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Original Research Article

Ameliorating Potential of *Annona muricata* on Testosterone Propionate-Induced benign Prostatic Hyperplasia in Male Wistar Rats

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Abstract

This study investigated the ameliorating potential of *Annona muricata* on Testosterone Propionate-Induced Benign prostatic hyperplasia (BPH) in male Wistar rats. Sixty (60) adult rats weighing 150 to 250g were grouped into six (6) of ten (10) rats. Group 1 (positive control) was fed with regular rat feeds and water. Group 2 (negative control), and Groups 3 - 6 were given regular feeds, water, and 3 mg/kg propionate for 28 days to induce benign prostatic hyperplasia. Group 3 to 6 were treated with 500, 1000, 1500, and 2000 mg/kg *A. muricata* extract respectively for 28 more days. Semen quality, Benign prostatic hyperplasia biomarkers, oxidative stress enzymes, Biomarkers of cardiac, renal, and hepatic function, Haematological indices as well as testis histology were investigated in treatment groups relative to controls. Results of Groups 3 to 6 show a significant ($p<0.05$) increase in sperm motility, viability, and count, with a significant decrease ($p<0.05$) in dead and abnormal sperm cells relative to the negative control. Prostate-specific antigen (PSA), a BPH indicator, showed a decrease and Carcinoembryonic antigen (CEA) an increase, however, both changes were non-significant ($p>0.05$). Cardiac biomarkers creatinine kinase MB (CKMB), D-dimer, and myoglobin, as well as liver, were not affected. The photomicrographs showing lesion in negative control were ameliorated by different concentrations of *A. muricata* extract and the tissues were restored to normal in treatment Groups 3 to 6. *Annona muricata* possesses ameliorative potentials on testosterone propionate-induced benign prostate hyperplasia. It has a positive effect on spermatogenesis and therefore, can boost semen quality.

Keywords: *Annona muricata*, Testosterone Propionate, Benign prostatic hyperplasia, Semen quality, Photomicrographs.

INTRODUCTION

Benign prostatic hyperplasia is a condition in which the prostate gland swells, becomes painful and inflamed. It can be excruciatingly painful and distressful. It is a common condition as men get older and are characterized by the proliferation of the cellular elements of the prostate, leading to an enlarged prostate gland. Sometimes chronic bladder outlet obstruction may set in as a result of BPH and this may lead to urinary retention, impaired kidney function, gross haematuria, bladder calculi, and recurrent urinary tract infections referred to as prostatitis.

Prostatitis is identified and characterized into acute bacterial prostatitis, persistent bacterial prostatitis, chronic prostatitis, and Chronic Pelvic Pain Syndrome (CPPS), and asymptomatic inflammatory prostatitis [1]. Acute and chronic bacterial prostatitis is both caused by bacterial infections in the prostate which are treated with antibiotics and supportive care [2, 3]. CPPS is characterized by urological discomfort symptoms in the absence of a urinary tract infection. Active urethritis, urogenital cancer, urinary tract disease, severe urethral stricture, or neurological disease affecting the bladder is excluded from this syndrome [4]. Asymptomatic inflammatory prostatitis is defined by the detection of prostatic inflammation without the presence of genitourinary symptoms [5]. This issue is discovered via a test for infertility or high Prostate-Specific Antigen (PSA) level in the prostate. This condition can cause an increase in white blood cells in the ejaculate (leukocytospermia) and male infertility.

Male infertility is responsible for about 40–50% of all infertility cases. It is characterized by low sperm production, abnormal sperm function, or blockages that prevent the delivery of sperm. Illnesses, injuries, chronic health problems, lifestyle choices, and other factors may also contribute to male infertility. Male infertility results in the inability to conceive a child which can be highly stressful and frustrating, but

some treatments are available for this male infertility. Environmental and occupational exposures to biological, physical, and chemical sources have an impact on male reproductive health [6]. These toxic sources threaten the male reproductive health, affecting it through cutaneous contact, inhalation, ingestion, and vertical or horizontal transmission [7].

Similar, modern lifestyle and long-term use of testosterone are causing enlarged prostate and decrease in sperm production. Benign prostatic hyperplasia and prostate cancer are also becoming very common. The adverse effect of this is increasing on daily basis, making it a health concern as cases of infertility are on the rise. Despite the prevalence of male infertility in Nigeria, not much effort has been made at tackling the problem; therefore, if adequate measures are not taken there will be an increasing impact of male factor infertility.

There are different effective treatments for BPH, including medications, minimally invasive therapies, and surgery. However, there is the need to investigate the ameliorating potential of some medicinal herbs as a natural and cost-effective remedy on testosterone propionate-induced benign prostatic hyperplasia in male Wistar rats. One such common medicinal plant with various health benefits is the leaf, fruit, seed, flower, bark, and root of *Annona muricata*[8]. Studies on *Annona muricata* show that it is antibactericidal, antiprotozoal, insecticidal, antistress, Larvicide, anxiolytic, selective cytotoxicity of tumoral cells, wound healing, anti-ulcer, hypoglycaemic, hepatoprotective, and antioxidant properties. However, the ameliorating potential of *Annona muricata* on testosterone propionate-induced benign prostatic hyperplasia in male rats is yet unknown and this necessitated the need for this study.

MATERIALS AND METHODS

Source and Preparation of Plant Sample

The fresh leaves and bark of *Annona muricata* were collected from Choba village, Rivers State, Nigeria. The plant was identified by Dr. Ekeke Chimezie of the Department of Plant Science Herbarium, Faculty of Science, University of Port Harcourt, and given the Voucher numbers UPH/V/1447. The fresh leaves of *Annona muricata* were washed with distilled water and then allowed to get dried in a dust-free environment for ten days. The dried leaves were blended using an electronic blender.

Source of Wistar rats

A total of 60 matured male Wistar rats of 13 weeks old weighing between 150 – 250 kg were used for this study. They were obtained from the Physiology Department animal house in the University of Port-Harcourt Rivers State. The rats were housed in conventional wire cages under standard laboratory conditions and allowed to acclimatize for two weeks before use. They were given standard feed and drinking water *ad libitum.*

Extraction of Plant Materials

The powdered material (300 g of *A. muricata*) was weighed and soaked in 650 ml distilled water and allowed to macerate at room temperature for 48 hours. The solution was filtered after 48 hours into a beaker using Whatman filter paper No 4. The suspension was filtered through Whatman filter paper No 4 and dried in water bath at 55°C. The dried extract was weighed and stored in a clean reagent bottle, then preserved in a refrigerator at 4 degrees Celsius until its use.

$$\%\ Recovery = \frac{crude\ extract\ obtained}{\text{Weight of grounded leaves}}\ X\ \frac{100}{1}$$
$$= \frac{10g}{300g}\ X\ \frac{100}{1} = 3.33\%$$

Aqueous concentration of 500 mg/kg, 1000 mg/kg, 1500 mg/kg and 2000 mg/kg of *A. muricata* extract was prepared using distill water and used as administrative doses.

Study design

The animals were weighed and divided into six (6) groups with ten (10) animals each. The six groups were: Group 1 (Normal control), Group 2 (negative control: induced benign prostatic hyperplasia for 28 days without treatment), Group 3 (administered 500 mg/kg of the aqueous extract of the *A. muricata*, Group 4 (administered 1000 mg/kg of the extract), Group 5 (administered 1500 mg/kg of the extract) and Group 6 (administered 2000 mg/kg of the extract). Benign prostatic hyperplasia was induced in Wistar rats using testosterone propionate for 28 days. Administration of extract was done for another 28 days, and then followed by accurate measurement of the changes. The animals were anesthetized using chloroform, and the blood samples of each animal were collected through the cardiac puncture method. The blood samples were aliquoted into the appropriate sample bottles (Plain and EDTA bottles), liver, kidney, and testes of Wistar rats were harvested into universal bottles containing formalin and transported to the laboratory.

Methods

Semen analysis was done using the WHO [9] manual procedures. Superoxide dismutase (SOD) and Malondialdehyde (MDA) were carried out according to the method of Usoh *et al.*, [10] lactate dehydrogenase (LDH) using the kinetic method [11]. For the liver function test, ALT, AST, and ALP were analyzed by kinetic methods kits from Randox (United Kingdom) using a double-beam spectrophotometer. Determination of Sodium and Potassium was done using a flame photometer as described by Chuang *et al*. [12] Urea, and Creatinine concentrations were analyzed using methods as described by Tietz [13] PSA and CEA were carried out using the sandwich enzyme-linked

immunosorbent assay (ELISA) kit (Boditech Med Incorporated, Republic of Korea), using the ichroma machine (Boditech: BOD13303, Korea). Hematology indices were done using the method described by Cheesbrough [14].

Histopathological examination

Histopathological slides were prepared at Anatomical Pathology Laboratory, University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, and Rivers State. The tissues were subjected to standard routine histological procedures as described by Kiernan [15].

STATISTICAL ANALYSIS

The results obtained were pooled and expressed as mean ± standard deviation. Triplicate reading was used for these calculations. Analysis of variance (for multiple comparisons) was used. Statistical Package for Social Science (SPSS) 22.0 of windows was used for the analysis. The significance level was taken at ($p < 0.05$).

RESULTS

Effect of aqueous leaf extract of *A. muricata* on semen parameters of testosterone propionate-induced benign prostatic hyperplasia in male rats

The results of the effect of aqueous leaf extract of *A. muricata* on semen parameters of testosterone propionate-induced benign prostatic hyperplasia in male rats are presented in Table 1. The semen volume of male rats in Groups 3 to 6 increased significantly ($p > 0.05$) when compared to the negative control. Groups 4 to 6 show a non-significant increase ($p > 0.05$) in viability, normal, activity, and sperm count compared to the negative control. Group 3 had a significant increase ($p > 0.05$) in viability (86.00 %), normal (86.00 %), activity (86.00 %) and sperm count (710.00 ml/10^6) compared to the negative control. Group 4 to 6 had a non-significant decrease ($p > 0.05$) in abnormal, Sluggish, and dead sperm cells analysed when compared to control while Group 3 had a significant decrease ($p > 0.05$) in abnormal (14.00 %), Sluggish (6.00 %), and dead (8.00 %) sperm cells compared to the negative control.

Effect of aqueous leaf extract of *A. muricata* on oxidative stress and hormone levels of testosterone propionate-induced benign prostatic hyperplasia in male rats

The results of the effect of aqueous leaf extract of *A. muricata* on oxidative stress and hormone levels of testosterone propionate-induced benign prostatic hyperplasia in male rats are presented in Table 2. Lactate Dehydrogenase (LDH) (u/l) of male rats in Groups 3 to 6 was non-significantly increased ($p > 0.05$) compared to the positive and negative control. Superoxide Dismutase (SOD) in Groups 3 to 6 was significantly decreased ($p > 0.05$) compared to the negative and the positive controls. Malondialdehyde (MDA) in Groups 3 to 6 was significantly increased ($p > 0.05$) when compared to the negative and the positive controls, while Group 5 showed a significant increase when compared to Groups 3, 4, and 6. Results showed that Follicle Stimulating Hormone (FSH) (miu/ml) of male rats in Groups 3 to 6 decreased significantly ($p > 0.05$) when compared to the positive and negative control. A significant increase ($p < 0.05$) in testosterone level was observed in Group 2. However, Groups 4 to 6 also showed a significant decrease ($p < 0.05$) in testosterone level compared to Group 2

Effect of aqueous leaf extract of *A. muricata* on hematological indices of testosterone propionate-induced benign prostatic hyperplasia in male rats

The results of the effect of aqueous leaf extract of *A. muricata* on hematological indices of testosterone propionate-induced benign prostatic hyperplasia in male rats presented in Table 4.5 showed that there was no significant difference ($p < 0.05$) in the PCV and HB levels of the test groups compared to the positive and negative control. The same non-significant decrease ($p < 0.05$) was obtained in PLT, MCH, MCV, MCH, RBC, Leucocyte, Neutrophils, Mesophiles, Basophils, and Eosinophils.

Table-1: Effect of aqueous leaf extract of *A. muricata* on semen parameters of testosterone propionate-induced benign prostatic hyperplasia in male rats

Groups	Volume (ml)	Viable (%)	Normal (%)	Abnormal (%)	Activity (%)	Sluggish (%)	Dead (%)	Sperm Count (ml x 10^6)
1	0.15 ± 0.05^{abdf}	82.50 ± 2.50^{abcdef}	75.00 ± 5.00^{abcdef}	25.00 ± 5.00^{abcdef}	67.50 ± 2.50^{abcdef}	10.00 ± 0.00^{abcdef}	22.50 ± 2.50^{abcdef}	450.00 ± 50.00^{abcdef}
2	0.10 ± 0.00^{ab}	68.33 ± 4.41^{abdef}	65.00 ± 2.89^{abdef}	35.00 ± 2.89^{abdef}	61.67 ± 1.67^{abdef}	11.67 ± 1.67^{abdef}	26.67 ± 3.33^{abdef}	300.00 ± 57.74^{abdef}
3	0.38 ± 0.02^{ce}	86.00 ± 1.87^{acef}	86.00 ± 1.87^{acde}	14.00 ± 1.87^{acde}	86.00 ± 1.87^{acde}	6.00 ± 1.00^{acde}	8.00 1.23^{acde}	710.00 ± 74.83^{acdef}
4	0.23 ± 0.05^{adef}	73.75 ± 5.54^{abdef}	73.75 ± 7.18^{abcdef}	26.25 ± 7.18^{abcdef}	71.25 ± 7.74^{abcdef}	8.75 ± 1.25^{abcdef}	20.00 ± 7.07^{abcdef}	537.50 ± 143.43^{abcdef}
5	0.30 ± 0.06^{cdef}	76.67± 4.41^{abcdef}	76.67 ± 4.41^{abcdef}	23.33 ± 4.41^{abcdef}	75.00 ± 7.64^{abcdef}	10.00 ± 2.89^{abcdef}	15.00 ± 5.00^{abcdef}	550.00 ± 104.08^{abcdef}
6	0.22 ± 0.04^{adef}	75.00 ± 4.47^{abcdef}	73.00 ± 5.39^{abdef}	27.00 ± 5.39^{abdef}	67.00 ± 6.44^{abdef}	11.00 ± 1.87^{abdef}	22.00 ± 4.90^{abdef}	510.00 ± 112.25^{abcdef}

Values are presented as mean ± SD of triplicate determinant (n = 3). Mean values with same superscript letters along the column are not statistically significant at $p < 0.05$

Table-2: Effect of aqueous leaf extract of *A. muricata* on oxidative stress and hormone levels of testosterone propionate-induced benign prostatic hyperplasia in male rats

GROUPS	LDH (u/l)	SOD (u/l)	MDA (u/l)	FSH (miu/ml)	TESTOSTERONE (ng/ml)
1	34.40 ± 7.90^{abcdef}	0.67 ± 0.12^{ab}	0.37 ± 0.06^{ab}	3.00 ± 0.40^{ab}	2.30 ± 0.30^{abcdef}
2	22.20 ± 0.50^{abcdef}	0.69 ± 0.01^{ab}	0.31 ± 0.02^{ab}	3.27 ± 0.24^{ab}	2.83 ± 0.20^{abcef}
3	22.15 ± 2.15^{abcdef}	0.29 ± 0.04^{cdef}	0.59 ± 0.02^{cdf}	1.98 ± 0.31^{cdef}	2.12 ± 0.20^{acf}
4	23.10 ± 2.90^{abcdef}	0.29 ± 0.01^{cdef}	0.62 ± 0.02^{cdf}	1.80 ± 0.24^{cdef}	1.68 ± 1.14^{de}
5	24.40 ± 0.80^{abcdef}	0.18 ± 0.01^{cde}	0.72 ± 0.00^{e}	1.47 ± 0.23^{cdef}	1.97 ± 0.29^{cef}
6	30.75 ± 4.05^{abcdef}	0.37 ± 0.06^{cde}	0.55 ± 0.04^{cdf}	1.80 ± 0.11^{cdef}	2.08 ± 0.15^{ac}

Values are presented as mean ± SD of the triplicate determinant (n = 3). Mean values with the same superscript letters along the column are not statistically significant at $p < 0.05$. **LDH** - Lactate Dehydrogenase, **SOD** - Superoxide Dismutase, **MDA** - Malondialdehyde, **FSH** - Follicle Stimulating Hormone

Effect of aqueous leaf extract of *A. muricata* on cardiac and cancer markers (ng/ml) of testosterone propionate-induced benign prostatic hyperplasia in male rats

The results of the effect of aqueous leaf extract of *A. muricata* on cardiac markers (ng/ml) of testosterone propionate-induced benign prostatic hyperplasia in male rats presented in Table 4 showed a non-significant decrease ($p < 0.05$) of CKMB concentration in Groups 3 to 6. D-DIMER concentration showed a non-significant increase ($p < 0.05$) in Groups 3 to 6. Myoglobin concentration showed a non-significant decrease ($p < 0.05$) in Groups 3 and 6, and a non-significant increase ($p < 0.05$) in Groups 4 and 5. Prostate-Specific Antigen (PSA) (ng/ml) of male rats in Groups 3 to 6 show a non-significant decrease ($p > 0.05$) when compared to the positive and the negative control. Similarly, there was a non-significant decrease ($p > 0.05$) in the level of Carcinoembryonic antigen of rats in Groups 3 to 6 when compared to the positive and negative controls.

Effect of aqueous leaf extract of *A. muricata* on liver markers of testosterone propionate-induced benign prostatic hyperplasia in male rats

Table 5 presents results of the effect of aqueous leaf extract of *A. muricata* on the liver of testosterone propionate-induced benign prostatic hyperplasia in male rats. There was a significant decrease ($p < 0.05$) in ALP concentration in Groups 3 to 6 of male rats compared to the negative control. The AST concentration had a significant increase ($p < 0.05$) in Groups 3 and 4 and a non-significant increase in Groups 5 and 6 when compared to the negative control. Results of ALT concentration showed a significant increase ($p < 0.05$) in Groups 3 and 4, and a non-significant decrease ($p > 0.05$) in Groups 5 and 6 compared to the negative control. Total-Protein concentration showed a non-significant increase ($p > 0.05$) in Groups 3 and 4, a significant increase in Groups 5, and a non-significant decrease in Group 6 compared to the negative control. Albumin concentration in Groups 3 and 4 showed a non-significant decrease ($p > 0.05$). However, there was a non-significant increase in Groups 5 and 6 when compared to the negative control.

Effect of aqueous leaf extract of *A. muricata* on the kidney of testosterone propionate-induced benign prostatic hyperplasia in male rats

The results of the Effect of aqueous leaf extract of *A. muricata* on the kidney of testosterone propionate-induced benign prostatic hyperplasia in male rats presented in Figure 1 shows a non-significant increase ($p > 0.05$) in creatinine and urea concentration in Groups 3 to 6 of male rats compared to the negative control. There was a non-significant increase ($p > 0.05$) in potassium concentration in Groups 3 to 4 compared to the negative control, and a non-significant decrease ($p > 0.05$) in potassium concentration in Groups 5 to 6. Results showed a non-significant decrease ($p > 0.05$) in sodium concentration in Groups 3 to 6 compared to the negative control.

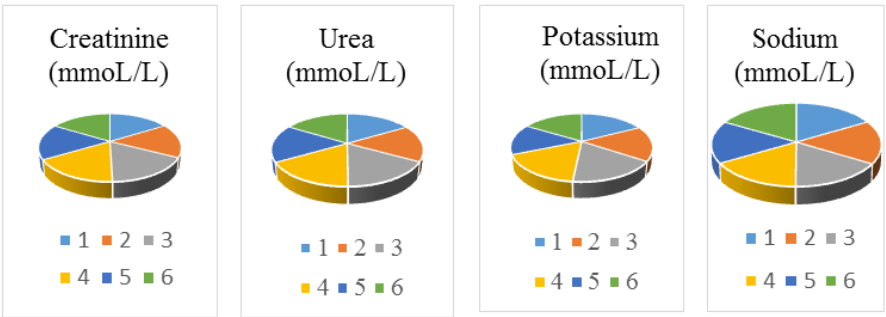

Fig-1: Effect of aqueous leaf extract of *A. muricata* on the kidney of testosterone propionate-induced benign prostatic hyperplasia in male rats from Groups 1-6.

Table-3A: Effect of aqueous leaf extract of *A. muricata* on haematological indices of testosterone propionate-induced benign prostatic hyperplasia in male rats

GROUPS	PCV (%)	HB (g/dl)	WBC (x 10^9/L)	PLT (x 10^9/L)	MCV (FL)	MCH (Pg)	MCHC (g/dL)
1	45.00 ± 3.00^{abcdef}	15.50 ± 0.50^{abcdef}	9.10 ± 0.90^{abcd}	383.50 ± 63.50^{abcdef}	53.35 ± 0.45^{abcdef}	17.80 ± 0.20^{abcdef}	33.35 ± 0.15^{abcdef}
2	42.50 ± 3.50^{abcdef}	14.15 ± 1.15^{abcdef}	10.90 ± 4.10^{abcdf}	482.00 ± 266.00^{abcdef}	53.30 ± 0.90^{abcdef}	17.60 ± 0.60^{abcdef}	33.23 ± 0.52^{abcdef}
3	46.50 ± 1.50^{abcdef}	15.50 ± 0.50^{abcdef}	13.10 ± 0.70^{abcdf}	376.00 ± 128.00^{abcdef}	53.42 ± 0.67^{abcdef}	17.68 ± 0.46^{abcdef}	32.62 ± 0.68^{abcdef}
4	43.50 ± 0.50^{abcdef}	14.45 ± 0.15^{abcdef}	16.90 ± 1.50^{abcdef}	353.00 ± 157.00^{abcdef}	53.45 ± 0.27^{abcdef}	16.83 ± 0.85^{abcdef}	32.58 ± 0.94^{abcdef}
5	42.50 ± 4.50^{abcdef}	14.05 ± 1.45^{abcdef}	23.30 ± 0.10^{def}	529.00 ± 109.00^{abcdef}	53.83 ± 0.97^{abcdef}	15.93 ± 0.72^{abcdef}	33.93 ± 0.47^{abcdef}
6	44.00 ± 2.00^{abcdef}	14.65 ± 0.65^{abcdef}	18.35 ± 4.05^{bcdef}	503.00 ± 59.00^{abcdef}	51.38 ± 1.28^{abcdef}	17.80 ± 0.39^{abcdef}	33.48 ± 0.49^{abcdef}

Values are presented as mean ± SD (n = 3). Mean values with same superscript letters along the column are not statistically significant at p < 0.05. ***PCV*** *- Packed Cell Volume,* ***HB*** *– Hemoglobin, WBC – White blood cell-*, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin content

Table-3B: Effect of aqueous leaf extract of *A. muricata* on haematological indices of testosterone propionate-induced benign prostatic hyperplasia in male rats

GROUPS	RBC (x 10^{12}/L)	N	L	M	E	B
1	8.55 ± 0.67^{abcdef}	62.00 ± 2.00^{abcd}	33.50 ± 3.50^{abcde}	2.50 ± 0.50^{abcdf}	4.50 ± 0.50^{abdef}	0.00 ± 0.00^{abcdef}
2	7.62 ± 0.64^{abcdef}	61.00 ± 2.00^{abcd}	35.00 ± 0.00^{abcde}	2.50 ± 0.50^{abcdf}	4.00 ± 0.00^{abcdef}	0.00 ± 0.00^{abcdef}
3	7.24 ± 0.40^{abcdef}	64.50 ± 5.50^{abc}	39.00 ± 1.00^{abcde}	2.50 ± 1.50^{abcdf}	1.50 ± 1.50^{abcdef}	0.00 ± 0.00^{abcdef}
4	7.56 ± 0.67^{abcdef}	50.50 ± 4.50^{abde}	53.50 ± 3.50^{abcde}	2.50 ± 0.50^{abcdf}	2.00 ± 0.00^{abcdef}	0.00 ± 0.00^{abcdef}
5	8.36 ± 0.68^{abcdef}	46.00 ± 2.00^{de}	36.00 ± 1.00^{abcde}	7.50 ± 1.50^{ef}	2.00 ± 0.00^{abcdef}	0.00 ± 0.00^{abcdef}
6	7.50 ± 0.59^{abcdef}	25.00 ± 5.00^{f}	67.50 ± 7.50^{abcdef}	5.50 ± 1.50^{abcdef}	2.00 ± 1.00^{abcdef}	0.00 ± 0.00^{abcdef}

Values are presented as mean ± SD of the triplicate determinant (n = 3). Mean values with the same superscript letters along the column are not statistically significant at p < 0.05.

Table-4: Effect of aqueous leaf extract of *A. muricata* on cardiac and cancer markers of testosterone propionate-induced benign prostatic hyperplasia in male rats

GROUPS	CKMB (ng/mL)	D-DIMER (ng/mL)	MYOGLOBIN (ng/mL)	PSA (ng/ml)	CEA (ng/ml)
1	2.70 ± 0.10^{abcdef}	50.20 ± 9.90^{abcdef}	7.10 ± 0.80^{abcdef}	1.60 ± 0.60^{abcd}	1.95 ± 0.15^{adf}
2	2.87 ± 0.43^{abcdefs}	33.67 ± 8.74^{abcdef}	7.43 ± 1.43^{abcdef}	1.23 ± 0.09^{abcdef}	0.93 ± 0.41^{bcdef}
3	2.84 ± 0.39^{abcdef}	47.44 ± 9.79^{abcdef}	5.74 ± 0.37^{abcf}	1.12 ± 0.06^{abcdef}	0.82 ± 0.15^{bcd}
4	2.75 ± 0.50^{abcdef}	42.88 ± 11.15^{abcdef}	7.73 ± 0.37^{abde}	1.15 ± 0.10^{abcdef}	1.08 ± 0.14^{bcdef}
5	2.17 ± 0.32^{abcdef}	53.80 ± 6.70^{abcdef}	7.83 ± 1.03^{abde}	1.07 ± 0.09^{bcdef}	1.50 ± 0.21^{abdef}
6	2.66 ± 0.40^{abcdef}	46.58 ± 2.97^{abcdef}	5.70 ± 0.45^{abcf}	0.92 ± 0.10^{bcdef}	1.48 ± 0.19^{abdef}

Values are presented as mean ± SD of the triplicate determinant (n = 3). Mean values with the same superscript letters along the column are not statistically significant at p < 0.05. CKMB - Creatine kinase-MB, **PSA** - Prostate-Specific Antigen, **CEA** - Carcinoembryonic Antigen

Table-5: Effect of aqueous leaf extract of *A. muricata* on liver markers of testosterone propionate-induced benign prostatic hyperplasia in male rats

GROUPS	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	T-PROTEIN (g/L)	ALBUMIN (g/L)
1	272.85 ± 10.75^{ab}	66.50 ± 10.50^{abdef}	53.00 ± 4.00^{abdef}	69.95 ± 0.65^{abcdef}	38.75 ± 0.55^{abcdef}
2	279.03 ± 0.88^{ab}	65.33 ± 4.18^{abef}	50.00 ± 1.73^{abef}	66.53 ± 2.34^{abcdf}	42.17 ± 1.37^{abcdef}
3	144.40 ± 4.88^{cef}	87.20 ± 6.59^{cd}	74.40 ± 6.70^{cd}	68.12 ± 2.39^{abcdf}	41.18 ± 1.00^{abcdef}
4	165.18 ± 8.74^{df}	82.50 ± 2.66^{acde}	68.50 ± 2.33^{acd}	69.43 ± 1.90^{abcdf}	40.70 ± 1.09^{abcdef}
5	139.97 ± 7.91^{cef}	68.33 ± 3.28^{abdef}	48.33 ± 2.73^{abef}	76.27 ± 1.21^{ae}	44.33 ± 1.21^{abcdef}
6	149.80 ± 8.09^{cdef}	65.80 ± 2.48^{abef}	45.80 ± 2.97^{abef}	66.44 ± 1.66^{abcdf}	43.18 ± 2.13^{abcdef}

Values are presented as mean ± SD of the triplicate determinant (n = 3). Mean values with the same superscript letters along the column are not statistically significant at p < 0.05. ***AST****=Aspartate aminotransferase,* ***ALT****=Alanine Aminotransferase,* ***ALP****= Alkaline Phosphatase*

Histology of testis section of male rats treated with aqueous leaf extract of *A. muricata* of testosterone propionate-induced benign prostatic hyperplasia x400

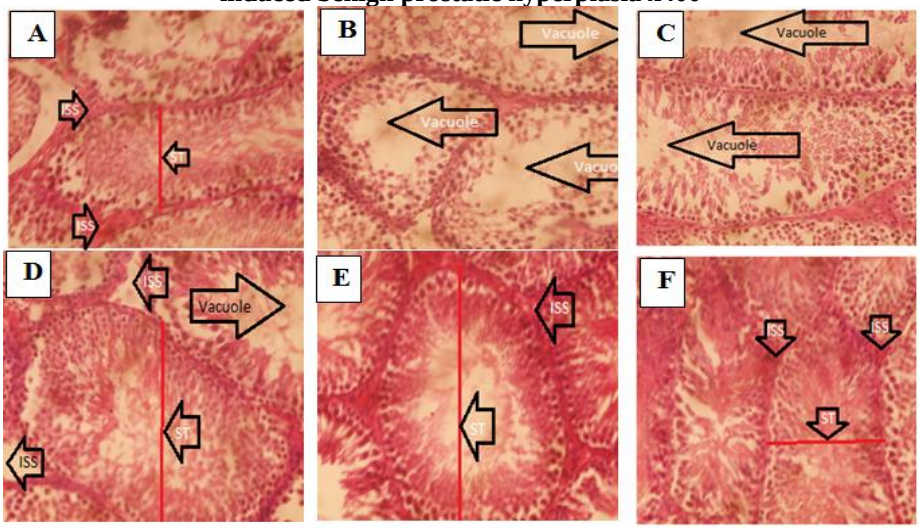

Plate 1: Photomicrographs of testis sections of rats from A: Group 1 (positive control) fed with regular feeds and water. Normal testes with somniferous tubules containing spermatogenic cells and spermatozoa. B: Group 2 (negative control) Showing distorted testes. Features include vacuolated seminiferous tubules. Terminal boards are empty resulting from disruption of mature spermatozoa. Interstitial spaces are smaller containing leydig cells. C: Group 3 shows normal testes with seminiferous tubules containing spermatogenic cells and spermatozoa. D: Group 4: showing stained interstitial spaces, and normal interstitial cells of Leydig. E: Group 5: Normal testes showing seminiferous tubule containing spermatogenic cells and spermatozoa. F: Group 6: Stained interstitial spaces are normal containing interstitial cells of Leydig.

DISCUSSION

Evaluation of aqueous leaf extract of *A. muricata* on the semen of testosterone propionate-induced benign prostatic hyperplasia in male rats revealed that *A. muricata* has no toxic effect on testes of male rats, corroborating the findings of Amadi *et al.* [16] who investigated the potential ameliorative effects of *A. muricata* (linn) on sodium fluoride-induced toxicity on hematological indices and fecundity of adult male Wistar rats, and discovered that leaf extracts of *A. muricata* caused an increase ($p<0.05$) in epididymal sperm count, sperm motility, and live spermatozoa along with a simultaneous decrease in dead spermatozoa as compared to the rats of the group treated with Sodium fluoride alone. *A. muricata* is antioxidative, anticancer, antimalarial, antihelmintic, piscicidal, antiviral, and antibacterial properties, implying a wide range of possible applications. *Asimicin*, *bullatacin*, and bullatalicin are three significant acetogenins found in ripe *A. muricata* pulp extract [17, 18].

Hormonal level of testosterone propionate-induced benign prostatic hyperplasia in male rats administered with aqueous leaf extract of *A. muricata* showed a significant decrease ($p > 0.05$) in FSH levels for male rats in Groups 3 to 6 treated with *A. muricata* compared to the negative control. This agrees with the study of Amadi *et al.* [16] who investigated the potential ameliorative effects of aqueous leaf extract of *A. muricata*. There was a decrease in testosterone and FSH concentrations in Groups 4 to 6 compared to the negative control. This decrease in FSH may be due to *A. muricata* toxic effect on the anterior pituitary gland, causing oxidative damage to the anterior pituitary gland's architecture, responsible for the generation of FSH (glycoprotein hormone) and luteinizing hormone. The decline in testosterone levels in groups administered with aqueous leaf extracts of *A. muricata* suggested that its phytochemical composition may reduce the effect of the pituitary gland and testosterone synthesis [19].

The effect of aqueous leaf extract of *A. muricata* on Oxidative stress of testosterone propionate-induced benign prostatic hyperplasia in male rats had a non-significant increase ($p > 0.05$) in Lactate Dehydrogenase (LDH) activity in Groups 3 to 6 compared to the positive and negative control. Superoxide Dismutase (SOD) concentration in Groups 3 to 6 was significantly decreased ($p > 0.05$) compared to negative and positive control. Malondialdehyde (MDA) in Groups 3 to 6 was significantly increased ($p > 0.05$) compared to the negative and positive control, and Group 5 had a significant increase compared to Groups 3, 4, and 6. This is in agreement with the findings of Agu *et al.* [20] who worked on "protective effect of ethanol extract of soursop (*annona muricata* linn) leaves on cycad induced oxidative stress in male albino wistar rats", and discovered an increase in LDH and MDA concentration in rats administered with *A. muricata* compared to that of the negative control, and

that the SOD concentration decreased compared to that of the negative control. The observed increase in LDH concentration of Groups 3 to 6 compared to negative control is owing to tissue damage produced by rats' exposure to testosterone propionate, a well-known toxin as described above. Malondialdehyde (MDA) is a measure of polyunsaturated lipid peroxidation caused by reactive oxygen radicals (ROS). These lipids are essential components of biological membrane structures. Membrane lipids are predominantly attacked by free radicals, resulting in their peroxidation [21]. This causes membrane instability, breakdown of membrane proteins and other macromolecules, and cellular damage [22]. The increased levels of oxidative stress caused by testosterone propionate were reduced in rats administered with *A. muricata.* This reduction was via the reduction in levels of SOD, LDH, and MDA in the rats, supporting the findings of Lolodi and Eriyamremu [23] who found a decrease in SOD activity in rats given Cycads.

There was a non-significant decrease ($p > 0.05$) in PSA level in male rats of Groups 3 to 6 compared to the positive and the negative control. Testosterone propionate-induced benign prostatic hyperplasia is a pointer to the ameliorative impact of testosterone propionate-induced benign prostatic hyperplasia. As a direct result of inhibiting 5-reductase, a decrease in PSA levels is linked to the reduction in prostatic hyperplasia [24] Furthermore, the fact that the PSA level in the negative control group remained high indicates that the observed decline in PSA in treated rats was due to the ameliorative effect of *A. muricata*. This corroborates the findings of Ogbu *et al.* [25] who discovered dutasteride and acetogenin-rich fraction isolated from *Annona muricata* leaves reduced the levels of PSA in rats with testosterone propionate-induced benign prostatic hyperplasia.

Carcinoembryonic Antigen (CEA) of rats in Groups 3 to 6 revealed a non-significant increase ($p > 0.05$) compared to the negative control, and with Group 3 having a significant decrease ($p > 0.05$) compared to Groups 5 and 6. Rajesh and Kala [26] reported a significant decrease in CEA levels in animals administered with benzo [a] pyrene and treated with leaf extract of *A. muricata* showed compared to benzo[a]pyrene induced untreated animals.

The Effect of aqueous leaf extract of *A. muricata* on hematological indices of testosterone propionate-induced benign prostatic hyperplasia in male rats showed normal hematology indices in the test groups when compared to the negative control. Previous studies have shown that some plant extracts including leaf extract of *A. muricata* have the potential to positively affect the erythropoietic system [16, 26, 27, 28].

The effect of aqueous leaf extract of *A. muricata* on cardiac markers of testosterone propionate-induced benign prostatic hyperplasia in male rats revealed there was a non-significant decrease ($p < 0.05$) of CKMB concentration in Groups 3 to 6 compared to that of the negative control. D-DIMER concentration had a non-significant increase ($p < 0.05$) in Groups 3 to 6 compared to the negative control. Myoglobin concentration had a non-significant decrease ($p < 0.05$) in Groups 3 and 6, and a non-significant increase ($p < 0.05$) in Groups 4 and 5. The result of CKMB, D-DIMER, and Myoglobin from this study indicates the aqueous leaf extract of *A. muricata* has no deleterious effect on the functional integrity of the cardiac system. This corroborates the findings of Niu *et al.* [29] who worked on "Hyperbaric oxygen improves survival in heatstroke rats by reducing multiorgan dysfunction and brain oxidative stress" and discovered there was an increase of D-dimer concentration in heatstroke rats.

The liver which is responsible for xenobiotic metabolism and detoxification is also susceptible to hepatotoxic chemicals [30]. The effect of aqueous leaf extract of *A. muricata* on the liver of testosterone propionate-induced benign prostatic hyperplasia in male rats revealed a significant decrease in ALP concentration in Groups 3 to 6 of male rats compared to the negative control, which agrees with the study of Syahida *et al.* [28] on "Soursop (*Anonna muricata*): Blood hematology and serum biochemistry of Sprague-Dawley rats" who discovered ALP concentration in rats treated with *A. muricata* was significantly decreased compared to their negative control. The AST concentration was significantly increased ($p > 0.05$) in Groups 3 and 4 and Groups 5 and 6 showed a non-significant increase compared to the negative control. The result showed that ALT concentration was significantly increased ($p > 0.05$) in Groups 3 and 4. However, there was a non-significant decrease ($p < 0.05$) in Groups 5 and 6 compared to the negative control. T-Protein had a non-significant increase ($p > 0.05$) in Groups 3 and 4, a significant increase in Group 5, and a non-significant decrease in group 6 compared to the negative control. Albumin concentration in Groups 3 and 4 had a non-significant decrease ($p < 0.05$) and a non-significant increase in Groups 5 and 6 compared to the negative control. This liver-function investigation revealed that aqueous leaf extract of *A. muricata* has no acute hepatotoxic effect. The enzymes AST and ALT are crucial in the breakdown of amino acids channeled into the Krebs cycle and electron transport chain [31] Because ALT and ALP are concentrated in the liver, they are considered accurate indicators of liver disease [27, 32]. Changes in membrane-bound ALP negatively impact membrane permeation, disrupting the transportation of metabolites [31]. When the liver is diseased, these enzymes are released into the system in excess of a critical concentration [33].

Renal dysfunction was investigated by checking the concentration of urea, creatinine, potassium, and sodium in the serum. According to Edmund and David [32] these markers occur above normal in the blood serum of patients with dysfunctional kidneys. The effect of aqueous leaf extract of *A. muricata* on kidneys of testosterone propionate-induced benign prostatic hyperplasia in male rats revealed a non-significant difference ($p > 0.05$) in urea, creatinine, sodium, and potassium in group 3 – 6 when compared to the positive and negative controls. The renal function investigation on groups treated with aqueous leaf extract of *A. muricata* compared to that of the negative control revealed a non-toxic effect on kidney and renal function. Onyegeme-Okerenta *et al.* [34] in their study on the ameliorating potential of *Annona muricata* on sodium Fluoride-induced toxicity on lives and kidneys of male Wistar rats concluded that concomitant treatment of NaF leaf extract of *Annona muricata* resulted in mild/moderate amelioration and generation of damaged hepatic and renal tissues.

CONCLUSION

The results obtained in this study showed that the aqueous leaf extract of *A. muricata* administered over an extended period is relatively safe with a tendency to ameliorate testosterone propionate-induced benign prostatic hyperplasia in male rats. The aqueous leaf extract of *A. muricata* on testosterone propionate-induced benign prostatic hyperplasia in male rats revealed increased sperm count, volume, normality, and viability, reducing the number of malformed, sluggish, and dead sperm cells. *A. muricata* has the potential to reduce to normal the sex hormones (Follicle Stimulating Hormone and Testosterone) in male Wistar rats. It also, has a positive effect on spermatogenesis and therefore, can boost semen quality. The administration of aqueous leaf extract of *A. muricata* also showed ameliorating potential on hematological indices, cardiac, kidneys, and cancer markers.

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Data accessibility statement

All relevant data are within the paper.

Conflicts of interest

The Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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