BIOCHEMICAL AND HISTOMORPHOLOGICAL EFFECTS OF AQUEOUS FRUIT EXTRACT OF *Annona muricata* ON TESTOSTERONE PROPIONATE INDUCED BENIGN PROSTATE HYPERPLASIA (BPH) IN MALE WISTAR RATS

ABSTRACT

Introduction: Benign prostate hyperplasia (BPH) is an age-related non-malignant prostate gland enlargement in men that leads to pain and difficulty voiding urine. The etiology of BPH is still unknown. Studies have been reported on the effects of *Annona muricata* (soursop) against benign prostatic hyperplasia (BPH) with little documentation.
Aim: This study aimed to determine the biochemical and histomorphological effects of aqueous fruit extract of *A. muricata* on testosterone propionate induced benign prostate hyperplasia (BPH) in male wistar Rats.
Methodology: The fruits of *A. muricata* were processed to obtain fruit extract and phytoconstituents. Acute toxicity study was conducted with six doses of *A. muricata* fruit extract (10, 100, 1000, 1500, 2900 and 5000 mg/kg) to determine the safety and tolerability dose limit. A total of 48 adult male wistar rats were used for the study. After 2 weeks of acclimatization, the animals were orchimetized. The rats were randomly divided into six groups of eight animals each; group 1 (normal control + sham treated + distilled water); groups 2 (positive control + orchiectomy + TP/day); group 3 (finasteride (3 mg/kg) + orchiectomy + TP/day); group 4 (100 mg/kg *AM* + orchiectomy + TP/day); group 5 (200 mg/kg *AM* + orchiectomy + TP/day) and group 6 (400 mg/kg *AM* + orchiectomy + TP/day) for 42 days. On 43rd day animals were euthanized, blood and prostate tissue samples were collected for biochemical and histological study.
Results: This study showed that, theextract significantly ($p < 0.05$) decreased both the prostate weight and testosterone levels in dose-dependent manner compared to finasteride treated rats. The effect of the extract on histology of the prostate had significant recovery and was able to restored enlarged prostate near-normal in dose-dependent manner. A significant ($p < 0.05$) recovery was observed at higher dose (400 mg/kg) of theextracts.
Conclusion: The effects of *Annona muricata* (soursop) against benign prostatic hyperplasia (BPH) showed inhibitory potentials via decreased prostate weight, prostate specific antigen, and testosterone levels in dose-dependent manner. At high dose (400 mg/kg) body weight of rats had significant recovery ($p < 0.05$) restoring prostatic histoarchitecture near-normal. This study suggests that *Annona muricata* fruit may be considered as treatment option for benign prostatic hyperplasia in men.

Keywords: *A. muricata,* Benign Prostate Hyperplasia (BPH), Testosterone propionate.

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Introduction

Benign prostate hyperplasia (BPH) is the most common neoplasm and a significant cause of urinary symptoms affecting adult males aged fifty years and above (1). The disease is marked by swollen prostate, pain, voiding dysfunction or weak urine stream (2) due to urethral constriction and bladder neck obstruction altering the morphology and physiology of the prostate leading to acute or chronic lower urinary tract symptoms (LUTs) which affects quality of life (3). The inflammatory process is associated with development and progression of BPH resulting from

increase in cell number, size as well as proliferation of the cellular elements of the prostate gland (4).

Several studies have linked the occurrence of BPH as a natural process of ageing though the actual mechanism is not fully understood but its believed to be under steroid hormone influence and ageing prostate (5).Testosterone propionate is a synthetic fast-acting form of testosterone that is often used in hormone replacement therapy for men with low testosterone levels. Several studies have linked testosterone therapy, including testosterone propionate and BPH. Higher levels of testosterone and prostate specific antigen could exacerbate BPH symptoms due to conversion of testosterone to dihydrotestosterone (DHT), which is a potent androgen that can stimulate prostate growth (6). This can be as a result of overproduction of oxidant molecules or depletion of the antioxidant system during prostate enlargement. An imbalance between oxidative stress and antioxidant system of the cells may play important role in development of prostate disease (6). The existing treatment options for BPH include drug therapy with α-blockers or 5α-reductase inhibitors, minimally invasive therapy and surgery (7). The mechanism of α-blocker involves the relaxation of the smooth muscles of the prostate and the bladder neck thereby relieving lower urinary symptoms (LUTs) and urinary obstruction caused by an inflamed prostate (8).

Management of BPH has been mainly to provide relief-treatment for the symptoms of the condition (9). The orthodox drugs presently in use are found to possess numerous side effects which made them not safe for therapeutic application. Hence, the need for alternative medicine with little or no side effects. Lots of side effects have been found associated with existing BPH drugs ranging from decreased libido, erectile dysfunction, dizziness to retrograde ejaculation (10). The use of phytotherapy for the prevention and treatment of BPH is gaining popularity (11) due to its promising efficacy, milder side effects and affordability compared to most other treatment options. *Annona muricata*, commonly known as soursop, belongs to the Annonaceae family. The plant is widely known for its anticancer properties (12). A wide range of ethnomedicinal activities have also been attributed to different parts of the plant owing to some of its properties including anti-inflammatory, antiproliferative, hypoglycemic, sedative, smooth muscle relaxant and antispasmodic effects (13). Some indigenous communities in Africa including Nigeria use *A. muricata* in their folk medicine. The Leaf extract of the plant is used to alleviate difficulty associated with urination in certain communities in Eastern part of Nigeria. Studies have linked *A. muricata* to cytotoxicity and inhibition of proliferation on variety of cancer cell (14). This plant is readily available, affordable and can be cultivated at low cost. There is pausity of data and no detailed investigation had been carried out to determine the effects of aqueous fruit extract of *A. muricata* on testosterone propionate induced benign hyperplasia (BPH) in male wistar rats.

Materials and Methods

Study Location

The study was carried out in Chemical pathology laboratory, School of Medical Laboratory Science, Pharmacognosy and Ethnopharmacy laboratory, Pharmacology and Toxicology laboratory, Faculty of Pharmaceutical Science and Centre for Advanced Medical Research and Training (CAMRET), UsmanuDanfodiyo University, Sokoto.

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Sample Collection and Identification

Fresh *A. muricata* fruits (soursop) were purchased from Central Market Sokoto, Nigeria. The sample of *Annona muricata* fruit was identified and authenticated at the Herbarium unit, Department of Pharmacology and Ethnomedicine, Faculty of Pharmaceutical Sciences, UsmanuDanfodiyo University Sokoto (UDUS), Nigeria. Specimen voucher number was assigned as PCG/UDUS/ANNONA/0004 and deposited in the Herbarium unit of the Department.

Extraction of Plant Material

The fresh *Annona muricata* fruits were washed with distilled water, chopped into pieces, seeds separated and air dried under shade in Pharmacognocy laboratory for 14 days. The dried fruits was blended using electronic blender (Binatone BLG 450, London, United Kingdom) and sieved through 40-mesh (0.4 mm) to powder. The powdered sample (500 g) was weighed, soaked in 3000 mL distilled water and allowed to macerate at room temperature for 24 hours. The mixture was filtered using Whatman filter paper (No.4). The filtrate was evaporated to dryness in an electric oven set at 55 ^{0}C. A dried brown paste was obtained. It was weighed, stored in wide mouth container and preserved in the refrigerator at 4^{0}C until use. The percentage (%) yield of the extract was calculated based on the formula;

$$\% \text{ Fruit extract yield} = \frac{\text{Weight of final extract}}{\text{Weight of powdered plant material}} \times 100$$

Experimental Animals Procurement and Management

A total of forty eight (48) male Wistar rats of 13 weeks old, weighing between 150-170 g were purchased from the Animal House, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria. The rats were housed in conventional well-ventilated wire cages under standard laboratory conditions in the Animal house, Faculty of Pharmaceutical Sciences, UsmanuDanfodiyo University, Sokoto (± 30^{o}C) and lighting period of about 12 hours daily. They were acclimatized for two weeks before use. They were fed standard commercial pelletized grower's feed and drinking water *ad libitum.* Principles of Laboratory Animal Care' was followed as well as specific National laws where applicable. All the experimental protocols followed institutional animal ethics committee guidelines.

Phytochemical Screening of *Annona muricata* Fruit

Phytochemical analysis was carried out in Department of Pharmacognocy and Ethnopharmacy, Faculty of Pharmaceutical Science, UsmanuDanfodiyo University, Sokoto using standard procedures to identify the phytochemical constituents as described by Harbone, (15); Trease and Evans, (16); Sofowora, (17).

Ethical Approval

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Ethical approval was obtained from the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences with ethical number (PTAC/Am/(Ae)/OT/70-24) assigned for the use and management of Animals.

Castration of Animals

To minimize the impact of endogenous testosterone during the study, the experimental rats were anaesthetized (ketamine: Xylaxine; 50:10 mg/mL). Orchiectomy was performed using Obisike*et al*., (18) approach, both testes of groups 2 – 6 rats were excised through the scrota sac. The negative control group (group 1) rats were treated as shams. The animals were given one week to recover before the study.

Acute Toxicity Study

Acute toxicity testing was conducted using Lorke's Method, (19). The aqueous *Annona muricata* extract was dissolved in distilled water and administered at doses of 10 mg/kg, 100 mg/kg, 1000 mg/kg 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. The rats were observed for 24 hours for clinical signs of toxicity and mortality.

Induction of Benign Prostate Hyperplasia (BPH)

Benign prostate hyperplasia was induced by subcutaneous injections of testosterone-propionate (TP) (10 mg/kg body weight) in the inguinal region of the animals for forty two (42) days. This proliferation stimulation with testosterone propionate resulted in the development of benign prostate hyperplasia in rats (20). The rats were weighed weekly and on the 43rd days the rats were sacrificed (21). The prostate weights, prostate specific antigen (PSA) levels and histological examination of the prostate tissue were pointers used to ascertain successful induction.

Chart 1. Experimental Design
n: 8 rats/Grp

GROUP	SUBCUTANEOUS INJECTION	TREATMENT (O/A)	DURATION/DAY
Grp1 Negative control	Distilled water	Rat pellet	42
Grp2 TPI + orchiectomy	TP (10mg/kg)	Rat pellet	42
Grp3 TPI + orchiectomy	TP (10mg/kg)	Finasteride (3mg/kg)	42
Grp4 TPI + orchiectomy	TP (10mg/kg)	Plant extract (100 mg/kg)	42
Grp5 TPI + orchiectomy	TP (10mg/kg)	Plant extract (200 mg/kg)	42
Grp6 TPI + orchiectomy	TP (10mg/kg)	Plant extract (400 mg/kg)	42

TPI: Testosterone-propionate induced; **Plant exract:**Annona muricata fruits; **n:**Number of rats per group; **Grp 1:** Negative control group; **Grp 2:**Positive control group; **Grp 3:**reference drug treatment group; **Grp 4-6:**Treatment group with varying doses of plant extract; **O/A:** Oral Administration

Sample Collection

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At the end of experiment, the rats were anaesthetized using ketamine: Xylaxine (50:10 mg/kg) beginning 10 to 15 minutes after simultaneous injection and lasting 15 to 30 minutes. The rats were sacrificed by lumbar dislocation (22) and blood samples were collected through cardiac puncture before abdominal incision. The blood samples were collected into Plain and EDTA bottles. Clear serum and plasma samples were obtained from the blood sample after centrifugation at 1200 rpm for 5 minutes. The prostate was carefully excised from dissected rats, weighed and kept in 10% formalin solution for histopathological examination.

Determination of Prostate Index

The excised prostate tissues of the rats were weighed to determine the prostate index. Prostate index (PI) (mg/g) was calculated base on the ratio of prostate weight (mg) to body weight (g) of the rats (23).

$$\text{Prostate Index (mg/g)} = \frac{\text{Total prostate weight}}{\text{Final body weight}} \times 1000$$

Laboratory Analysis

Biochemical Analysis: Prostate specific antigen (PSA) and testosterone were determined using sandwich enzyme-linked immunosorbent assay (ELISA). AccubindELisa microwells, (Monobind Inc. Lake Forest, CA 92630, USA) (UT-6550 Microplate reader).

Histopathological Examination: Histopathological slides were prepared at Histopathology Laboratory, UsmanuDanfodiyo University, Teaching Hospital (UDTH), Sokoto State. The tissues were subjected to standard routine histological procedures as described by Kiernan, (24).

Data Analysis

Data generated from this study were analyzed using Statistical Package for Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± Standard Error of Mean (±SEM). Analysis of Variance (one way ANOVA) was performed followed by Tukey's *post-hoc* test for comparison and results with $p < 0.05$ values were considered significant.

Results

Table 1: Phytochemical Constituents of Aqueous fruit extract of *Annona muricata*

Compound	Test	Observation	Results
Carbohydrates	Molich's	Purple colour	++
Saponins	Froth's	Persistent frothing	++
Tannins	Lead acetate	Blue-greenish colour	+
Alkaloids	Wagner's	Reddish-brown colour	++
Cardiac glycosides	Killer-Killiani's	Green-blue colour	++
Steroids	Salkowski	Reddish-brown interface	++

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Phenols	Ferric chloride	Bluish-green	++
Flavonoids	Ferric chloride	Dark green	++

(++): moderate present; **(+):** present

Table 2: Acute Toxicity Study of *Annona muricata* Extract

S/N	DOSE (mg)	OBSERVATION	
		First Phase	Second Phase
1	10	0/3	-
2	100	0/3	-
3	1000	0/3	-
4	1600	-	0/1
5	2900	-	0/1
6	5000	-	0/1

Acute toxicity study after 24 hours was ≤ 5000 mg/kg.
0: no death; **n:** number

Table 3: Prostate Index (PI) in Benign Prostate Hyperplasia (BPH) wistar Rats

GROUP	PROSTATE INDEX (PI) (mg/g)
1. NC	0.81 ± 0.18^{a}
2. PC	6.00 ± 0.32^{d}
3. FIN (3mg/kg)	4.23 ± 0.26^{bc}
4. 100 mg/kg *AM*	4.97 ± 0.41^{bc}
5. 200 mg/kg *AM*	4.39 ± 0.29^{bc}
6. 400 mg/kg *AM*	3.82 ± 0.32^{b}

Values were expressed as mean ± sem. values with different superscript on the same column differ significantly at $P < 0.05$. **NC:** normal control; **PC:** positive control; **FN:** finasteride; **AM:** *Annona muricata*.

Table 4: Effects of Aqueous Fruit Extract of *Annona muricata* on PSA and Testosterone levels in Benign Prostate Hyperplasia (BPH) wistar Rats

GROUP	PSA (ng/mL)	TESTOSTERONE (ng/mL)
1. NC	1.09 ± 0.02^{a}	1.23 ± 0.04^{a}
2. PC	2.58 ± 0.01^{e}	2.81 ± 0.01^{bcd}
3. FIN (3mg/kg)	1.25 ± 0.02^{ac}	2.43 ± 0.04^{bc}
4. 100 mg/kg *AM*	1.86 ± 0.07^{bcd}	2.51 ± 0.11^{bcd}
5. 200 mg/kg *AM*	1.68 ± 0.08^{bcd}	2.42 ± 0.09^{bc}
6. 400 mg/kg *AM*	1.57 ± 0.11^{bcd}	2.08 ± 0.01^{bc}

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Values were expressed as mean ± SEM. Values with different superscript on the same column differ significantly at $P < 0.05$. **PSA:** Prostate specific antigen; **NC:** Normal control; **PC:** Positive control; **FIN**: Finasteride; **AM:** *Annona muricata.*

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Plate 1: Photomicrograph of the prostate section showing effects of aqueous fruit extract of *Annona muricata* on TP-Induced BPH in Male Wistar Rats H and E X100

Grp 1: Control; **Grp 2:** Positive control; **Grp3:** 3mg/kg Finasteride; **Grp 4:** 100 mg/kg AMF extract; **Grp 5:** 200 mg/kg AMF extract; **Grp 6:** 400 mg/kg AM extract**; X:** Thin epithelia lining; **Y:** Thick epithelial lining; **N:** Normal glandular Lumen; **Z:** Intraglandular epithelia convolution.

Discussion

Despite advancement in the diagnosis and management of BPH, the disease still pose urologic health challenge affecting adult men worldwide (25). This study investigates the efficacy of *Annona muricata* fruit on testosterone propionate induced benign prostate hyperplasia in male wistar rats.

The results of phytochemical screening showed the presence of flavonoids, alkaloids, tannins, saponins, phenols, carbohydrates, cardiac glycosides and steroids (Table 1). The findings from this study agrees with study conducted by Chikezie *et al.,* (26) on the effect of methanol leaf extract of *Annona muricata* on antioxidant enzymes in dihydrotestosterone estradiol valerate induced benign prostatic hyperplasia in male albino rats. The findings also corroborates with study carried out by Siti *et al*., (27) assessing the acute toxicological effects of *A. muricata* leaf ethanol extract on rats: biochemical, histopathological and metabolomics analyses. Phytoconstituents are known to alleviate the detrimental effect of oxidative stress- a factor implicated in the development of age-related diseases such as BPH (29). Studies have reported *Annona muricata* as a rich source of bioactive compound, acetogenin. These natural compounds have been shown to exhibit cytotoxic properties and are believed to have potential anti-cancer effects.Phytoconstituents are plant metabolites that are pharmacologically active (26). The presence of phytochemicals such as; flavonoid, alkaloid, tannin and sterol might explain the acclaimed nutritional and medicinal properties of the plant. The presences of alkaloids suggest possible annonaine, muricatine and coreximine effects which have been studied for their pharmacological activities (26). Soursop fruit is rich sources of flavonoids suggest possible quercetin, kaempferol and catechin effects which have antioxidant and anti-inflammatory properties. Flavonoids are biologically good for the management of cardiovascular diseases, cancer and oxidative stress (33). The presence of tannins also may suggest the anti-inflammatory, antioxidant and antimicrobial potentials of the plant material (26). The presence of Phytosterols is a good indication as sitosterol and stigmasterol effect have been studied for their potential health benefits, including cholesterol-lowering effects were stated to show anti-cancer properties (34). Saponins also serve as anti-cancer agent and as antioxidants as they prevent degeneration of DNA (35). The result of acute toxicity study showed that there was no toxicity recorded after 24 hours of extract administration. The safety dose of the extract administered orally was safe at dose $\leq$ 5000 mg/kg (Table 2). This result agrees with study (36) in which no severe clinical signs of toxicity and death recorded.

In this study TP-induced BPH causes increase in relative prostate weights and prostatic epithelial hyperplasia in rats that received the exogenous hormone alone compared to normal control rats. The prostatic weight increase is considered as one of the pointer of successful BPH induction (37). The enlargement of the prostate is characterized by proliferation of the cellular elements of the prostate gland which involves epithelial and stromal cellular components (38). A dose-dependent reduction in prostate weight was observed in group 3, 4 and 5 treated with the aqueous fruit extract when compared to untreated rats (positive control) (Table 3). Also, there was a non-significant difference ($p < 0.05$) in relative prostate weights between rats administered highest dose (400 mg/kg) of the extract compared to the finasteride group. This explains the remedial potentials of the fruit material on BPH.Previous research studies had established an increase in the prostate size as vital indicator of BPH progression (39), and this study confirmed the findings since BPH rats' recorded significant enlargement of the prostate gland. It is well understood that when the prostate enlarges, the urethral canal constricts, resulting in partial or complete urinary canal obstruction (40). The findings from this study also agrees with research conducted by Patience *et al.,* (41) on theeffect of acetogenin fraction of *Annona muricata* leaves

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on antioxidant status and some indices of benign prostatic hyperplasia in rats who reported significant decrease ($p < 0.05$) in prostate weight in rats.

The increase PSA level was relative to increased prostate weights of rats that received the exogenous hormone alone. A significant decrease in PSA level was observed in test group administered aqueous fruit extract of *A. muricata*in dose-dependent manner. The decrease level of PSA is associated with reduction of prostatic hyperplasia which had direct consequences on 5α-reductase inhibition (42). At highest treatment dose (400 mg/kg body weight), similar inhibitory effect as finasteride treated rats on induced BPH was demonstrated, possibly through 5α-reductase inhibitory activity (Table 4). The PSA, a glycoprotein found in serum act as a semi-quantitative indicator of prostatic hyperplasia and also a predictor of BPH (30). The level of free testosterone in the blood is considered to be important in BPH progression. Testosterone is known to promote the proliferation of prostate cells through the activity of type II 5α-reductase, an enzyme responsible for conversion of testosterone to a more potent androgen dihydrotestosterone (DHT) (43). The increase in testosterone levels shown in TP-induced BPH rats was significantly inhibited by theextracts in dose-dependent manner (Table 4). This suggests inhibitory potential of *Annona muricata* fruitextracts on down regulation of DHT production in serum and prostate. *Annona muricata* extracts can be a viable alternative to finasteride by enhancing mopping up of free testosterone in the system to prevent its conversion to a more potent DHT by 5α-reductase found mainly within the stromal cells(1). The findings from this study agrees with study conducted by Ibukun *et al.*, (44) on *Annona muricata* (Soursop) mitigated testicular toxicity and prostatic impairment in testosterone-propionate induced BPH in male rats. Testosterone and DHT are involved in the pathogenesis of BPH playing an important role in the development and maintenance of male reproductive organ (45). The serum concentrations of testosterone and DHT may vary with age (46). The levels of DHT in the serum of BPH patients are significantly higher than those of unaffected men of comparable age (47). DHT is primarily synthesized from circulating testosterone in the prostate, hair follicles, and testes via enzymatic action of 5α-reductase. Interestingly, DHT binds to androgen receptors more strongly than testosterone and adrenal androgens. This is due to the greater affinity of DHT towards androgen receptors when compared to that of testosterone and adrenal androgens (45).

The prostate histoarchitecture of TP-induced BPH rats upon H & E staining showed thick epithelial lining and intraglandular epithelial convolution when compared to normal control rats had thin epithelial, normal glandular lumen and no hyperchromasia(Plate 1). The abnormal characteristics found in the prostate of TP-induce BPH (positive control) rats could be attributed dosing effect of testosterone propionate. Several literatures have associated oxidative stress with BPH development (28); this could arise as a result of overproduction of oxidant molecules or possible prostate tissue damage by the reactive oxygen species, thereby worsening prostate morphology (28).The histological findings from this study agrees with study carried out by Patience *et al.,* (41) on theeffect of acetogenin fraction of *Annona muricata* leaves on antioxidant status and some indices of benign prostatic hyperplasia in rats affirming a possible anti-BPH effect of the acetogenin-rich fraction of *A. muricata* leave extract. The efficacy of the extract exhibited could be attributed to phytoconstituents and antioxidant capacity of the plant material.

Conclusion

The findings from this study showed that aqueous fruit extract of *Annona muricata* had inhibitory via reduction of prostate weight, prostate specific antigen, and testosterone levels in dose-dependent manner. The study further revealed, TP-induced group administered 400 mg/kg

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body weight of rats had significant recovery ($p < 0.05$) restoring prostatic histoarchitecture near-normal. This study suggests that *Annona muricata* fruit may be considered as an affordable and treatment option for benign prostatic hyperplasia in men.

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