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

I.U. Leje^{1*}, U.Wali², M.H. Yeldu², H.M. Alhassan³, I.Z. Wasagu², U. Abubakar⁴, B.H. Abubakar⁵, H.A. Evuti⁶, and M. Bello⁷

¹Department of Medical Laboratory, General Hospital Minna, Niger State, 920101, Nigeria.

²Department of Chemical Pathology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, 840001, Nigeria.

³Department of Immunology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, 840001, Nigeria.

⁴Department of Histopathology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, 840001, Nigeria.

⁵Drugs and Medical Supply Management, Ministry of Health, Sokoto State, 840001, Nigeria.

⁶Africa Centre of Excellence for Mycotoxins and Food Safety, Federal University of Technology, Minna, P.M.B 65, Minna, Niger State, 920001, Nigeria.

⁷Department of Chemical Pathology, Specialist Hospital Sokoto, Sokoto State, 840001, Nigeria.

*Corresponding author:

I.U. Leje

Department of Medical Laboratory,
General Hospital Minna,
Niger State, 920101,
Nigeria.

Email: isahumarleje@gmail.com

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Abstract

The urological condition benign prostatic hyperplasia (BPH) affects 50 years and older men with histological evidence present in 50% of this age group. The clinical knowledge about BPH remains incomplete despite its widespread occurrence. The therapeutic properties of *Annona muricata* (sour sop) have been documented but researchers have not thoroughly studied its effects on BPH. This research examined how aqueous *A. muricata* fruit extract affects BPH in orchiectomized Wistar rats that received testosterone-propionate. The acute toxicity test evaluated extract safety through testing various doses from 10 mg/kg to 5000 mg/kg. The research involved 48 adult male rats who received six different treatments including normal control and positive control (testosterone propionate-induced) as well as finasteride-treated and three extract-treated groups at 100 mg/kg, 200 mg/kg and 400 mg/kg. The 42-day treatment period ended with euthanasia followed by tissue collection on day 43 for biochemical and gene expression analyses. *A. muricata* extract demonstrated significant ($p < 0.05$) MDA reduction while activating antioxidant enzymes SOD, GPx, and CAT in a dose-dependent pattern. The extract did not cause any major adverse effects on kidney or liver function. The 400 mg/kg extract dose produced major gene expression reductions of androgen receptor (AR) and tumor necrosis factor (TNF). The research indicates *A. muricata* reduces BPH through antioxidant effects which decrease oxidative stress and inflammation. The research demonstrates that *A. muricata* fruit extract has therapeutic value as a natural BPH treatment modulator.

INTRODUCTION

Benign prostate hyperplasia (BPH) denotes a non-cancerous enlargement of the prostate gland in middle-aged and older aged men [1]. It's characterized by the proliferation of the cellular components of the prostate into discrete mass [2]. This abnormal increase in prostate size compresses the urethra canal, leading to increased urgency, terminal dribbling of urine, pain and urine incontinence [3]. Although the pathophysiology of the disease remains incompletely elucidated, androgens [4], obesity, diabetes, renal dysfunction, systemic inflammation and

hyperlipidemia play central role in its development. The disease increases the risk of lower urinary tract symptoms (LUTs), hinted as irritative and obstructive symptoms, affecting quality of life [5]. Several literatures have shown dihydrotestosterone (DHT), an androgen derived from testosterone through 5 α -reductase activity, are involved in glandular proliferation with nodular hyperplasia [6]. This mechanism explains the implication of 5 α -reductase inhibitors, such as finasteride and dutasteride, used in the treatment of BPH [7]. Surgery and medical therapy such as dutasteride and finasteride have been used to treat BPH and improve lower urinary tract symptoms [7]. These medications

have been implicated as they result to undesirable side effects linked to sexual and reproductive dysfunction associated with the use of these drugs [9].

In recent years, herbal medicine has gained enormous use in research due to their nutritional value, cheap and assessable in development of new treatment strategies that is cost-effective in the treatment of BPH [10]. *A. muricata* belonging to the Annonaceae family [10] is consumed fresh, and most communities in the Southern part of Nigeria use it in their folk medicine to treat difficulty associated with urination, diabetes, cancer, insomnia and cystitis [11]. Studies have linked *A. muricata* to antioxidative and regulatory potentials that can help manage BPH [12]. There is limited information on the antioxidative and regulatory effects of *Annona muricata*'s aqueous fruit extract on testosterone propionate-induced BPH in orchietomized Wistar rats.

MATERIALS AND METHODS

Study Site

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.

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, Usmanu Danfodiyo University Sokoto (UDUS), Nigeria. The specimen voucher number was logged as PCG/UDUS/ANNONA/0004 and the sample deposited in the Herbarium unit.

Extraction of Plant Material

The fresh *Annona muricata* fruits were washed with distilled water, chopped into pieces, seeds separated and air dried under dust-free environment in a Pharmacognosy laboratory for 14 days until constant weight. Using an electronic blender (Binatone BLG 450, London, United Kingdom) the dried fruits were blended and sieved through 40-mesh (0.4 mm) to obtain fine powder. About 500g powdered sample was weighed, soaked in 3000 mL distilled water and was 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 °C. A dried brown paste was obtained. It was weighed, stored in wide wide-mouth labeled container and preserved in the refrigerator at 4°C until use. The percentage (%) yield of the extract was calculated;

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

Experimental Animals Procurement and Management

Forty-eight [48] male Wistar rats of 13 weeks old, weighing 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 ($\pm 30^\circ\text{C}$) and a lighting period of about 12 hours daily.

The animals acclimatized for two weeks and were fed standard commercial pelletized grower's feed and drinking water *ad libitum*. Principles of Laboratory Animal Care' -were 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 the 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 [13]; Trease and Evans, [14]; Sofowora, [15] to determine the presence or absence of flavonoids, tannins, carbohydrates, alkaloids, cardiac glycosides, saponins and phenols.

Ethical Approval

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

Orchiectomy of rats

To minimize the impact of endogenous testosterone during the study, the experimental rats were anesthetized (ketamine: Xylaxine; 50:10 mg/mL). Orchiectomy was performed using Obisike *et al.*, [16] 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.

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 was 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, [17]. 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. 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.

Sample Size Calculation

The sample size was determined using G power analysis software [22]. G Power (software) calculates sample size based on pre-designed effect size at the 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

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 the rats [18]. The rats were weighed weekly and on the 43rd days the rats were sacrificed. The increased 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 animals is presented in the Table below;

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 (10 mg/kg)	Rat pellet	42
Grp2 TPI + orchiectomy	TP (10 mg/kg)	Finasteride (3 mg/kg)	42
Grp2 TPI + orchiectomy	TP (10 mg/kg)	Plant extract (100 mg/kg)	42
Grp2 TPI + orchiectomy	TP (10 mg/kg)	Plant extract (200 mg/kg)	42
Grp2 TPI + orchiectomy	TP (10 mg/kg)	Plant extract (400 mg/kg)	42

Note: 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:** reference drug treatment group; **Grp 4-6:** Treatment group with varying doses of plant extract; **O/A:** Oral Administration

Sample Collection

At the end of the experiment, the rats were anaesthetized using ketamine: Xylazine (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 using 5 mL syringe into Plain tube. Clear serum was obtained from the blood sample after centrifugation at 1200 rpm for 5 minutes used for biochemical analysis of MDA, SOD, CAT and GPx. Following euthanasia, the rats were sacrificed through lumbar dislocation [19]. The prostate was carefully excised from dissected rats using a surgical blade and dissecting forceps. The prostate was harvested and weighed. About 50 g of the prostate was kept in lysate buffer solution for gene expression study.

Determination of Prostate Index

The excised prostate tissues were weighed to determine the prostate index. Prostate index (PI) (mg/g) was calculated;

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

Laboratory Analysis

Biochemical Analysis: Serum MDA was determined using colorimetric method. This method was estimated using thiobarbituric acid reactive substance assay (TBARS) as described by Shah and Walker's, [20]. Glutathione Peroxidase activity was measured the colorimetric method [21]. T-SOD was carried out based on colorimetric method [22]. Catalase was measured based on colorimetric method [23]. The liver function test, ALT, AST, and ALP were analyzed using kinetic methods and TP, ALB were analysed using kits from spectrum (United Kingdom).

The kidney function test, Na, K, CL, Urea, and Creatinine were analysed using analytical kits, products of Spectrum and Teco.

Molecular Study

Reverse Transcriptase-Polymerase Chain reaction (RT-PCR)

Quantitative RT-PCR reactions were carried out in triplicates using Qiagen, Rotor-Gene Q software 2.3.1.49- (SN 031515) on a Bio-Rad Thermocycler. Amplification was carried out in a total volume of 20 µL in a cycler IQ™ PCR 96 well plate (Bio-Rad, Hercules, CA). Reaction mixture was composed of *Trans Script* Green One-Step RT/RI Enzyme Mix (0.4 µL), 2 x *PerfectStart* Green One-Step qRT-PCR SuperMix (10 µL), RNase-free Water (6 µL), RNA template (4 µL), Forward Primer (0.4 µL) and Reverse Primer (0.4 µL). qRT-PCR conditions (two-step) was denaturation:55 °C for 10 minute, annealing: 94 °C for 1 minute and 45 cycles (cycling repeats) of 95 °C for 10 second and 55°C for 30 second. Extension: from 50 °C to 99°C, hold for 90 seconds on the 1st step and hold for 5 seconds on the next step. Melting point analysis started at 55 °C. The results were expressed as a ratio to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Primer Design

Nucleotide sequences for rats (*Rattus norvegicus*) *AR* and *TNF* were obtained from the NCBI database using Nucleotide search (<http://ncbi.nlm.nih.gov/nucleotide/>). Primers specific for rats *AR* and *TNF* were designed using Primer Tool Quest (<http://ncbi.nlm.nih.gov/tools/primers-blast/>) and Net primer (<http://biotools.ncbic.northwestern.edu/oligoCalc.html>) programs. Using an mRNA sequence as a guide, the Primer software selects primers and suggests the optimal substitutes. The Net primer program examined the primer sequence for % GC, hairpin formation, and dimerization. The criteria followed when designing the primer pairs was compatibility of most, if not all, confirmed transcripts; an amplicon size lower than 150 base pairs; a GC% lower than 65%; spanning at least one exon-exon junction; and each primer pair was designed to span a different exon-exon junction. Primer pairs were designed for *AR* and *TNF* to ensure that they satisfy the specific criteria required for this experiment.

Table 2. Primers sequence, length and GC content.

Target Gene	Primers Sequence	Length	Gc % Min/Max	Tm Min/Max
AR-F (5'→3')	GCGGAAGGGAAACAGAAGTA	20	50	51.78
AR-R (5'→3')	AGAGTCATCCCTGCTTCATAAC	22	45	52.97
TNF-F (5'→3')	GCAGATGGGCTGTACCTTATC	21	52	54.86
TNF-R (5'→3')	GAAATGGCAATCGGCTGAC	20	50	51.78
GAPDH-F (5'→3')	GCCTCTCTCCCAATTCAACCCT	20	50	51.78
GAPDH-R (5'→3')	CTCGTGGTTCACACCATCA	21	45	52.97

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. The Delta-Delta Ct algorithm (Livak method) was used to calculate the log-fold change between Ct values of target genes and reference gene (GAPDH) expressed.

RESULTS

The phytochemical investigation of the aqueous fruit extract of *Annona muricata* revealed the presence of several bioactive compounds, each identified using standard qualitative tests. These results, summarized in **Table 3**, indicate that the fruit extract contains a rich profile of secondary metabolites known for their diverse therapeutic potential. The Molisch's test confirmed the presence of carbohydrates by developing a characteristic purple color at the interface, indicating that monosaccharides or polysaccharides are moderately present (++). This suggests that the fruit may serve as an energy source and contribute to cell osmoregulation and structural roles.

Saponins, known for their surfactant and antimicrobial properties, were detected using the Froth's test, which showed persistent frothing—indicating moderate presence (++). The presence of tannins, which have astringent and antioxidant properties, was confirmed using the lead acetate test, as evidenced by the formation of a blue-greenish precipitate (+). Alkaloids, a class of nitrogen-containing compounds often associated with analgesic and cytotoxic activities, were identified through the Wagner's test, which produced a reddish-brown precipitate, indicating moderate presence (++). The Keller-Kiliani's test for cardiac glycosides, known for their role in heart function modulation, resulted in a green-blue coloration, indicating their presence (+). Steroids, which may contribute to anti-inflammatory and hormonal activities, were moderately detected (++) through the Salkowski's test, as indicated by the appearance of a reddish-brown interface. The Ferric chloride test was used to identify phenolic compounds and flavonoids, both known for their antioxidant and free radical-scavenging properties. Phenols gave a bluish-green color (++), while flavonoids yielded a dark green coloration (++), suggesting their significant presence in the extract.

Table 3. 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	++

Note: (++) : moderate present; (+): present

The acute toxicity evaluation of the aqueous fruit extract of *Annona muricata* was conducted to assess its safety profile in vivo, following the OECD guidelines for testing of chemicals (commonly Guideline 425 or 423). This test is essential for determining the lethal dose (LD₅₀) and detecting any signs of toxicity or mortality in experimental animals after a single extract administration. As shown in **Table 4**, two phases of the study were carried out using increasing oral doses: Phase 1 included lower doses (10 mg/kg to 1000 mg/kg body weight), while Phase 2 tested higher doses (1600 mg/kg, 2900 mg/kg, and 5000 mg/kg body weight).

No deaths (0/3 animals) were recorded 24 hours post-administered in all groups. In addition, cage-side observations indicated no signs of toxicity or behavioral abnormalities at any tested dose. The animals seemed normal or calm, with no signs of distress such as piloerection, tremors, lethargy, or abnormal posture. The absence of mortality or observable adverse effects up to the maximum tested dose of 5000 mg/kg suggests that the extract is non-toxic and safe for acute oral consumption at high concentrations. According to established toxicological thresholds, a substance with an LD₅₀ greater than 5000 mg/kg is considered practically non-toxic. These findings support the preclinical safety of *Annona muricata* fruit extract and validate its traditional use, warranting further studies for chronic toxicity and therapeutic efficacy.

Table 4. Acute toxicity study of *Annona muricata* extract.

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

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

The impact of *A. muricata* fruit extract on malondialdehyde (MDA) concentration and the activities of antioxidant enzymes were estimated as indices for oxidative stress status of the rats. Subcutaneous injection of testosterone propionate significantly increased the lipid peroxidation marker (MDA) of the group that received the hormone alone and as well decreased antioxidant activities compared to the normal control group. The results of the effect of *A. muricata* aqueous leaf extract on MDA and GPx, SOD and CAT activity on testosterone propionate-induced benign prostatic hyperplasia in male rats are shown in **Table 5** below. The results obtained were equivalent to the normal control, and the group was administered the standard treatment; administration of the extract (400 mg/kg) reversed the effect of the exogenous hormone as the MDA levels significantly decreased and the antioxidant activities of the rats were raised. The result of the effect of aqueous leaf extract of *A. muricata* on urea, sodium, potassium, chloride and creatinine levels on testosterone propionate-induced benign prostatic hyperplasia in male rats is presented in **Table 6** below. The effects of aqueous *Annona muricata* fruit extract on the kidney function of testosterone propionate-induced BPH show non-significant differences in urea, sodium, potassium, chloride and creatinine levels of treated and untreated rats. This result revealed a non-toxic effect on renal function.

The result of the effect of aqueous leaf extract of *A. muricata* on urea, sodium, potassium, chloride and creatinine levels on testosterone propionate-induced benign prostatic hyperplasia in male rats is presented in **Table 7** below. The effects of aqueous *Annona muricata* fruit extract on some ALP, AST, ALT, TP and ALB levels on testosterone propionate induced BPH shows non-significant difference in treatment and untreated group. This result revealed a non-toxic hepatocellular effect of the extract.

Table 5. Effects of Aqueous Fruit Extract of *muricata* on Oxidative stress and Antioxidant Enzymes Activity in BPH Rats.

GROUP	MDA (NM/ML)	GPX (U/L)	CAT (U/L)	SOD (U/L)
NC	0.35 ± 0.01 ^{BC}	6.27 ± 1.01 ^{DE}	13.86 ± 0.82 ^{DE}	1.66 ± 0.02 ^B
PC	1.42 ± 0.01 ^A	2.64 ± 1.33 ^A	9.42 ± 2.99 ^A	0.63 ± 0.02 ^A
FIN (3MG/KG)	0.47 ± 0.02 ^{BC}	5.92 ± 1.71 ^{BCD}	12.75 ± 5.04 ^{BCD}	0.82 ± 0.04 ^C
100 MG/KG AM	0.62 ± 0.01 ^{BCD}	3.61 ± 0.79 ^{AB}	10.37 ± 3.39 ^{AB}	0.70 ± 0.02 ^B
200 MG/KG AM	0.56 ± 0.01 ^{CD}	4.08 ± 0.91 ^{BC}	11.58 ± 4.03 ^{BCD}	0.73 ± 0.03 ^B
400 MG/KG AM	0.52 ± 0.03 ^{CD}	4.40 ± 1.41 ^{BC}	11.98 ± 5.45 ^{BCD}	0.78 ± 0.02 ^{BC}

Note: Values were expressed as mean ± sem. Values with different superscript on the same column differ significantly at P < 0.05. MDA: malondialdehyde; GPx: glutathione peroxidase; CAT: catalase; SOD: superoxide dismutase; 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.

Table 6. Effects of aqueous fruit extract of *A. muricata* on kidney function markers in BPH Rats.

GROUP	UREA (MMOL/L)	NA (MMOL/L)	K (MMOL/L)	CL (MMOL/L)	CRE (MG/ DL)
NC	5.57 ± 0.23 ^{AB}	136.98 ± 1.16 ^{AB}	4.32 ± 0.19 ^{AB}	99.60 ± 1.78 ^A	0.80 ± 0.03 ^A
PC	5.64 ± 0.24 ^{AB}	138.21 ± 0.86 ^{AB}	4.22 ± 0.26 ^{BC}	98.20 ± 1.32 ^A	0.80 ± 0.06 ^A
FIN (3MG/KG)	5.48 ± 0.22 ^{AB}	138.11 ± 1.07 ^{AB}	4.88 ± 0.34 ^{AB}	99.20 ± 1.16 ^A	0.79 ± 0.03 ^A
100 MG/KG AM	5.48 ± 0.18 ^{AB}	137.48 ± 4.12 ^{AB}	4.96 ± 0.17 ^{AB}	97.80 ± 1.39 ^A	0.80 ± 0.04 ^A
200 MG/KG AM	5.30 ± 0.22 ^{AB}	137.87 ± 4.45 ^{AB}	4.78 ± 0.33 ^{AB}	97.80 ± 1.28 ^A	0.80 ± 0.08 ^A
400 MG/KG AM	5.28 ± 0.13 ^{AB}	138.49 ± 1.79 ^{AB}	4.14 ± 0.12 ^{AB}	97.80 ± 1.19 ^A	0.80 ± 0.21 ^A

Note: Values were expressed as mean ± SEM. Values with different superscripts on the same column differ significantly at (P < 0.05). NC: Normal control; PC: Positive control; FIN: Finasteride; AM: *Annona muricata*; Superscript a, b and c: expression of significant levels in a statistical sense.

Table 7. Effects of aqueous fruit extract of *Annona muricata* on some liver function markers in BPH Rats.

Group	ALP (U/L)	AST (U/L)	ALT (U/L)	TP (G/ DL)	ALB (G/ DL)
NC	172.66 ± 1.54 ^{AB}	86.20 ± 0.49 ^{AB}	64.99 ± 1.33 ^{AB}	6.62 ± 0.14 ^{AB}	4.78 ± 0.08 ^{AB}
PC	176.27 ± 1.29 ^{AB}	83.69 ± 2.57 ^{AB}	66.75 ± 2.17 ^{AB}	6.49 ± 0.08 ^{AB}	4.17 ± 0.10 ^{AB}
FIN (3MG/KG)	179.16 ± 2.15 ^{AB}	85.85 ± 4.00 ^{AB}	64.94 ± 1.83 ^{AB}	6.73 ± 0.07 ^{AB}	4.99 ± 0.05 ^{AB}
100 MG/KG AM	174.67 ± 0.65 ^{AB}	82.12 ± 1.05 ^{AB}	67.30 ± 0.68 ^{AB}	6.76 ± 0.09 ^{AB}	4.43 ± 0.10 ^{AB}
200 MG/KG AM	171.87 ± 3.26 ^{AB}	85.41 ± 3.00 ^{AB}	65.06 ± 1.46 ^{AB}	6.65 ± 0.13 ^{AB}	4.17 ± 0.11 ^{AB}
400 MG/KG AM	172.78 ± 4.12 ^{AB}	86.61 ± 1.09 ^{AB}	65.72 ± 0.47 ^{AB}	6.60 ± 0.09 ^{AB}	4.17 ± 0.15 ^{AB}

Note: Values were expressed as mean ± SEM. Values with different superscript on the same column differ significantly at P < 0.05. AST: Aspartate aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase; TP: Total protein; ALB: Albumin; NC: Normal control; PC: Positive control; FIN: Finasteride; AM: *Annona muricata*; Superscript a, b and c: expression of significant levels in a statistical sense.

The result of the effects of aqueous fruit extract of *Annona muricata* on the levels of androgen receptor (AR) and tumor necrosis factor (TNF) expressed on TP-Induced BPH-induced male Wistar rats is presented in Figs. 1 and 2 below. In this study, aqueous *A. muricata* fruit extracts at dose-dependent reduced the expression levels of AR and TNF with decreased relative log-fold change near to standard drug (finasteride) treated groups. The positive control group displayed double folds' increase in the expression levels of the target genes compare to the control and treatment groups. The remarkable effect of the extract was observed at high dose (400 mg/kg) when compared to the positive group.

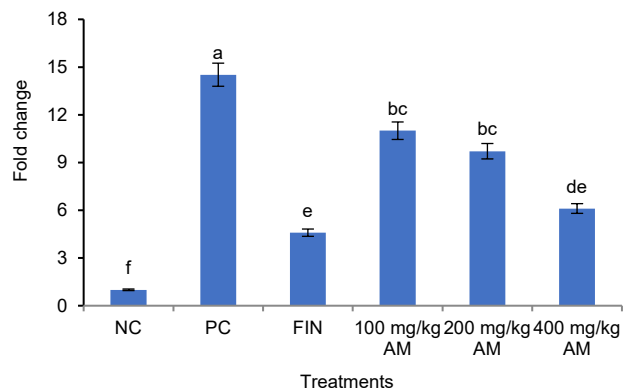


Fig. 1. Effects of Aqueous fruit extract of *Annona muricata* on expression of Androgen receptor (AR) in TP-induced BPH in male wistar rats. The extract significantly reduces the expression of AR gene in a dose-dependent manner compared to PC group. NC: Negative control; PC: Positive control; FIN: Finasteride, 3mg/kg; AMF: *Annona muricata* fruit extract; Superscript a, b, c, d, e and f: expression of significant levels statistically.

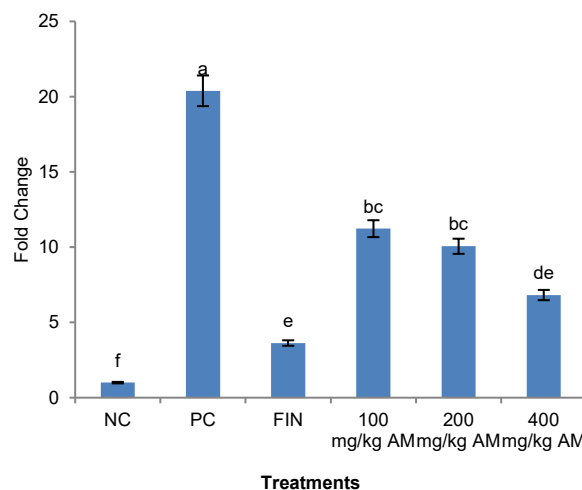


Fig. 2. Effects of Aqueous fruit extract of *Annona muricata* on tumor necrosis factor (TNF) expression in TP-induced BPH in male wistar rats. The extract significantly reduces the expression of TNF gene in a dose-dependent manner compared to PC group. NC: Negative control; PC: Positive control; FIN: Finasteride, 3mg/kg; AMF: *Annona muricata* fruit extract; Superscript a, b, c, d, e and f: expression of significant levels in a statistical sense.

DISCUSSION

The results of the phytochemical screening showed the presence of flavonoids, alkaloids, tannins, saponins, phenols, carbohydrates, cardiac glycosides, and steroids (Table 3). The findings corroborate with a study carried out by Siti *et al.*, [24] assessing the acute toxicological effects of *A. muricata* leaf ethanol extract on rats: biochemical, histopathological, and

metabolomics analyses. *A. muricata* are known to contain annonaceous acetogenins [25] as bioactive compounds found in the leaves, seeds, and stems that have been studied for their potential anti-cancer and anti-parasitic properties. The presence of these phytochemicals in *A. muricata* might explain the acclaimed potential health benefits, including its antioxidant, anti-inflammatory and cytotoxic properties [26]. The quercetin in soursop has shown flavonoids containing plants to exhibit anti-inflammatory and antioxidant properties [12]. Alkaloids, such as isoquinoline alkaloids, show analgesic and anti-inflammatory properties. High tannins and phenol content suggests *A. muricata* could act as an antimicrobial agent, anti-cancer as the presence of phenol is known to inhibit pathogens [12]. The presence of carbohydrates in *A. muricata* fruit extract, such as glucose and fructose, may provide energy and support various metabolic functions in the body.

The result of acute toxicity study showed no death recorded after 24 hours of extract administration. The safety and tolerability of the aqueous fruit extract administered orally was safe at dose ≤ 5000 mg/kg (Table 4). This finding corroborates a study [27] in which no physical signs of toxicity and death was observed. In this study, a significant increase in the concentration of MDA with a corresponding decrease in the antioxidant activities was observed in rats that received the exogenous hormone (TP-induced) alone (Table 5). This explains the association between oxidative stress and antioxidant depletion in BPH development. This decrease could be due to the impact of exogenous hormone and physiological changes associated with prostate enlargement. However, administration of *A. muricata* significantly ($p < 0.05$) decreased the MDA concentration and restored CAT, SOD and GPx function in a dose-dependent manner when compared to untreated rats.

The reinforcement of the antioxidant system and phytoconstituents could be one of the possible mechanisms through which the plant material was able to attenuate the BPH progression. This finding agrees with a study carried out by Agu *et al.*, [28] who worked on "protective effect of ethanol extract of soursop (*Annona muricata* linn) leaves on cycad-induced oxidative stress in male albino Wistar rats" who recorded marked increase in the levels of these antioxidant enzymes while there was diminished peroxidative activity in treated rats. Reactive oxygen radicals (ROS) produce polyunsaturated lipid peroxidation, which is measured by malondialdehyde. These fats play a crucial role in the formation of cellular membranes. Free radicals are the main attackers of membrane lipids, which causes their peroxidation [29].

This results in cellular damage, membrane instability, and the disintegration of other macromolecules and membrane proteins [30]. Accordingly, it has been noted that BPH exhibits lower antioxidant levels as a consequence of elevated peroxidative activity [31]. Numerous academic studies have linked the development of BPH to oxidative stress [32]; this could be due to the overproduction of oxidant molecules or the antioxidant system being depleted during prostate enlargement. In addition to the potential for reactive oxygen species to injure prostate tissue, compensatory cellular growth may also occur, exacerbating the enlargement of the prostate [32]. Prostate disease development, however, is significantly influenced by the equilibrium between oxidative stress and the antioxidant system of the cells [33]. Renal dysfunction was conducted by determining the concentration of urea, creatinine, chloride, potassium and sodium in serum to evaluate the overall function of the kidney. Edmund and David [34] state that these indicators are seen in blood serum above normal in patients with kidney

impairment. *A. muricata*'s aqueous leaf extract was found to have no significant effect ($p < 0.05$) on the levels of urea, creatinine, sodium, and potassium in the kidneys of male Wistar rats with testosterone propionate-induced benign prostatic hyperplasia when compared to the normal control group (Table 6). The renal function indicated a non-toxic influence on renal function. This result is in line with a study by Siti *et al.*, [24] that evaluated the acute toxicological effects of ethanol extract from *Annona muricata* leaves on rats. The study included biochemical, histological, and metabolomics investigations, and it found no evidence of renal failure based on urea and creatinine levels [35]. Urea and creatinine are waste products excreted through the kidney in which high level of these substances in blood indicates abnormalities in renal function. The kidney removes about 85% of urea, but serum urea levels rise when renal clearance declines [36].

A liver function test was performed to determine the degree of organ damage that the extract had caused. This is true because many endogenous and exogenous compounds that are found in the body are absorbed, metabolized, conjugated, and excreted by the liver. As a result, any issue or toxicity associated with the extract will result in elevated AST, ALP, and ALT enzyme levels. These enzymes are helpful for hepatocellular damage markers since they are only secreted in the event of a liver injury [37]. The normal level of ALP, ALT, AST, TP and ALB shown in TP-induced BPH and treatment rats suggests that no injury occurred in the liver when compared with the normal control group (Table 7). Siti *et al.*, [24] on assessing the acute toxicological effects of *Annona muricata* leaf ethanol extract on rats: Biochemical, histopathological and metabolomics analyses observed a normal level of AST, ALT and ALP in treated group suggestive of non-hepatocellular injury. The enzymes AST and ALT are crucial in the breakdown of amino acids channeled into the Krebs cycle and electron transport chain as such ALT is more concentrated in the liver, and thus, its elevation in the blood is considered an accurate indicator of liver disease [37]. Changes in membrane-bound ALP negatively impact membrane permeation, disrupting the transportation of metabolites. When the liver is diseased, these enzymes are released into the system in excess of a critical concentration [38].

Annona muricata play an important role in the inflammation and hypertrophy of prostatic tissues observed on TP-induced BPH in male Wistar rats. In this study, aqueous *A. muricata* fruit extracts at dose-dependent reduced the expression levels of AR and TNF with a decrease in relative log-fold change when compared with standard drug (finasteride) treated rats (Fig 1 & 2). The positive control rats display double folds increase in the target genes' expression levels compared with the control and treatment rats. A remarkable effect of the extract was observed at the highest dose (400 mg/kg) compared with the positive group.

This finding agrees with the study conducted by Gwan *et al.* [39] on the anti-inflammatory effects of graviola stem bark extracts on testosterone propionate-induced benign hyperplasia in model rats who reported that AR, 5 α -R, inflammatory molecules, and growth factors play a critical role in the inflammation and hypertrophy of prostatic tissues in BPH. Testicular secretion serves as the main source of androgens, specifically testosterone. When androgens enter prostatic tissue, they are converted to dihydrotestosterone (DHT) by three five α reductase isozymes (made by the SRD5A1, SRD5A2, and SRD5A3 genes). DHT binds to the receptor in prostate cells with two to five times greater affinity than testosterone and increases AR signaling ten times more than testosterone [39].

CONCLUSION

The findings from this study showed that the aqueous fruit extract of *Annona muricata* had a modulatory effect, which was able to significantly down-regulated the expression of AR and TNF via decreased MDA and increased CAT, SOD, and GPax levels in a dose-dependent manner. The non-significant levels of ALP, ALT, AST, TP, ALB, urea, sodium, chloride, potassium, and creatinine shown are suggestive of non-liver and kidney injury. This finding supports the therapeutic use of *Annona muricata* fruit in folk medicine and may be considered as a treatment strategy for BPH in men.

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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AUTHORS' CONTRIBUTION

The conceptualization of the study was led by I. U. Leje and Wali U. Data analysis was conducted by I.U. Leje and Wali. U. The initial draft of the manuscript was prepared by I. U. Leje, Wali U and with the remaining authors contributing to the review and editing.

DATA AVAILABILITY STATEMENT

Data available on request

AI USAGE DECLARATION

During the preparation of this work, the authors used generative AI technologies for language editing and refinement. All of the authors reviewed and edited the content. The authors take full responsibility for the final content of the publication.

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