in the aqueous extract. The extract demonstrated the presence of several important phytochemicals, including tannins, phenols, flavonoids, steroids, and alkaloids, while showing the absence of saponins and terpenoids. (Table 1.3).

| Phytochemical | Test used | Qualitative Test Result |
|---|---|---|
| Tannins | Ferric chloride test | + |
| Phenols | Lead acetate test | + |
| Flavonoids | Shinoda test | + |
| Saponins | Foam test | - |
| Terpenoids | Bontrager's test | - |
| Steroids | Salkowski's test | + |
| Alkaloids | Wagner's test | + |

**Table 1.3:** Phytochemical composition of Artocarpus heterophyllus aqueous seed extract.

**Note:** + indicates presence; - indicates absence

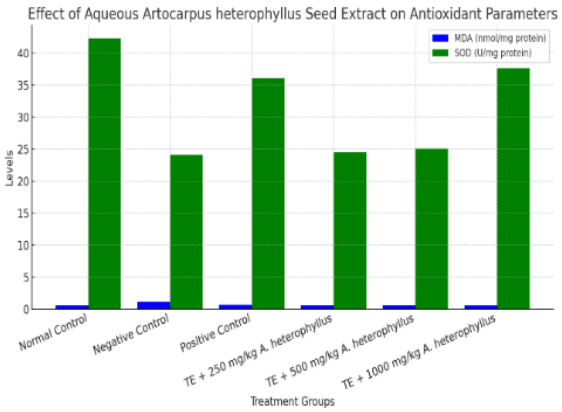


**Figure 1.1:** Showing the effect of aqueous extract of *Artocarpus Heterophyllus* seed extracts on antioxidant parameters.


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### Effect of the aqueous Artocarpus heterophyllus seed extract on the antioxidant parameters in testosterone enanthate-induced BPH in male wistar rats

Testosterone enanthate administration led to a significant elevation in prostatic MDA levels in the negative control group (1.15 ± 0.13 nmol/mg protein) compared to normal controls (0.55 ± 0.05 nmol/mg protein; $p< 0.0001$). Treatment with *A. heterophyllus* extract produced dose-dependent reductions in MDA levels; 250 mg/kg: 0.62 ± 0.06 nmol/mg protein ($p = 0.0001$ vs negative control), 500 mg/kg: 0.61 ± 0.08 nmol/mg protein ($p = 0.0001$ vs negative control) and 1000 mg/kg: 0.54 ± 0.03 nmol/mg protein ($p< 0.0001$ vs negative control). The study also indicated that finasteride treatment significantly lowered prostate tissue MDA levels (0.54 ± 0.003 nmol/mg protein; $p = 0.0003$) when compared to the negative control. (Figure 1.1).

SOD activity showed significant perturbation in BPH condition and subsequent modulation by treatments. The negative control group exhibited markedly reduced SOD activity (24.12 ± 3.13 U/mg protein) compared to normal controls (42.31 ± 2.65 U/mg protein; $p = 0.0012$). 250 and 500 mg/kg of *A. heterophyllus* treatment groups showed an increase in SOD activity (24.51 ± 3.46 and 25.1 ± 2.08 U/mg protein) compared to the negative control group ($p = 0.0014$ and $p = 0.0020$) respectively. Furthermore, the study indicated that there was a significant increase in the prostate tissue SOD concentration in animals treated with the positive control ($p = 0.0375$) and those treated with 1000 mg/kg of *A. heterophyllus* ($p = 0.0165$) as opposed to those in the negative control group. The study also noted a significant decrease in the prostate tissue SOD concentration of animals treated with 250 mg/kg of *A. heterophyllus* ($p =0.0458$) as compared to those in the positive control.

### Effect of the aqueous Artocarpus heterophyllus seed extract on the levels of the serum prostate-specific antigen in testosterone enanthate-induced BPH in male Wistar rats

The study indicated that in comparison with the normal control group, there were significantly higher serum prostate-specific antigen levels among animals in the negative control group ($p = 0.0015$), positive control group (finasteride - 10 mg/kg) ($p = 0.0031$) and 250 mg/kg of *A. heterophyllus* group ($p = 0.0033$) respectively. (Figure 1.2).

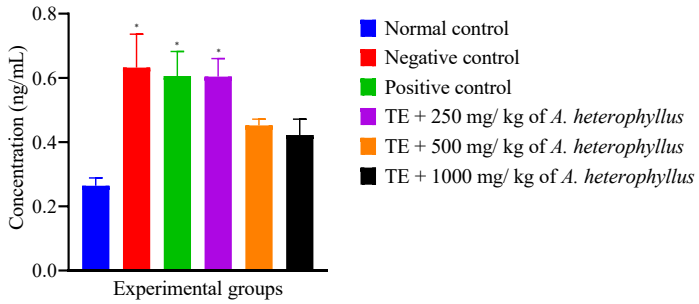


**Figure 1.2:** Serum prostate-specific antigen levels in testosterone enanthate-induced BPH in male Wistar rats.


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### Effect of aqueous *Artocarpus heterophyllus* seed extract on the body weight, prostate weight, prostatic index, and percentage inhibition of the prostatic index in testosterone enanthate-induced BPH in male Wistar rats

Initial body weights showed no significant differences among the experimental groups, ranging from 205.8 ± 21.89 g to 226.18 ± 4.51 g, indicating successful randomization of animals at the study's commencement. However, final body weights demonstrated notable treatment-related effects. The negative control group showed a significant increase in body weight (268.5 ± 12.4 g, $p$ = 0.0004) compared to the normal control group (242.8 ± 16.6 g), suggesting that testosterone enanthate administration influenced overall body mass. Treatment with both the positive control and *A. heterophyllus* extract exhibited a dose-dependent modulation of this weight gain, with the 500 mg/kg and 1000 mg/kg groups showing significant attenuation of weight gain (234.6 ± 10.2 g; 238.4 ± 9.8 g and 235.2 ± 8.9 g, $p$< 0.0001) versus negative control respectively. (Table 1.4).

Prostate weight measurements revealed pronounced effects of both testosterone enanthate induction and subsequent treatments. The negative control and low-dose (250 mg/kg) extract groups demonstrated a significant increase in prostate weight (1.48 ± 0.14 g and 1.12 ± 0.12 g, $p$ = 0.0001) compared to the normal control group (0.69 ± 0.11 g), confirming successful induction of prostatic hyperplasia. Treatment responses showed clear dose-dependency with the positive control group, medium and high-dose extract groups (500 mg/kg and 1000 mg/kg) reducing the prostate weight to 0.58 ± 0.09 g, 0.82 ± 0.1 g, and 0.65 ± 0.08 g, respectively ($p$ = 0.0050) versus the negative control. The low-dose (250 mg/kg) caused a modest increase in prostate weight with 1.12 ± 0.12 g ($p$ = 0.0031) versus the positive control group. (Figure 1.3).

The study indicated that the negative control group and low dose (250 mg/kg) extract group had a significantly elevated prostatic index (5.51 ± 0.4 × $10^{-3}$ and 4.56 ± 0.5 × $10^{-3}$, $p$ = 0.002 and $p$ = 0.0004) compared to the normal control group (2.82 ± 0.3 × $10^{-3}$). The study also indicated that there was a significant reduction in the prostatic index among animals treated with the positive control (2.47 ± 0.3 × $10^{-3}$; $p$ = 0.0001), medium (3.44 ± 0.4 × $10^{-3}$; $p$ = 0.0035) and high dose extract groups (2.76 ± 0.3 × $10^{-3}$; $p$ = 0.0020) when compared to animals in the negative control group. (Table 1.5).

### Effect of Aqueous Artocarpus heterophyllus seed extract on the histomorphology of prostate tissue in testosterone enanthate-induced BPH in male Wistar rats

The results of the histopathological examination of the prostate, as shown in **Figure 1.3,** revealed normal histomorphological features of the prostate gland, with round, flat glands with peripheral enfolding and no apparent epithelial hyperplasia in the normal control group Figure 1.3A. Testosterone enanthate caused hyperplasia with central and peripheral enfolding alongside cystic features in the negative control group **(Figure 1.3B**). The prostate of animals that were treated with 10 mg/kg of finasteride and both the intermediate and high doses of Artocarpus heterophyllus aqueous seed extract (500 and 1000 mg/kg) completely prevented these pathological changes and restored the histology of the prostate to the near normal level (Figure 1.3C, 1.3E and 1.3F). However,Testosterone enanthate-

induced pathological changes of similar grade were found in the prostate of rats treated with the lowest dose of the Artocarpus heterophyllus aqueous seed extract (250 mg/kg) (Figure 1.3D).

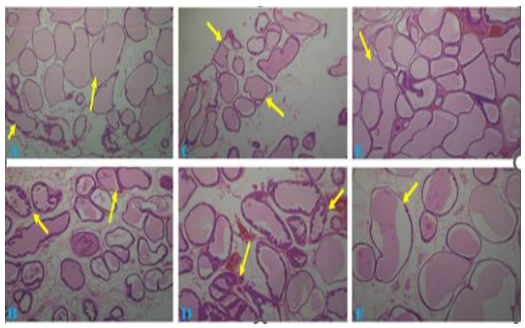

**Figure 1.3:** Photomicrograph showing a section of the prostate from different treatment groups (H&E; x 40).

The percentage inhibition of prostatic index indicated that the animals treated with finasteride, the positive control, achieved the highest percentage inhibition (55.17%), while the *A. heterophyllus* extract showed a dose-dependent effect, with the highest dose (1000 mg/kg) having the highest inhibitory effect (49.91%). (Table 1.4).

| | **Treatment groups** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Study variables** | Normal control | Negative control | Positive control | TE + 250 mg/kg of *A. heterophyllus* | TE + 500 mg/kg of *A. heterophyllus* | TE + 1000 mg/kg of *A. heterophyllus* | F – value | P – value |
| **Initial B.W (g)** | 205.8 ± 21.89 | 208.32 ± 11.44 | 207.6 ± 16.21 | 205.86 ± 8.19 | 206.46 ± 9.97 | 226.18 ± 4.51 | 0.8268 | 0.6772 |
| **Week 1** | 220.6 ± 6.88 | 232.6 ±5.77 | 220 ±5.00 | 221 ±4.30 | 220.4 ± 4.41 | 230 ± 1.70 | 1.306 | 0.2946 |
| **Week 2** | 232 ± 5.39 | 249 ± 4.30 | 229 ± 4.30 | 230 ± 3.54 | 229.6 ± 3.83 | 232 ± 1.41 | 3.687 | 0.0129 |
| **Week 3** | 242 ± 5.39 | 264 ± 4.30 | 235 ± 3.54 | 240 ± 3.54 | 238 ± 2.98 | 234 ± 1.41 | 8.875 | < 0.0001 |
| **Final B.W (g)** | 242.8 ± 16.6 | 268.5 ± 12.4 * | 234.6 ± 10.2 [a] | 245.3 ± 11.8 | 238.4 ± 9.8 [a] | 235.2 ± 8.9 [a] | 5.79 | 0.0012 |

**Table 1.4:** Effect of aqueous Artocarpus heterophyllus seed extract on the bodyweight.

Data are expressed as Mean ± SEM, n = 5; * - $p < 0.05$ when compared to the normal control group, [a] – $p < 0.05$ when compared to the negative control group, [b] – $p < 0.05$ when compared to the positive control group; TE – Testosterone enanthate; B.W – Body weight.


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| | Treatment groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Study variables | Normal control | Negative control | Positive control | TE + 250 mg/kg of *A. heterophyllus* | TE + 500 mg/kg of *A. heterophyllus* | TE + 1000 mg/kg of *A. heterophyllus* | F – value | P – value |
| Prostate index (x $10^{-3}$) | 2.82 ± 0.3 | 5.51 ± 0.4 * | 2.47 ± 0.3 a | 4.56 ± 0.5 *, b | 3.44 ± 0.4 a | 2.76 ± 0.3 a | 5.335 | 0.002 |
| % inhibition of PI | - | - | 55.17 | 17.24 | 37.57 | 49.91 | - | - |

**Table 1.5:** Effect of aqueous Artocarpus heterophyllus seed extract on the prostatic indices.

Data are expressed as Mean ± SEM, n = 5; * - $p < 0.05$ when compared to the normal control group, a – $p < 0.05$ when compared to the negative control group, b – $p < 0.05$ when compared to the positive control group; TE – Testosterone enanthate; B.W – Body weight; P. I – Prostate index.

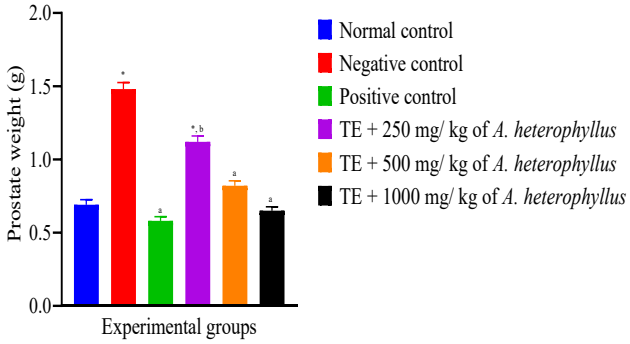


**Figure 1.4:** Prostate weights in testosterone enanthate-induced BPH in male Wistar rats.

## Discussion

This study investigated the effects of aqueous *Artocarpus heterophyllus* seed extract in mitigating testosterone enanthate-induced benign prostatic hyperplasia in adult male Wistar rats. The findings demonstrated that the aqueous seed extract, particularly at higher doses (500 and 1000 mg/kg), exhibited significant anti-BPH activity, comparable to the standard drug finasteride. These effects were mediated through antioxidant modulation, restoration of prostate histoarchitecture, and normalization of prostate-specific biomarkers.

Table 1.2 shows that the use of water as a solvent effectively extracted a larger proportion of the plant's phytochemicals possessing a higher concentration of bioactive compounds which could enhance the pharmacological potency of (anti-inflammatory, antiandrogenic, and antioxidant effects) of the extract in treating in BPH (characterized by reduction in prostate enlargement and BPH symptoms). Secondly, a higher yield means small quantity of raw materials may be required to achieve the same therapeutic effects, making the treatment more economically viable for e large-scale production and lastly it may indicate that more diverse bio active compounds are present potentially leading to synergistic effect that improves the extract's ability to counteract testosterone inducing BPH.


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The extraction process yielded 10.42% of extract from the seed powder. This is consistent with findings from studies, which demonstrated that aqueous extracts typically yield around 10 – 20% when extracted using optimized methodology [16,17]. The phytochemical analysis of the extract revealed the presence of tannins, phenols, flavonoids, steroids, and alkaloids, which are compounds widely recognized for their antioxidant and anti-inflammatory properties [18]. These findings are consistent with previous reports on the phytochemical composition of *Artocarpus heterophyllus* [18]. Flavonoids and phenols, in particular, are known to scavenge free radicals and reduce oxidative stress, a key contributor to BPH pathogenesis [18].

The presence of flavonoids is especially significant, as previous research has demonstrated their capacity to inhibit 5α-reductase activity, a crucial enzyme in BPH pathogenesis [18]. Similarly, the phenolic compounds identified may contribute to the extract's therapeutic efficacy through their known antioxidant and anti-inflammatory properties [18]. The absence of saponins and terpenoids suggests that the observed therapeutic effects may stem from synergistic interactions among the detected phytochemicals rather than isolated compounds [18]. This aligns with studies on other plant-derived polyphenols, such as those in *Serenoa repens* and *Urtica dioica*, where multi-component interactions enhance therapeutic efficacy in BPH management [18].

The presence of tannins, phenols, flavonoids, saponins, steroids, and alkaloids in *Artocarpus heterophyllus* seed extract suggests a multitargeted therapeutic potential, making it relevant in managing BPH, reducing oxidative stress, modulating hormonal balance [19].

Results of our study, as shown in (Figure 1.1) indicated a highly significant reduction ($p \leq 0.0001$) in MDA levels, suggesting that the extract is effective in reducing oxidative stress and lipid peroxidation.

MDA is a marker for cellular damage and its decrease indicates that the extract’s ability to protect cells from oxidative damage. These findings support the extract’s potential as a natural antioxidant that can help reduce oxidative damage, particularly in conditions like benign prostatic hyperplasia (BPH) where oxidative stress plays a significant role [19].

On the other hand, there was a concomitant increase in the levels of SOD ($p \leq 0.0003$). Increase level of SOD associated with enhancing the body’s antioxidant defense system. SOD neutralizes super oxide radicals in the body reducing oxidative damage. By implication, this shows that the extract can stimulate the body’s natural antioxidant mechanisms, providing a strong defense against reactive oxygen species (ROS). This could be beneficial in preventing tissue damage and inflammation, both of which are key factors in BPH [20]. Both MDA reduction and SOD increase were observed to be dose-dependent, indicating that higher doses of the extract led to greater anti-oxidant effect, however it highlights the importance of optimal dosing to maximize therapeutic benefits while avoiding potential toxicity.

Testosterone enanthate administration induced significant oxidative stress, evidenced by elevated prostatic malondialdehyde levels and suppressed superoxide dismutase activity in the negative control group. This study finding aligns with several findings where testosterone enanthate was used to induce BPH in Albino Wistar rats [18]. The dose-dependent reduction in MDA levels and restoration of SOD

activity by *Artocarpus heterophyllus* extract highlight its antioxidant efficacy [17,21,19]. Notably, the 1000 mg/kg dose normalized MDA and SOD levels to near-baseline values, mirroring the effects of finasteride. This aligns with studies linking polyphenol-rich extracts to enhanced antioxidant enzyme activity and reduced lipid peroxidation in BPH models [9,22,7,23,24]. Furthermore, quercetin (a flavonoid) has been shown to inhibit 5α-reductase activity, a key enzyme in androgen-driven prostate enlargement, suggesting a potential mechanism for the observed effects.

Additionally, *in vitro* studies on jackfruit seed extracts have been shown to inhibit lipid peroxidation and modulate inflammatory markers, suggesting a possible role in managing hormone-related disorders [7. Furthermore, research by Yahfoufi *et al.*, (2018)), noted that cycloheterophyllin and artonins A and B are phenols isolated from *Artocarpus heterophyllus* serve as powerful antioxidants against lipid peroxidation when bio membranes are exposed to oxygen radicals. Nirmal, Chaudhary and Amir, (2024) as well as Okolo *et al*., (2023) opined that those three phenolic compounds, including artocarpesin isolated from *Artocarpus heterophyllus* suppressed the liposaccharide-induced production of nitric oxide and prostaglandin E2 through the down-regulation of inducible nitric oxide synthase and cyclooxygenase 2 protein expressions. Thus, artocarpesin from *Artocarpus heterophyllus* may provide a potential therapeutic approach for inflammation-associated disorders including BPH.

Serum prostate-specific antigen, a biomarker for prostate pathology, was significantly elevated in TE-induced rats. However, treatment with 500 and 1000 mg/kg of the extract reduced PSA levels, corroborating histopathological findings [23]. The reduction in PSA caused by the higher doses of *Artocarpus heterophyllus* can be attributed to the presence of phytochemicals such as flavonoids, tannins, saponins, and lignans which have demonstrated anti-BPH properties through antioxidant, anti-inflammatory, and hormonal modulation mechanisms [7]. Furthermore, Kumar, (2022) notes that extracts from related *Moraceae* family members, such as *Morus alba* and *Artocarpus altilis,* have exhibited significant anti-BPH activity in animal models which further validates the anti-BPH activity of *Artocarpus heterophyllus* seed extract. Other plants such as *Serenoa repens*, *Pygeum africanum*, *Urtica dioica*, and *Curcuma longa* have also been extensively studied for their ability to inhibit 5-alpha reductase, reducing DHT synthesis, and modulating inflammatory pathways and hormonal activity in BPH [18].

The restoration of normal glandular architecture and inhibition of epithelial hyperplasia in high-dose groups further validate the extract's anti-hyperplastic properties [22]. In contrast, the low dose (250 mg/kg) failed to mitigate histological changes, suggesting a threshold concentration of bioactive compounds is required for efficacy. This dose-dependency parallels findings in *Cucurbita pepo* seed oil studies, where higher doses were necessary to achieve significant anti-proliferative effects.

The prostatic index and % inhibition, as shown in our study (table 4.5), was found to increase with dose. This indicates that the extract has a dose-dependent effect on reducing prostate enlargement significantly ($p \leq 0.002$). The possible mechanism of action may be that the extract exerts anti-androgenic or anti-proliferative effects, thus potentially reducing the factors that contribute to prostate enlargement, such as testosterone levels or cell proliferation [24].


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The reduction in prostate weight and prostatic index in treated groups further underscores the extract's therapeutic potential. The 1000 mg/kg dose achieved a 49.91% inhibition of prostatic index, nearing the 55.17% inhibition by finasteride. These results are consistent with prior reports on plant-derived antioxidants attenuating androgen-driven prostate enlargement. The modulation of body weight in treated groups, particularly the attenuation of testosterone-induced weight gain, may also reflect systemic anti-androgenic or metabolic effects warranting further exploration.

## Conclusion

The findings of this study demonstrate that the aqueous *Artocarpus heterophyllus* seed extract has potent anti-BPH activity in TE-induced rats, mediated through antioxidant mechanisms and modulation of prostate-specific parameters. Higher doses (500–1000 mg/kg) effectively reverse oxidative damage, normalize PSA levels, restore prostate histology, and reduce prostate enlargement. The efficacy of the extract at these doses did not significantly differ from that of finasteride, positioning it as a promising natural alternative for BPH management.

The 10% increased yield of raw material suggests that the aqueous extraction method used was efficient in isolating active compound for *Artocarpus heterophyllus* seeds. This could enhance the therapeutic potential of the extract against testosterone-induced BPH in adult Wistar rats, making a promising natural alternative for managing prostate enlargement, however, further dose-response studies and clinical trials are necessary to validate its effectiveness and safety for humans.

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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