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Effects of Aqueous Fruit Extract of *Annona muricata* on Testosterone Propionate Induced Benign Prostate Hyperplasia (BPH) in Male Wistar Rats

I.U. Leje [a*], Wali. U. [b], M.H. Yeldu [b], H.M. Alhassan [c], I. Z. Wasagu [b], U. Abubakar [d], B.H. Abubakar [e], H. A. Evuti [f] and M. Bello [g]

[a] Department of Medical Laboratory, General Hospital Minna, Niger State, Nigeria.
[b] Department of Chemical Pathology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
[c] Department of Immunology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
[d] Department of Histopathology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.
[e] Drugs and Medical Supply Management, Sokoto State, Nigeria.
[f] Africa Center of Excellence for Mycotoxins and Food Safety, Federal University of Technology, Minna, Niger State, Nigeria.
[g] Department of Chemical Pathology, Specialist Hospital, Sokoto, Sokoto State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: isahumarleje@gmail.com, isahumarleje@yahoo.com;*

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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 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. An 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 + castrated + TP/day); group 3 (finasteride (3 mg/kg) + castrated + TP/day); group 4 (100 mg/kg *AM* + castrated + TP/day); group 5 (200 mg/kg *AM* + castrated + TP/day) and group 6 (400 mg/kg *AM* + castrated + TP/day) for 42 days. On the 43rd day animals were euthanized, blood and prostate tissue samples were collected for biochemical and histological study.
Results: This study showed that, the extract significantly ($p < 0.05$) decreased both the prostate weight and testosterone levels in a dose-dependent manner compared to finasteride-treated rats. The effect of the extract on the histology of the prostate had significant recovery and was able to restore the enlarged prostate to near-normal in a dose-dependent manner. A significant ($p < 0.05$) recovery was observed at a higher dose (400 mg/kg) of the extracts.
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 a dose-dependent manner. At a high dose (400 mg/kg) body weight of rats had significant recovery ($p < 0.05$) restoring prostatic histoarchitecture to near-normal. This study suggests that *Annona muricata* fruit may be considered a treatment option for benign prostatic hyperplasia in men.

Keywords: A. muricata; benign prostate hyperplasia (BPH); testosterone propionate.

1. INTRODUCTION

Benign prostate hyperplasia (BPH) is a non-malignant enlargement that cause a significant urinary symptoms affecting adult males aged fifty years and above [1]. The disease is marked by a 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 linked to the onset and advancement of BPH, which is brought on by an increase in the quantity and size of prostate gland cells as well as their proliferation [4].

Though the precise mechanism is unknown, it is thought to be related to the ageing of the prostate and the action of steroid hormones. Numerous studies have connected the onset of BPH with the natural ageing process [5]. Men with low testosterone levels are frequently treated with hormone replacement treatment, which includes the synthetic fast-acting version of testosterone known as testosterone propionate. Numerous researches have connected BPH to testosterone medication, particularly testosterone propionate. Because testosterone is converted to dihydrotestosterone (DHT), a powerful androgen that can drive prostate development, higher levels of testosterone and prostate specific antigen may make BPH symptoms worse [6]. This may be brought on by the overproduction of oxidant molecules or the antioxidant system being depleted as a result of prostate enlargement. Prostate illness may occur as a result of an imbalance between oxidative stress and the cell's antioxidant system [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 and, -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 the difficulty associated with urination in certain communities in the Eastern part of Nigeria. Studies have linked *A. muricata* to cytotoxicity and inhibition of proliferation in variety of cancer cell [14]. This plant is readily available, affordable and can be cultivated at low cost. There is paucity of data and no detailed investigation has been carried out to determine the effects of aqueous fruit extract of *A. muricata* on testosterone propionate-induced BPH in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Study Location

The study was carried out in the 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), Usmanu Danfodiyo University, Sokoto.

2.2 Plant 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, Usmanu Danfodiyo University Sokoto (UDUS), Nigeria. Specimen voucher number was assigned as PCG/UDUS/ANNONA/0004 and deposited in the Herbarium unit of the Department.

2.3 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 a Pharmacognosy laboratory for 14 days under same condition until constant weight. The dried fruits were blended using an 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 a 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;

% Fruit extract yield = Weight of final extract / Weight of powdered plant material X 100

2.4 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, Usmanu Danfodiyo University, Sokoto (± 30^{o}C) and a 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.

2.5 Phytochemical Screening of *Annona muricata* Fruit

Phytochemical analysis was carried out in Department of Pharmacognosy and

Ethnopharmacy, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto using standard procedures to identify the phytochemical constituents as described by Harbone, [15]; Trease and Evans, [16]; Sofowora, [17].

2.6 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 scrotal sac. The negative control group (group 1) rats were treated as shams. The animals were given one week to recover before the study.

2.7 Acute Toxicity Study

A total of 12 female 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 were used for the study and randomly divided into two groups (Group 1: 4 groups of 3 animals each and Group 2: 3 groups of 1 animal each). The use of twelve female rats for the study and the rats' groupings were in accordance with Lorke's Method, [19]. The dose was chosen based on a limit test from Organization for Economic Co-operation and Development (OECD) guidelines 423 to determine the range of lethal dose [20]. The aqueous *Annona muricata* extract was dissolved in distilled water and administered via oral gavage at doses of 10 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. Observations of physical state and behavioral changes were conducted to assess any signs of toxicity at intervals of 30, 60, 120, 240 minutes and once daily for 14 days [21].

2.8 Sample Size Calculation

Sample size was determined using G power analysis software [22]. G Power (software) calculates sample size based on pre-designed effect size at small, medium and large difference between the groups based on Cohen's principles [23].

Where;

Sample size calculated by software = 8 animals per 6 groups

Supposed expected attrition = 10%

Corrected sample size = Sample size/ (1 – {% attrition/100})

Therefore: N = 8/0.9

N = 8 rats per group

The total number of rats/6 groups is 48

2.9 Induction of Benign Prostate Hyperplasia (BPH)

A total of forty (40) male Wistar rats of 13 weeks old, weighing between 150-170 g were induced-BPH 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 [24]. The rats were weighed weekly and on the 43rd days the rats were sacrificed [25]. The prostate weights, prostate specific antigen (PSA) levels and histological examination of the prostate tissue were pointers used to ascertain successful induction. The grouping of the animal is presented in the Table 1.

Table 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 extract:*** *Annona muricata fruits;* ***n:*** *Number of rats per group;* ***Grp 1:*** *Negative control group;* ***Grp 2:*** *Positive control group;* ***Grp 3:*** *Standard drug treatment group;* ***Grp 4-6:*** *Treatment group with varying doses of plant extract;* ***O/A:*** *Oral Administration*

2.10 Sample Collection

At the end of experiment, the rats were anaesthetized given ketamine: Xylaxine (50:10 mg/kg) beginning 10 to 15 minutes after simultaneous injection and lasting 15 to 30 minutes. The blood samples were collected through cardiac puncture before abdominal incision [26] using 5 mL syringe into Plain tubes. Clear serum was obtained from the blood sample after centrifugation at 1200 rpm for 5 minutes used for biochemical analysis of PSA and testosterone levels. Following euthanasia, the prostate was immediately and carefully excised from dissected rats using surgical blade and dissecting forceps. The prostates were weighed and kept in 10% formalin solution for histopathological examination using hematoxylin and eosin stain (H & E).

2.11 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 based on the ratio of prostate weight (mg) to body weight (g) of the rats [27].

Prostate Index (mg/g) = Total prostate weight / Final body weight X 1000

2.12 Laboratory Analysis

2.12.1 Biochemical analysis

Prostate specific antigen (PSA) and testosterone were determined using sandwich enzyme-linked immunosorbent assay (ELISA). Both kits were product of AccuBind ELISA microwells, Monobind Inc. Lake Forest, CA 92630, United State of America; LOT: EIA-21K1C4 (REF 2125-300A) and LOT: EIA-37k1G1 (REF 3725-300A) respectively.

Histopathological Examination: Histopathological slides were prepared at Histopathology Laboratory, Usmanu Danfodiyo University, Teaching Hospital (UDTH) Sokoto, State. The tissues were subjected to standard routine histological procedures as described by Kiernan, [28].

2.12.2 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.

3. RESULTS

The result of phytochemical study is presented in Table 2. The results of phytochemical screening studied were carbohydrates, saponins, tannins, alkaloids, cardiac glycosides, steroids, phenols and flavonoids.

The result of acute toxicity study is presented in Table 3. The acute toxicity result showed that there was no toxicity/mortality observed at doses less than or equal to 5000 mg/kg body weight after 14 days of extract administration. This implies that the fruit extract is safe for consumption and is non-toxic up to a dose of 5000 mg/kg body weight of Wistar rats.

The result of the effect of aqueous fruit extract of *A. muricata* on prostate index in BPH male Wistar rats is presented in Table 4. The result showed that experimental BPH-induction with testosterone propionate increased the average prostatic weight of the Wistar rats. The treatment with the extract caused a dose-dependent reduction in the prostatic weight when compared to the positive control rats (untreated). At a high dose of 400 mg/kg body weight of rats showed a significant ($p < 0.05$) reduction in the prostate weight when compared to the finasteride-treated rats.

Table 2. 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	++
Phenols	Ferric chloride	Bluish-green	++
Flavonoids	Ferric chloride	Dark green	++

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

Table 3. Acute Toxicity Study of *Annona muricata* Extract

S/N	Dose (mg)	Observation		
		First Phase	Second Phase	Cage side
1	10	0/3	-	Animal appeared normal
2	100	0/3	-	Animal appeared normal
3	1000	0/3	-	Animal appeared calm
4	1600	-	0/1	Animal showed no distress
5	2900	-	0/1	Animal showed no distress
6	5000	-	0/1	Animal showed no distress

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

Table 4. Prostate Index (PI) in 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 (a, b, c and d) on the same column differ significantly at P < 0.05. ***NC:** normal control; **PC:** positive control; **FN:** finasteride; **AM:** Annona muricata; **Superscript a, b, c and d:** expression of significant levels in a statistical sense*

Table 5. Effects of Aqueous Fruit Extract of *Annona muricata* on PSA and Testosterone levels in 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}

Values were expressed as mean ± SEM. Values with different superscript (a, b, c and d) 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, **Superscript a, b, c and d:** expression of significant levels in a statistical sense*

The results of the effect of aqueous fruit extract of *A. muricata* on PSA and testosterone levels in BPH male rats is presented in Table 5. At the end of the 42 days of subcutaneous injection of testosterone propionate, there was a significant increase ($p < 0.05$) in PSA and testosterone levels in untreated rats (positive control) when compared to the normal control rats. Daily administration of the extract, at a dose of 400 mg/kg body weight rats, caused a significant ($p < 0.05$) decrease in the PSA and testosterone levels when compared to untreated rats (positive group). This result was non-significantly different ($p < 0.05$) from rats treated with the standard drug (finasteride). The plant exerts its effect on the induced BPH in a dose-dependent manner.

In this study, all animals administered with exogenous testosterone exhibited prostastic hyperplasia. The photomicrograph of a cross section of normal prostatic tissue histology (GRP 1) stained with H & E showed normal glandular stroma and thick glandular epithelial lining that appeared convoluted. The photomicrograph (GRP 2) of the positive control indicated hyperplasia of the stroma unlike in the normal prostatic tissue. The cross section of prostactic tissue of the animals treated with a low dose of the extract (GRP 4) showed a reduction in glandular stromal size and decreased density. At a higher dose of the extract (GRP 6) showed more profound recovery and restoration of glandular stroma. The epithelial lining showed thick intraglandular convolution that was comparable to normal control (GRP 1).

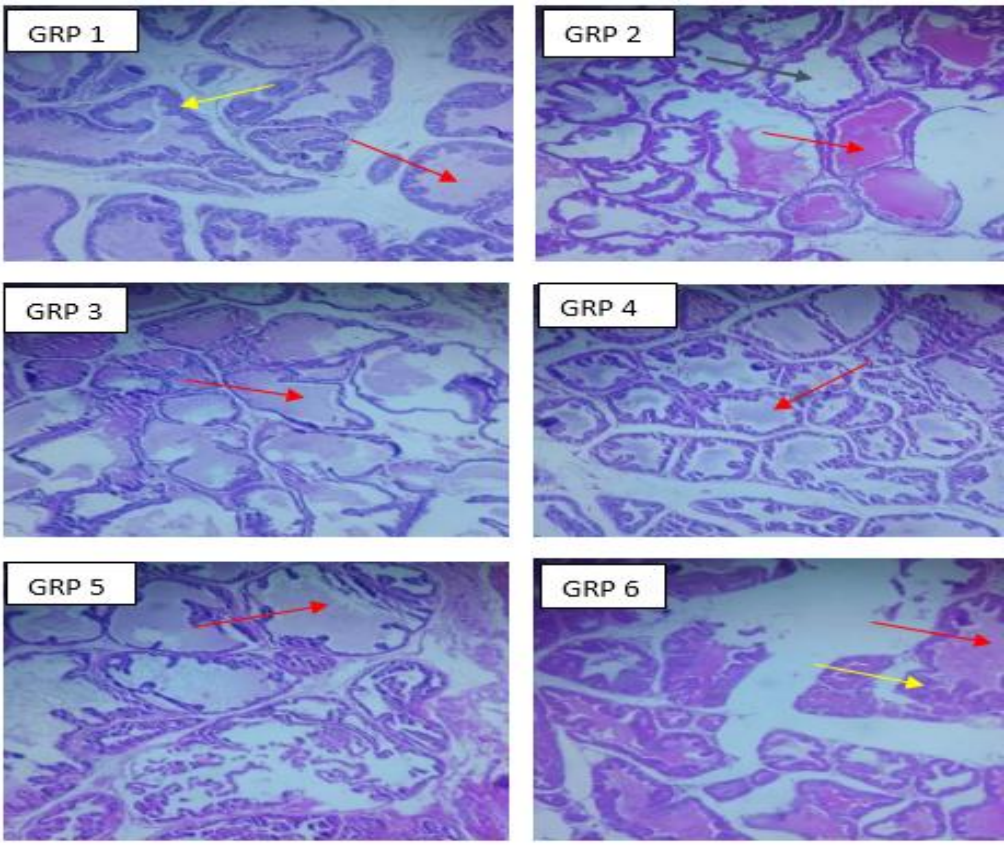

Plate 1. Photomicrograph of the prostate section showing effects of aqueous fruit extract of *Annona muricata* on TP-Induced BPH in Male Wistar Rats H and E X 400

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****; Yellow:*** *Thick intraglandular epithelia convolution;* ***Red:*** *glandular stroma;* ***Black:*** *Extensive hyperplastic gland*

4. DISCUSSION

BPH continues to cause urologic health problems for adult men globally, despite improvements in diagnosis and treatment [29]. This study examines the effects of *Annona muricata* fruit on male Wistar rats' benign prostatic hyperplasia caused by testosterone propionate.

Flavonoids, alkaloids, tannins, saponins, phenols, carbohydrates, cardiac glycosides, and steroids were detected based on the results of the phytochemical screening (Table 2). The results of this investigation are consistent with a study by Chikezie et al., [30] on the impact of *Annona muricata* leaf extract on antioxidant enzymes in male albino rats that had benign prostatic hyperplasia caused by dihydrotestosterone, estrogen, and valerate. The results also support the investigation conducted by Siti et al., [31] which evaluated the acute toxicological effects of ethanol extract from *A. muricata* leaves on rats by biochemical, histopathological, and metabolomics investigations. It is well recognized that phytoconstituents mitigate the deleterious impact of oxidative stress, which is a factor linked to the onset of age-related illnesses like BPH [32]. Studies have shown that acetogenin, a bioactive molecule [33], is abundant in *Annona muricata*. These organic substances are thought to have possible anti-cancer actions since they have been demonstrated to have cytotoxic qualities. Plant metabolites with pharmacological activity are known as phytoconstituents [30]. Phytochemicals such flavonoids, alkaloids, tannins, and sterols may be responsible for the plant's well-known therapeutic and nutritional qualities. The pharmacological activities of annonaine, muricatine, and coreximine have been examined; the existence of these alkaloids suggests potential effects of these substances [30]. Fruits such as soursop are high in flavonoids, which may have anti-inflammatory

and antioxidant benefits through kaempferol, quercetin, and catechin. According to biology, flavonoids are beneficial in the treatment of cancer, oxidative stress, and cardiovascular illnesses [33]. Tannins may also indicate that the plant material has anti-inflammatory, antioxidant, and antibacterial properties [34]. The presence of phytosterols is a positive sign because studies on the possible health advantages of sitosterol and stigmasterol have shown that they have anti-cancer characteristics in addition to decreasing cholesterol [33]. Because they stop DNA deterioration, saponins also function as antioxidants and anti-cancer agents [34]. A safe limit oral dose of the extract and no harm was observed, based on the findings of the acute toxicity study. Oral administration of the extract at a dose ≤ 5000 mg/kg was shown to be the safe dose (Table 3). This outcome is consistent with study [35] where no significant clinical symptoms of toxicity or mortality were noted. In this study, rats who received the exogenous hormone alone showed increased relative prostate weights and prostatic epithelial hyperplasia in comparison to normal control rats with TP-induced BPH. One indicator of a successful BPH induction is thought to be an increase in prostatic weight [36]. Prostate gland enlargement is defined by the proliferation of the gland's biological components, including stromal and epithelial cells [37]. When groups 3, 4, and 5 receiving the aqueous fruit extract were compared to the untreated rats (positive control), a dose-dependent decrease in prostate weight was noted (Table 4). Additionally, between rats given the maximum dose (400 mg/kg) of the extract and the finasteride group, there was a non-significant difference ($p < 0.05$) in the relative prostate weights. This clarifies how the effects of the extract on BPH can be remedied. Based on the substantial growth of the prostate gland in BPH rats, this study validated the findings of previous research investigations that established an increase in prostate size as a critical predictor of BPH development [38]. It is commonly known that the urethral canal constricts when the prostate grows, obstructing the urine canal partially or completely [39]. The results of this investigation align with those of Patience et al., [40] study regarding the impact of *Annona muricata* leaf acetogenin fraction on antioxidant status and certain indicators of benign prostatic hyperplasia in rats, which revealed a noteworthy reduction ($p < 0.05$) in the rats' prostate weight. Rats who received the exogenous hormone alone had larger prostate weights, which were correlated with the elevated PSA level [41]. The test group that received an aqueous fruit extract of *A. muricata* in a dose-dependent manner saw a notable reduction in PSA levels. Reduced prostatic hyperplasia is linked to lower PSA levels, and this has a direct impact on 5α-reductase inhibition [41]. A comparable inhibitory impact to finasteride-treated rats on induced BPH was shown at the highest treatment dose (400 mg/kg body weight), maybe due to 5α-reductase inhibitory activity (Table 5). A glycoprotein called PSA, which is present in serum, is a semi-quantitative marker of prostatic hyperplasia and a predictor of BPH [38]. Blood levels of free testosterone are thought to have a significant role in the development of BPH. Through the action of type II 5α-reductase, an enzyme that changes testosterone into the more potent androgen dihydrotestosterone (DHT), testosterone is known to encourage the growth of prostate cells [42]. In TP-induced BPH rats, the extracts significantly and dose-dependently inhibited the increase in testosterone levels (Table 5), suggesting the potential of *Annona muricata* fruit extracts to inhibit the production of DHT in serum and the prostate. *Annona muricata* extracts can be a good substitute for finasteride because they improve the system's ability to absorb free testosterone and prevent 5α-reductase, which is primarily found in stromal cells, from converting it into a more potent form [42]. The results of this investigation corroborate those of a study by Ibukun et al., [43] on *Annona muricata* (Soursop), which reduced testicular toxicity and prostatic impairment in male rats with BPH induced by testosterone propionate. The pathophysiology of BPH involves testosterone and DHT, which are crucial for the growth and maintenance of the male reproductive organ [44]. Age-related changes in DHT and increased testosterone blood concentrations have been observed in BPH [45]. Compared to males of similar age who are not affected, BPH patients have significantly higher serum levels of DHT [46]. Through the enzymatic action of 5α-reductase, DHT is largely produced from circulating testosterone in the testes, hair follicles, and prostate. It's interesting to note that DHT attaches to androgen receptors more firmly than both adrenal and testosterone do. This is because, in contrast to testosterone and adrenal androgens, DHT has a higher affinity for androgen receptors [44]. When compared to normal control rats, the prostate histoarchitecture of TP-induced BPH animals revealed large hyperplastic gland with thick intraglandular epithelial convolution, normal glandular stroma, and no hyperchromasia (Plate 1). *Annona*

muricata fruit extract showed a considerable recovery ($p < 0.05$) at a higher dose of 400 mg/kg, returning prostatic histoarchitecture to almost normal. The dosage effect of testosterone propionate may be responsible for the aberrant features observed in the prostate of TP-induced BPH (positive control) rats. Several study have linked the development of BPH to oxidative stress [32]; this might be due to the over synthesis of oxidant molecules or potential injury to prostate tissue by reactive oxygen species, which would affect prostate morphology. The histological findings from this study agrees with a study carried out by Patience et al., [40] on the effect 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 the antioxidant capacity of the plant material [47-50].

5. CONCLUSION

The findings from this study showed that aqueous fruit extract of *Annona muricata* had inhibitory potentials via reduction of prostate weight, prostate-specific antigen, and testosterone levels in dose-dependent manner. The study further revealed, the TP-induced group administered 400 mg/kg body weight of rats had a significant recovery ($p < 0.05$), restoring prostatic histoarchitecture to near-normal. This study suggests that *Annona muricata* fruit may be considered as an affordable and treatment option for benign prostatic hyperplasia in men.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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