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Xylopia aethiopica Attenuates Oxidative Stress and Hepatorenal Damage in Testosterone Propionate-Induced Benign Prostatic Hyperplasia in Rats

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Abstract

Objectives *Xylopia aethiopica* (XAE), commonly known as African pepper or Ethiopian pepper, is a plant native to West Africa and known for its aromatic and medicinal properties. It was used to investigate the antioxidative, antihepatotoxic, and antinephrotoxic potentials of XAE in benign prostatic hyperplasia (BPH) in Wister albino rats.
Methods The proximate, and vitamin composition, oxidative stress indicators, and indices of kidney and hepatic functions were performed by standard methods.
Results The proximate composition of the XAE leaf showed varied concentrations of Mg, Ca, Na, Zn, Se, and Cl, as well as vitamins A, E, B3, D, C, K, B2, and Bi. The activities of catalase glutathione, superoxide dismutase, malondialdehyde levels, K, Na, Cl^-, urea, uric acid, and creatinine in the kidney were increased in testosterone propionate (Tp)-induced BPH compared with the control groups. Total protein levels significantly decreased in Tp-induced BPH compared with XAE-treated groups increased on XAE treatment. The aspartate transaminase, alanine aminotransferase, and alkaline phosphatase activities were not significantly different in Tp-induced BPH, XAE, and normal controls.
Conclusion The study revealed that XAE can be used in the management of oxidative stress and hepatorenal damage in BPH condition.

Keywords
- benign prostatic hyperplasia
- *Xylopia aethiopica*
- oxidative stress
- hepato-renal damage
- minerals

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Introduction

Benign prostatic hyperplasia (BPH) is a common condition that affects older men, involving the noncancerous enlargement of the prostate gland. It is believed to be caused by hormonal changes, such as an increase in dihydrotestosterone levels, as well as genetics and lifestyle. It can cause bothersome urinary symptoms.[1] Oxidative stress has been suggested to play a role in the development and progression of BPH, and it may also contribute to hepatorenal implications associated with the condition. The liver and kidneys are important organs for detoxification and elimination of reactive oxygen species and their by-products.[2] However, excessive oxidative stress can overwhelm their antioxidant defense mechanisms, leading to hepatic and renal damage.[2] Studies have shown that patients with BPH have increased levels of liver enzymes, such as alanine aminotransferase and aspartate aminotransferase, indicating liver dysfunction. In addition, BPH has been associated with an increased risk of chronic kidney disease and renal dysfunction, possibly due to oxidative stress and inflammation.[2]

Xylopia aethiopica (XAE) is a plant commonly found in West Africa and is known for its medicinal properties.[3] Tall, slender, aromatic, and evergreen, *XAE* is of the family Annonaceae, and can reach heights of 15 to 30 m with a diameter of 60 to 70 cm.[4] The plant naturally grows in Savanna region of Africa.[4] The fruit of *XAE*, sometimes known as "Guinea pepper" or "Negro pepper," has a variety of recognized uses in folk medicine.[4] It has been traditionally used to treat various ailments such as malaria, fever, and gastrointestinal disorders.[5,6] Recent studies have shown that *XAE* has unique properties that make it useful in the management of metabolic complications.[7] The antioxidant properties of *XAE* are based on the rich presence of antioxidants, such as flavonoids and phenolic compounds.[7] These antioxidants help to scavenge free radicals and prevent oxidative stress, which can lead to various diseases, including cancer, diabetes, and cardiovascular diseases.

XAE has earlier been used in the management of BPH.[3] *XAE* has anti-inflammatory properties, which help to reduce inflammation in the body.[7] Chronic inflammation is associated with various diseases including cancer, diabetes, and cardiovascular disease.

Oxidative stress is linked to the development and progression of BPH, and this research has identified antioxidative and hepatorenal protective effects of *XAE* in animal models.

Methodology

Reagents and Chemicals

Chemicals and reagents used were from Sigma-Aldrich, Span Diagnostics, UWI, MONZA, Plasmatec and Elab Science.

Collection of *Xylopia aethiopica*

The leaves of *XAE* were harvested from the wild in the early hours of May 9, 2022 at the onset of wet season at 8.30 AM at Enugu State, Nigeria's Obukpa, Nsukka, Igboeze South Local Government Area. Prof. J.C. Okafor verified the harvested *XAE* leaves, and it was then processed into fine powder before sieving after drying at room temperature varying between 25 and 29°C. The extract was then filtered and concentrated using a rotary evaporator for 3 days at 65°C. The yield of the extract was 15% of the original sample used for extraction. The product was stored in a refrigerator until use. The time and season of plant leaf collection reported in this study are vital for accurate interpretation of data, understanding the effects of environmental factors on biological processes, and ensuring the reproducibility and reliability of scientific studies in plant biology.

Procurement of Drugs and Chemicals

Renhoks Pharmaceuticals supplied testosterone propionate (Tp) and finasteride of analytical grade.

Proximate Analysis of Plant Extract

The following proximate analyses were performed using standard procedures.

The Association of Official Agricultural Chemists (AOAC) method was used to determine crude fat, and crude fat content was obtained using gravimetric measurement of N-hexane or petroleum ether.[8] Moisture content was determined by the method of Aguinaldo et al.[9] Ash was determined using the method of Harbone.[10] However, crude proteins were determined by the method of Hussain et al.[11] Crude fiber was determined according to the method of Alberts et al.[12] The gravimetric method was used to determine the percent of indigestible carbohydrate in a sample. However, Clegg's method was used to calculate total carbohydrate, which involved perchloric acid digestion and colorimetric measurement of soluble sugars using the Anthronic method of Clegg.[13] The total available glucose was determined as % of glucose.

Determination of Vitamins Content in *X. aethiopica*

Fat-soluble and water-soluble soluble vitamins were determined using standard procedures following the methods as listed below:

Vitamin D was estimated by the modified method of AOAC,[8] vitamin A according to the method of Kumura and Itokawa,[14] tocopherol (vitamin E) following the method of Jargar et al,[15] ascorbic acid (vitamin C) and thiamine (vitamin B1) to the method of AOAC,[16] riboflavin (vitamin B2), and phylloquinone (vitamin K) as described in AOAC,[17] vitamin B3 and B6 according to the method of Zhang et al.[18]

Determination of Mineral Content

The mineral content of *XAE* was determined using the method described by AOAC.[16] The atomic absorption spectrophotometer quantitatively measured the concentration of elements present in the liquid sample. *XAE* extract was placed into a crucible and incinerated in a muffle furnace. After boiling, it was cooled and filtered into a 200 mL volumetric flask. The absorbance values of the minerals were recorded and the percentage of elements in the extract was calculated.

Experimental Animals

A total of 200 male Wistar albino rats were procured from the animal house at the Department of Biochemistry, University of Nigeria, Nsukka. The rats that ranged in weight from 250 to 400 g were 16 weeks old. They were kept in regular laboratory conditions and had unrestricted access to food and water.

Experimental Design and Animal Treatment

Ibiam et al[3] studied how *XAE* can modulate Tp)-induced BPH in rats. The study was performed in phases as shown in ►**Table 1**. In this phase, the rats were acclimatized for 2 weeks and they were divided into 12 groups with 12 rats in each group. They were weighed weekly during the experiment. Group A1 served as control and they received only the vehicle, olive oil. Tp, 14 mg kg^{-1} body weight, was administered intraperitoneally daily to group A2 for 4 weeks to induce BPH. For preventive studies Finasteride (F), a potent and specific 5α-reductase inhibitor, 10 mg kg^{-1} was administered along with Tp (14 mg kg^{-1}) for 8 weeks to group B_1 rats. Groups B_2, B_3, B_4, and B_5 were pretreated with ethanolic extract of *XAE* doses of 100, 200, 300, and 400 mg kg^{-1} respectively for four weeks. It was followed by Tp(14 mg kg^{-1}) along with (XAE), doses of 100, 200, 300, and 400 mg kg^{-1} treatments for 8 weeks.

In the curative studies, Tp(14 mg kg^{-1}) was administered to group C_1 for 4weeks to induce BPH after which F (10 mg kg^{-1}) was given for 8 weeks. BPH was induced in group C_2, C_3, C_4, and C_5 with Tp(14 mg kg^{-1}) for 4 weeks after which (XAE) doses of 500, 600, 700, and 800 mg kg^{-1} treatments for 8 weeks. They were all fed with water and feeds.[3]

Preparation of Tissue Homogenate

Rats were sacrificed and the prostates were washed, blotted, and weighed. Ten percent of the homogenate was prepared in a 0.05M phosphate buffer and centrifuged. The supernatant was used to measure oxidative stress indicators.

Oxidative Stress Indicators

The superoxide dismutase activity was determined by the method of McCord and Firdorick.[19] The catalase activity was determined by the method of Beers and Sizer.[20] The reduced glutathione in the tissue was determined by the method of Moron et al.[21] The malondialdehyde of the tissue was determined as earlier reported by Udeozor et al.[22]

Renal Function Tests

The following renal function tests were performed.

Serum urea was determined using the diacetyl monoxide urea method described by Wybenga.[23] Serum creatinine was determined by the alkaline picrate creatinine method described by Husdan and Rapoport.[24] The potassium ion was determined using the method of sodium tetraphenyl boron described by Chessbrough.[25] The concentration of Na^{+} was determined by the method of Chessbrough.[25] The concentration of chloride ion (Cl^{-}) was determined by the method of Chessbrough.[25] The carbon dioxide content was estimated by a method of Forester et al.[26]

Liver Function Indices

The following liver function parameters were performed. Serum protein was determined by means of biuret reaction as described by Sánchez et al.[27] Aspartate transaminase (AST) also known as serum glutamate oxaloacetate transaminase activity was determined by the method of Huang et al.[28] Alanine transaminase (ALT) also called serum glutamate pyruvic transaminase activity was determined by the method of Huang et al.[28] Alkaline phosphatase (ALP) activity was determined by the method of Talib and Khurana[29]

Table 1 Animal grouping and treatment

Group	Pre-treatment/Inducement	Treatment	Purpose
A1		Olive oil, vehicle only	Control
A2	Inducement of BPH (4 weeks) Tp (14 mg kg^{-1})		
B1		Tp(14 mg kg^{-1}) + F(10 mg kg^{-1})	Treatment with standard drug
B2	Pretreatment with *XAE* (4 weeks) (*XAE*) 100 mg kg^{-1}	Tp (14 mg kg^{-1}) + (*XAE*) 100 mg kg^{-1}(8 weeks)	Preventive studies
B3	(*XAE*) 200 mg kg^{-1}	Tp (14 mg kg^{-1}) + (*XAE*) 200 mg kg^{-1}	“
B4	(*XAE*) 300 mg kg^{-1}	Tp (14 mg kg^{-1}) + (*XAE*) 300 mg kg^{-1}	“
B5	(*XAE*) 400 mg kg^{-1}	Tp (14 mg kg^{-1}) + (*XAE*) 400 mg kg^{-1}	“
	Inducement of BPH (4 weeks)	**Treatment 8 weeks**	**Curative potential**
C1	Tp (14 mg kg^{-1})	F(10 mg kg^{-1})	Treatment with standard drug
C2	Tp(14 mg kg^{-1})	(*XAE*) 500 mg kg^{-1}	Curative studies
C3	Tp (14 mg kg^{-1})	(*XAE*) 600 mg kg^{-1}	“
C4	Tp (14 mg kg^{-1})	(*XAE*) 700 mg kg^{-1}	“
C5	Tp (14 mg kg^{-1})	(*XAE*) 800mg kg^{-1}	“

Abbreviations: BPH, benign prostatic hyperplasia; F, finasteride; Tp, testosterone propionate; XAE, *Xylopia aethiopica* ethanol extract.

Table 2 Proximate composition, vitamin and mineral content of ethanolic leaf extract of *Xylopia aethiopica*

Proximate	Mean ± SD (%)	Vitamins	Mean ± SD (%)	Minerals	Mean ± SD (%)
Moisture	8.59 ± 0.04	A	4.82 ± 1.16	Ca	0.87 ± 0.02
Ash	14.02 ± 0.06	E	2.31 ± 0.05	Mg	2.68 ± 0.04
Fiber	12.39 ± 0.04	D	1.24 ± 0.33	Zn	0.12 ± 0.01
Protein	7.09 ± 0.01	K	$2 \times 10^{-3} \pm 2 \times 10^{-4}$	Se	0.10 ± 0.01
Fat	3.01 ± 0.15	B_1	$1 \times 10^{-4} \pm 1 \times 10^{-5}$	Na	0.15 ± 0.01
Carbohydrates	54.90 ± 0.18	B_2	$1 \times 10^{-3} \pm 1 \times 10^{-4}$	Cl	0.02 ± 0.01
		B_3	1.30 ± 0.16	K	ND
		C	0.13 ± 0.06		
		B_6	ND		

The results are presented as mean ± standard deviation (SD) of replicate measurements. ND, not detected.

Determination of Some Metals in Prostate Homogenate

The processes utilized by Alexaris and Lazos[30] to digest the homogenate sample are the most essential elements in this work. The flask was heated, diluted with deionized water, and analyzed using an atomic absorption spectrophotometer. The standard curve was then used to read the metal concentrations.

Statistical Analysis

The results were presented as mean ± standard deviation, and the distinctions between the groups receiving treatment and the control groups were assessed through one-way analysis of variance, in conjunction with a paired one-sample *t*-test. All statistical computations were conducted with SPSS 20.0 at *p*-value less than 0.05

Results

Proximate Composition, Vitamins, and Mineral Content of the *Xylopia aethiopica* Ethanol Leaf Extract

The results of the proximate composition, vitamins, and mineral content of *XAE* ethanol leaf extract are shown in ►**Table 2**. The results show that carbohydrate content recorded highest value (54.90 ± 0.15%), while fat was found to be lowest (3.01 ± 0.18%). The values of ash (14.02 ± 0.16%), fiber (12.39 ± 0.04%), and protein (7.09 ± 0.01%) were also recorded. The results of the vitamins content showed that vitamin A recorded the highest value (4.82 ± 1.16%) and vitamin B1 was found to be lowest ($1 \times 10^{-4} \pm 1.10^{-5}$%). Vitamins E (2.31 ± 0.05), D (1.24 ± 0 × 33%), K ($2 \times 10^{-3} \pm 2 \times 10^{-4}$), B_2($1 \times 10^{-3} \pm 1 \times 10^{-4}$%), B_3(1.30 ± 0.16%), and vitamin C (0.13 ± 0.06%) were recorded. The mineral contents of showed that Mg was highest (2.68 ± 0.04%), while Cl was found to be lowest (0.02 ± 0.01%). The values of Ca (0.87 ± 0.02%), Zn (0.12 ± 0.01%), Se (0.10 ± 0.01%), and Na (0.15 ± 0.01%) were also shown.

Effect of *Xylopia aethiopica* on Prostate Tissue Minerals Levels in Tp-Induced Benign Prostatic Hyperplasia in Rats

The result of *XAE* on prostate tissue minerals in Tp-induced BPH in rats are shown in ►**Tables 3** and **4**. The level of the prostate minerals, Zn, Ca, Mg, and Se decreased significantly ($p < 0.05$) in Tp-induced BPH in rats. However, this was increased significantly ($p < 0.05$) in finasteride and *XAE*

Table 3 Effect of *XAE* on prostate tissue mineral levels in Tp-induced benign prostatic hyperplasia in rats (preventive studies)

Group	Zn mg/L	Ca mg/L	Mg mg/L	Se mg/L
A1	6.60 ± 1.00^a	4.80 ± 1.35^a	9.50 ± 2.25^a	5.69 ± 2.20^a
A_2	4.25 ± 1.50^c	2.60 ± 0.72^c	5.15 ± 1.25^c	2.50 ± 0.40^c
B_1	5.25 ± 1.15^b	3.90 ± 1.91^b	7.18 ± 1.45^c	3.10 ± 1.50^b
B_2	5.90 ± 1.20^b	3.98 ± 1.45^b	7.85 ± 2.14^c	3.75 ± 0.75^b
B_3	6.25 ± 1.45^b	4.20 ± 0.48^b	8.10 ± 1.25^b	4.10 ± 7.10^b
B_4	6.75 ± 0.35^b	4.75 ± 1.30^b	8.70 ± 1.50^b	4.95 ± 1.20^b
B_5	7.10 ± 0.55^b	5.00 ± 1.25^a	9.25 ± 1.15^a	5.50 ± 0.50^a

Abbreviations: F, finasteride; Tp, testosterone propionate; XAE, Xylopia aethiopica ethanol leaf extracts.
The results are presented as mean ± standard deviation of three replicate measurements.
A_1—Olive oil (vehicle only), A_2—Tp (14 mg kg^{-1}), B_1—Tp (14 mg kg^{-1}) + F (10 mg kg^{-1}), B_2—(*XAE*) 100 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 100 mg kg^{-1}, B_3—(*XAE*) 200 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 200 mg kg^{-1}, B_4—(*XAE*) 300 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 300 mg kg^{-1}, B_5—(*XAE*) 400 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 400 mg kg^{-1}.
Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Table 4 Effect of *XAE* on prostate tissue mineral levels in Tp-induced benign prostatic hyperplasia in rats (curative studies)

Group	Zn mg/L	Ca mg/L	Mg mg/L	Se mg/L
A1	6.60 ± 10^{a}	4.80 ± 1.35^{a}	9.50 ± 2.25^{a}	5.69 ± 2.20^{a}
A_2	4.25 ± 1.50^{c}	2.60 ± 0.72^{c}	5.15 ± 1.25^{c}	2.50 ± 0.40^{c}
C_1	4.95 ± 0.55^{c}	2.90 ± 1.20^{c}	7.60 ± 1.35^{c}	3.45 ± 1.20^{c}
C_2	5.20 ± 1.15^{c}	3.30 ± 1.22^{c}	8.00 ± 1.25^{c}	4.00 ± 1.15^{c}
C_3	5.95 ± 1.25^{c}	3.75 ± 1.50^{c}	8.90 ± 76^{b}	4.75 ± 1.50
C_4	6.20 ± 0.75^{c}	4.50 ± 1.30^{b}	4.25 ± 0.80^{b}	5.10 ± 0.75^{b}
C_5	6.90 ± 1.20^{a}	5.10 ± 1.25^{b}	9.85 ± 1.75^{a}	6.10 ± 1.25^{a}

Abbreviations: AI, after induction for 4 weeks; F, finasteride; Tp, testosterone propionate; *XAE*, Xylopia aethiopica ethanol leaf extract.
The results are presented as mean ± standard deviation of three replicate measurements.
A_1—Olive oil (vehicle only), A_2—Tp(14 mg kg^{-1}), C_1—Tp (14 mg kg^{-1}) AI + F(10 mg kg^{-1}), C_2—Tp (14 mg kg^{-1}) AI + (*XAE*) 500 mg kg^{-1}, C_3—Tp (14 mg kg^{-1}) AI + (*XAE*) 600 mg kg^{-1}, C_4—Tp (14 mg kg^{-1}) AI + (*XAE*) 700 mg kg^{-1}, C_5—T p(14 mg kg^{-1}) AI + (*XAE*) 800 mg kg^{-1}.
Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

treatment groups. Also the levels of the prostate minerals were higher in the *XAE* groups than in the finasteride treated group.

Effect of *Xylopia aethiopica* on Antioxidant Activities in Tp-Induced Benign Prostatic Hyperplasia in Rats

The results of *XAE* on antioxidant activities in Tp-induced BPH in rats are shown in ►**Tables 5** and **6**. The activities of catalase (CT), glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) decreased significantly ($p < 0.05$) in Tp-induced BPH group compared with control group. Thus, the trend was reversed (significantly ($p < 0.05$) increased) in finasteride and *XAE* treatment groups. However, the activities increased more significantly ($p < 0.05$) in *XAE* treated groups than in finasteride group

Effect of *Xylopia aethiopica* on Kidney Biochemical Parameters in Tp-Induced Benign Prostatic Hyperplasia in Rats

The results of *XAE* on kidney biochemical parameters in Tp-induced BPH in rats are shown in ►**Tables 7** and **8**. The results showed that the levels of K, Na, Cl, HCO_3^-, urea, uric acid, and creatinine were significantly ($p < 0.05$) higher in the TP only group compared with the control group. However, the levels were reversed significantly ($p < 0.05$) lower in the finasteride and *XAE* treatment groups relative to the Tp only group for both studies. At high doses of *XAE* 700,800 mg kg^{-1}, the values were quite lower compared with values obtained in the control group.

Effect of *X. aethiopica* on Liver Function Parameters in Tp-Induced Benign Prostatic Hyperplasia in Rats

The result of *XAE* on liver function parameters in Tp-induced BPH in rats are shown in ►**Tables 9** and **10**. The results showed a significant ($p < 0.05$) decrease in the total protein level in the Tp-induced BPH rats relative to control group. The trend changed on treatment with finasteride and *XAE*; it significantly ($p < 0.05$) increased in finasteride and *XAE* treatment groups compared with the Tp only group in both studies. The levels of AST, ALT, and ALP were not significantly ($p < 0.05$) different in the Tp-induced BPH group, finasteride, and *XAE* treatment groups compared with control in both studies (preventive and curative)

Table 5 Effect of *XAE* on antioxidant activities in Tp-induced benign prostatic hyperplasia in rats (preventive studies)

Group	CT u/mg Protein	GSH µmol/mg protein	SOD u/mg protein	MDA µmol/g protein
A_1	149.14 ± 0.11^{a}	43.32 ± 0.11^{a}	55.94 ± 2.50^{a}	8.25 ± 0.45^{a}
A_2	110.57 ± 2.76^{c}	35.93 ± 1.25^{c}	35.94 ± 1.95^{c}	7.25 ± 0.17^{b}
B_1	145.14 ± 0.65^{b}	39.96 ± 0.56^{b}	37.81 ± 2.40^{c}	6.40 ± 0.31^{c}
B_2	147.25 ± 0.30^{b}	42.65 ± 0.11^{a}	38.37 ± 2.17^{c}	5.90 ± 0.39^{c}
B_3	148.50 ± 0.11^{b}	42.95 ± 0.42^{a}	43.45 ± 1.90^{b}	7.69 ± 0.42^{b}
B_4	149.64 ± 0.80^{b}	43.50 ± 0.51^{a}	45.70 ± 1.95^{b}	6.79 ± 0.27^{b}
B_5	153.14 ± 1.14^{b}	44.35 ± 0.54^{a}	46.50 ± 1.76^{b}	6.50 ± 0.30^{b}

Abbreviations: CT, catalase; GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; Tp, testosterone propionate; XAE, Xylopia aethiopica ethanol leaf extract.
Results are presented as mean ± standard deviation of three replicate measurements.
A_1—Olive oil (vehicle only), A_2—Tp(14 mg kg^{-1}), B_1—Tp (14 mg kg^{-1}) + F(10 mg kg^{-1}), B_2—(*XAE*) 100 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 100 mg kg^{-1},B_3—(*XAE*) 200 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 200 mg kg^{-1}, B_4—(*XAE*) 300 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 300 mg kg^{-1}, B_5—(*XAE*) 400 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 400 mg kg^{-1}.
Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Table 6 Effect of *XAE* on antioxidant activities in Tp-induced benign prostatic hyperplasia in rats (curative studies)

Group	CT u/mg protein	GSH µmol/mg protein	SOD u/mg protein	MDA µmol/g protein
A_1	149.14 ± 0.11^a	43.32 ± 0.11^a	55.94 ± 2.50^a	8.25 ± 0.45^a
A_2	110.57 ± 2.76^c	35.93 ± 1.25^c	35.94 ± 1.75^c	7.25 ± 0.17^b
C_1	105.14 ± 2.20^c	34.60 ± 0.42^c	48.65 ± 1.81^b	6.55 ± 0.23^c
C_2	116.00 ± 0.63^c	43.10 ± 0.51^a	50.73 ± 1.80^b	5.50 ± 0.1^b
C_3	132.50 ± 2.04^c	45.34 ± 0.7^a	52.50 ± 1.84^b	4.25 ± 0.71^c
C_4	151.00 ± 0.14^b	44.66 ± 0.20^a	53.54 ± 1.17^b	4.60 ± 0.1^c
C_5	178.86 ± 2.15^a	45.67 ± 1.50^a	55.22 ± 1.15^b	4.75 ± 0.12^c

Abbreviations: CT, catalase; GSH, glutathione; SOD, superoxide dismutase; MDA, malondialdehyde; Tp, testosterone propionate; XAE, Xylopia aethiopica ethanol leaf extract.
Results are presented as mean ± standard deviation of three replicate measurements.
A_1—Olive oil (vehicle only), A_2—Tp(14 mg kg^{-1}), C_1—Tp(14 mg kg^{-1})AI + F(10 mg kg^{-1}), C_2—Tp (14 mg kg^{-1}) AI + (*XAE*) 500 mg kg^{-1}, C_3—Tp(14 mg kg^{-1}) AI + (*XAE*) 600 mg kg^{-1}, C_4—Tp (14 mg kg^{-1}) AI + (*XAE*) 700 mg kg^{-1}, C_5—Tp(14 mg kg^{-1}) AI + (*XAE*) 800 mg kg^{-1}. Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Table 7 Effect of *XAE* on kidney biochemical parameters in Tp-Induced benign prostatic hyperplasia in rats (preventive studies)

Group	K mmol/L	Na mmol/L	Cl mmol/L	HCO_3 mmol/L	Urea mmol/L	Uric acid Mmol/L	Creatinine mmol/L
A_1	3.00 ± 0.02^c	136.50 ± 0.25^c	101.40 ± 0.64^b	22.90 ± 2.35^b	9.60 ± 1.40^b	1.28 ± 0.12^c	89.10 ± 2.35^b
A_2	4.92 ± 0.04^a	146.40 ± 1.20^a	105.95 ± 1.65^a	27.60 ± 1.50^a	11.40 ± 1.35^a	3.90 ± 0.01^a	111.35 ± 1.25^a
B_1	3.92 ± 0.15^b	145.30 ± 1.28^a	102.60 ± 1.25^b	24.80 ± 1.15^b	9.80 ± 1.85^b	3.60 ± 0.15^b	97.50 ± 2.20^b
B_2	3.70 ± 1.15^b	144.15 ± 1.25^b	101.52 ± 1.15^b	23.40 ± 0.95^b	7.10 ± 1.45^c	2.90 ± 0.18^b	77.90 ± 1.50^b
B_3	3.60 ± 0.13^b	141.30 ± 0.30^b	100.10 ± 0.95^b	22.70 ± 1.75^b	5.40 ± 1.25^c	2.20 ± 0.05^c	50.40 ± 2.20^C
B_4	3.30 ± 0.15^b	140.40 ± 1.66^b	97.25 ± 0.10^b	21.40 ± 0.85^C	4.80 ± 1.15^c	1.90 ± 0.02^c	42.50 ± 2.25^C
B_5	3.10 ± 0.75^b	139.20 ± 0.40^b	90.00 ± 1.25^b	19.30 ± 0.85^C	3.80 ± 0.30^c	1.50 ± 0.01^c	33.70 ± 1.25^C

Abbreviations: AI, after inducement; F, finasteride; Tp, testosterone propionate; *XAE*, *Xylopia aethiopica* ethanol leaf extract.
The results are presented as mean ± standard deviation of three replicate measurements.
A_1—Control, A_2—Tp (14 mg kg^{-1}), B_1—Tp (14 mg kg^{-1}) + F(10 mg kg^{-1}), B_2—(*XAE*) 100 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 100 mg kg^{-1}, B_3—(*XAE*) 200 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 200 mg kg^{-1}, B_4—(*XAE*) 300 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 300 mg kg^{-1}, B_5—(*XAE*) 400 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 400 mg kg^{-1}.
Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Table 8 Effect of *XAE* on kidney biochemical parameters in Tp-induced benign prostatic hyperplasia in rats (curative studies)

Group	K mmol/L	Na mmol/L	Cl mmol/L	HCO_3 mmol/L	Urea mmol/L	Uric acid mmol/L	Creatinine mmol/L
A_1	3.00 ± 0.02^c	136.50 ± 0.25^c	101.40 ± 0.64^b	22.90 ± 1.35	9.60 ± 1.40^b	1.28 ± 0.12^c	89.10 ± 2.35^b
A_2	4.92 ± 0.04^a	146.40 ± 1.20^a	105.95 ± 1.65^a	27.60 ± 1.50^a	11.40 ± 1.35^a	3.90 ± 0.01^a	111.35 ± 1.25^a
C_1	3.60 ± 0.25^b	144.90 ± 1.25^b	104.90 ± 1.44^b	23.90 ± 2.40^b	9.30 ± 1.50^b	3.60 ± 0.16^b	92.60 ± 0.58^b
C_2	3.40 ± 0.05^b	138.95 ± 0.95^b	98.00 ± 2.50^b	21.10 ± 1.30^b	5.50 ± 0.73^c	3.20 ± 0.13^b	70.50 ± 1.25^b
C_3	3.10 ± 0.15^b	136.00 ± 0.48^b	94.50 ± 1.10^b	19.30 ± 1.25^b	4.75 ± 0.15^c	1.40 ± 0.18^c	45.50 ± 0.95^c
C_4	2.95 ± 0.03^b	133.45 ± 2.25^b	90.20 ± 2.15^b	18.85 ± 1.15^b	3.60 ± 0.16^c	1.30 ± 0.12^c	35.60 ± 1.50^c
C_5	2.80 ± 0.16^b	130.45 ± 0.95^c	88.20 ± 2.25^b	18.00 ± 2.25^c	2.40 ± 0.40^c	1.20 ± 0.06^c	30.45 ± 2.15^c

Abbreviations: AI, after inducement; F, finasteride; Tp, testosterone propionate; *XAE*, *Xylopia aethiopica* ethanol leaf extract.
The results are presented as mean ± standard deviation of three measurements.
Groups—A1—Control, A2—Tp (14 mg kg-1), C1—Tp (14 mg kg-1) AI + F(10 mg kg-1), C2—Tp (14 mg kg-1) AI+ (*XAE*) 500 mg g-1, C3—Tp (14 mg kg-1) AI + (*XAE*) 600 mg kg-1, C4—Tp (14 mg kg-1) AI + (*XAE*) 700 mg kg-1, C5—Tp (14 mg kg-1) AI + (*XAE*) 800 mg kg-1.
Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Table 9 Effect of *XAE* on liver function parameters in Tp-induced benign prostatic hyperplasia in rats (preventive studies)

Group	Total protein mg/dL	AST u/L	ALT u/L	ALP u/L
A_1	7.65 ± 0.55^b	45.82 ± 1.45^b	65.45 ± 0.75^b	93.85 ± 0.75^b
A_2	3.05 ± 0.50^c	60.27 ± 1.25^a	70.10 ± 1.15^a	94.18 ± 1.15^a
B_1	6.08 ± 0.75^b	50.50 ± 1.40^b	71.75 ± 1.35^a	92.79 ± 0.75^c
B_2	7.60 ± 1.00^b	60.75 ± 1.35^a	75.45 ± 1.10^a	92.80 ± 2.25^c
B_3	8.43 ± 0.65^b	51.85 ± 2.50^b	74.75 ± 1.05^a	92.55 ± 1.25^c
B_4	9.65 ± 1.10^a	50.95 ± 1.25^b	73.65 ± 0.90^a	92.85 ± 1.25^c
B_5	10.45 ± 0.75^a	52.00 ± 2.15^b	72.00 ± 2.15^b	92.80 ± 1.75^c

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Tp, testosterone propionate; *XAE*, *Xylopia aethiopica* ethanol leaf extract.
Results are presented as mean ± standard deviation of three measurement.
A_1—Control (vehicle only), A_2—Tp (14 mg kg^{-1}), B_1—Tp (14 mg kg^{-1}) + F(10 mg kg^{-1}), B_2—(*XAE*) 100 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 100 mg kg^{-1}, B_3—(*XAE*) 200 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 200 mg kg^{-1}, B_4—(*XAE*) 300 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 300 mg kg^{-1}, B_5—(*XAE*) 400 mg kg^{-1} + Tp (14 mg kg^{-1}) + (*XAE*) 400 mg kg^{-1}.
Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Table 10 Effect of *XAE* on liver function parameters in Tp-induced benign prostatic hyperplasia in rats (curative studies)

Group	Total protein mg/dL	AST u/L	ALT u/L	ALP u/L
A_1	7.65 ± 0.55^b	45.82 ± 1.45^b	65.45 ± 0.75^b	93.85 ± 0.75^b
A_2	3.05 ± 0.50^c	60.27 ± 1.25^a	70.10 ± 1.15^a	94.18 ± 1.15^a
C_1	6.65 ± 0.50^b	51.55 ± 1.65^b	71.50 ± 2.50^a	92.25 ± 1.35^c
C_2	8.35 ± 0.45^b	50.50 ± 1.35^b	70.45 ± 1.15^a	93.15 ± 1.35^b
C_3	9.45 ± 1.15^b	49.25 ± 1.50^b	67.25 ± 0.75^a	93.95 ± 1.65^b
C_4	11.00 ± 1.50^a	48.45 ± 2.00^b	65.15 ± 1.15^b	93.30 ± 2.30^b
C_5	11.65 ± 0.25^a	46.30 ± 1.30^b	63.50 ± 1.08^b	93.25 ± 1.35^b

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Tp, testosterone propionate; *XAE*, *Xylopia aethiopica* ethanol leaf extract.
Results are presented as mean ± standard deviation of three readings.
A_1—Control, A_2—Tp (14 mg kg^{-1}), C_1—Tp (14 mg kg^{-1}) AI + F (10 mg kg^{-1}), C_2—Tp(14 mg kg^{-1}) AI+ (*XAE*) 500 mg kg^{-1}, C_3—Tp (14 mg kg^{-1}) AI + (*XAE*) 600 mg kg^{-1}, C_4—Tp (14 mg kg^{-1}) AI + (*XAE*) 700 mg kg^{-1}, C_5—Tp (14 mg kg^{-1}) AI + (*XAE*) 800 mg kg^{-1}.
Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Discussion

The results of the proximate composition of *XAE* showed the following order of occurrence; carbohydrates, ash, fiber, moisture, protein, and fat. The result is consistent with the report researchers in Europe, Japan, India, and Nigeria who found that polysaccharide in *XAE* reduced tumor and cancers.[31] This finding is in line with earlier report that dietary fiber through the consumption of fruits and vegetables has been shown to be preventive and associated with decreased incidence of BPH.

XAE ethanol leaf extract contains minerals such as Mg, Ca, Na, Zn, Se, and Cl that can be administered to Tp-induced BPH in albino rats to reduce the levels of BPH. Mg levels were found to be significantly lowered in patients with BPH, while Ca levels in BPH patients have not been associated with the condition.[32] *XAE* ethanol leaf extract contains vitamins A, E, B3, D, C, K, B2, and B that are anti-BPH and protect against growth and viability of BPH cells. National Health and Nutritional Examination survey showed that vitamin D deficiency is associated with lower urinary track symptoms, while intake of vitamin D supplement and vitamin D analog has been shown to decrease BPH prevalence and prostate size.[33] It has been widely reported that some antioxidants such as vitamin A, E, and C found also in *XAE* play key roles in the prevention of BPH by ameliorating oxidative stress, which otherwise results in DNA damage and increases the risk of mutation and malignant transformation.[34]

BPH decreased minerals in prostate homogenates compared with the normal control group, while these were significantly increased in finasteride and *XAE* treatment groups. *XAE* treatments can enhance the levels of prostatic fluid minerals to promote prostate health. Zinc is an important constituent of prostatic fluid and is known to play an important role in the development and functioning of prostate.[35] It is suggested that the ameliorative effects of selenium against the histological and histochemical changes of the prostate induced by carbimazole may be due to its antioxidant properties from a previous study[36] this could have been responsible for the observed therapeutic potentials of *XAE* in this study due to the significant presence of Se. Ca is an important nutrient for prostate health, with a positive correlation between it and Mg and Zn in hyperplasia of the prostate gland. Age-related changes in Ca levels are linked to BPH.[32] Calcium (Ca) is an essential mineral that plays a crucial role in various bodily functions, including bone health, muscle contraction, blood clotting, and nerve transmission. While calcium is important for overall health, its relevance specifically to prostate health is not as direct as it is for other areas of the body. Prostate health is more closely associated with other nutrients and factors

The results of *XAE* on kidney parameters in Tp-induced BPH in rats showed that the levels of K, Na, Cl, HCO_3^-, urea, uric acid, and creatinine were significantly higher in the TP only group compared with the control group. However, the levels were lowered significantly in the finasteride and *XAE* treatment groups relative to the Tp only group for both studies. Basically, potassium, sodium, chloride, and bicarbonate are electrolytes that play crucial roles in maintaining the balance of fluids and ions in the body. Abnormal levels of these electrolytes can indicate disruptions in kidney function, fluid balance, or other physiological processes. If these levels are consistently elevated in individuals with BPH, it might suggest an underlying kidney or metabolic issue. However, electrolyte imbalances are not typically directly associated with BPH itself. More so, urea, uric acid, and creatinine are waste products that are eliminated by the kidneys. Elevated levels of these substances can indicate impaired kidney function. If individuals with BPH have consistently higher levels of urea, uric acid, and creatinine, it could suggest that BPH is impacting kidney function. However, it is important to note that these markers are not specific to BPH and can be influenced by various factors, including diet, hydration, and other medical conditions.

BPH-induction significantly decreased antioxidant activities and increased lipid peroxidation, consistent with previous reports.[37] In this study, the activities were, however, found to be significantly ($p < 0.05$) increased in finasteride and *XAE* treatment groups in both studies. Thus, the initial decline in the activities of CT, SOD, GSH and the increased concentration of MDA in Tp-induced BPH rats were substantially restored by *XAE* treatments well above the control values. This indicates protection of prostate glands against free radicals damage thereby suppressing the development of BPH. This finding can be attributed to the antioxidant properties of *XAE* based on its phytochemical constituent.

XAE studies showed that Tp-induced BPH had higher levels of potassium, sodium, chloride, bicarbonate, urea, uric acid, and creatinine than control groups. Finasteride and *XAE* treatment groups had lower levels. Without relief, BPH can lead to asphyxiation, hydroureters, hydronephrosis, obstructive nephropathy, renal insufficiency, and kidney failure.[38] *XAE* and finasteride treatment in both studies exhibited a significant improvement in these major pathological problems observed in Tp-induced BPH rats. Reduction in the levels of serum urea, uric acid, electrolytes, and creatinine by *XAE* and finasteride treatments in both studies may be due to decreased prostate size, return of urinary flow rate to control values, and recovery of kidney from BPH damage. The findings of this study are in agreement with previous reports that urine output decreased drastically in testosterone treatment group due to enlargement of prostate gland.[39]

The total protein level in rats with Tp-induced BPH was found to have decreased compared with a control group. However, finasteride and *XAE* treatment groups showed a significant increase in protein levels. Most plant extracts possess antioxidant, hepatoprotective, and nephroprotective functions. Oxidative stress can lead to tissue damage, including damage to organs involved in protein metabolism. Certain plant extracts are rich in antioxidants that can help reduce oxidative stress, potentially leading to normalized serum protein levels as observed in *XAE* treatments in this study. The liver plays a crucial role in protein synthesis and metabolism. Some plant extracts are known to have hepatoprotective effects, supporting liver health and function. Improved liver function could positively affect serum protein levels. While kidneys are also involved in maintaining proper protein levels in the blood, some plant compounds possess diuretic and nephroprotective properties, which might impact kidney function and subsequently influence serum protein levels. These could be attributed to the observations with *XAE* and finasteride in this study. Carbimazole and selenium treatment restored to normal the total protein levels of the prostate epithelial cells in previous studies.[36] *XAE* treatments in this study restored the depleted protein content level caused by Tp-induced BPH, and did not affect the activities of AST, ALT, and ALP in the Tp-induced BPH group, finasteride and *XAE* treatment groups. Thus, *XAE* aside from inhibiting BPH development was well tolerated by the rats in both studies.

Authors' Contributions

U.A.I. conceived the study; D.E.U., C.C.E., O.U.O conducted literature searches; P.M.A., E.N.N., E.U.A. conducted data analysis; C.C., C.A., M.O.I., S.A.A., G.U.U., and V.N. wrote the original draft; U.A.I., D.E.U., and P.M.A. read and approved the final draft before submission. All authors have critically reviewed and approved the final draft of the manuscript.

Ethical Approval

The animal experimental methodology was performed in compliance with the precautions for the care and use of laboratory animals that Ebonyi State University Abakaliki, Ebonyi State (EBSU/ET/18/001) prescribed and approved.

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Conflict of Interest

None declared.

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