Sphenostylis Stenocarpa Seed Ethanol Extract Normalizes Redox imbalance and other Alterations Associated with Benign Prostatic Hyperplasia in Rat Model

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ABSTRACT

Background: *Natural products are gaining increasing interest in the management of prostate diseases due to their promising efficacy and milder side effect when compared to the conventional drugs used in treating them.*

Objective: *This study investigated the effect of ethanol extract of Sphenostylis stenocarpa seeds (EESS) on markers of Benign Prostatic Hyperplasia (BPH) and antioxidant activities in Wistar rats.*

Methods*: Twenty-five male Wistar rats were randomized into 5 groups of 5 rats each. Rats in groups 2-5 were subcutaneous injection with testosterone propionate (TP) for 28 days to induce BPH. The Wistar rats in group 1 served as the normal control, group 2 and 3 were the model and positive control groups respectively. The EESS was concurrently administered to the rats in the test groups (4 and 5) at different doses for the 28 days. BPH markers, hormonal profile, oxidative stress indices, kidney and liver function parameters of the rats were determined.*

Results*: The subcutaneous injection of TP caused a significant increase ($p < 0.05$) in the prostate-specific antigen level (PSA) (2.24 ± 0.28 ng/ml), prostate weight (0.96 ± 0.08 g) and prostate protein content (5.04 ± 0.15 g/tissue) of rats in the model group when compared to their respective normal control (1.54 ± 0.01 ng/ml, 0.62 ± 0.05 g, 4.04 ± 0.07 g/tissue). The administration of EESS to the rats exhibited a remarkable reversal of the induced BPH evidenced by a significant decrease in PSA level (1.70 ± 0.05 ng/ml), prostate weight (0.52 ± 0.04 g) and prostate protein content (4.02 ± 0.37 g/tissue) which were compromised in the model group. A significant increase ($p < 0.05$) in antioxidant activities was equally observed in groups treated with the plant extract compared to the model group.*

Conclusion: *The result of this study suggests that S. stenocarpa seeds may be useful in BPH management.*

Keywords: Antioxidant, benign prostatic hyperplasia, ethanol extract, *Sphenostylis stenocarpa*, testosterone propionate.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is an abnormal increase in the size of the prostate common among elderly men. This condition leads to constriction of the urethra thereby causing difficulty in the flow of urine from the bladder (1). Globally, the prevalence of BPH was estimated to

be 5.48 million in 1990 and has increased to 11.26 million in 2019 (2). In Nigeria, research has shown that 25% of adult males aged 40 and above have BPH (3). The prevalence of the disease increases with age (3, 4). Oxidative stress is a significant factor in the aging process and is believed to play an important role in the development of BPH (5). Among the clinical manifestations of BPH are severe urine retention, lower urinary tract symptoms, bladder stones and urinary tract infections. Urine retention has been shown to increase the risk of urinary tract infections (6). Although BPH is noncancerous, it could increase the risk of prostate cancer when left untreated (7), and in worse cases could lead to permanent damage of the bladder or death (8).

The cause of BPH is not yet fully understood however, several studies have linked the development of the disease to some factors including tissue remodeling in the ageing prostate, hormonal alterations, metabolic syndrome and inflammation (9, 10). Existing drugs for the treatment of BPH which are mainly based on synthetic 5α-reductase inhibitors and α-adrenoceptor antagonists have been associated with several side effects ranging from impotence, decreased libido, cardiac and endocrine-related complications (11). Thus, an alternative option of prevention and treatment of the disease with a lesser side effect is of high interest.

Plant-derived medication has been shown to be a promising alternative for the management of BPH due to its established efficacy, low cost and fewer side effects (12). Anti-BPH properties of some plant materials such as soybean, saw palmetto (*Serenoa repens*) and pumpkin (*Cucurbita pepo*) has been widely reported (13, 14) and their efficacy are attributed to the presence of certain phytoconstituents including isoflavones (13), beta-sitosterol (15) and polyphenols (16). Dietary vitamins C and E (17, 18), and minerals such as selenium and zinc (17, 19) have equally been linked to anti-BPH properties of plant-derived medications. Several studies suggest that these phytotherapeutic agents can act against BPH through different mechanisms including 5-α-reductase inhibition, blockade of α-adrenergic receptors, anti-inflammatory, antiproliferative and antioxidant activities (10, 19, 20).

Sphenostylis stenocarpa (African yam bean) is a close relative of soybean belonging to the Fabaceae family. The plant thrives in Nigeria and some other tropical African countries (21). In Nigeria, *S. stenocarpa* seed is commonly known as Ijiriji, Azama, Nsama or Kutonoso. The plant is an underutilized traditional food crop in Nigeria with rich nutritional value (22). It has a superior amino acids profile compared to most African leguminous crops (23) and the fatty acids constituent comprises 66% unsaturated fatty acids (24, 25) are suitable for human consumption. The plant seed has been recommended as a healthy food supplement due to its nutritional value (26).

The potential of *S. stenocarpa* seed in the management of some disease conditions including anemia (21) and dyslipidemia associated with BPH (27) has been reported. Enujiugha et al. (28) showed that the plant seed possesses a radical scavenging capacity due to its high phenolic content. The usefulness of the protein hydrolysate of the plant as antioxidants in the management of oxidative stress-related metabolic disorders has been reported (23). The anti-fibrotic and hepatoprotective potentials of the plant seed against carbon tetrachloride-induced liver injury in male Wistar rats via antioxidant and anti-inflammation properties have also been reported (29). The aqueous extract from the cooked seeds is traditionally used in some Eastern parts of Nigeria to alleviate the difficulty associated with urination, however, there is no or dearth information on its possible anti-BPH properties. Preliminary studies indicated that *S. stenocarpa* seeds contain isoflavones (30), which exhibit anti-BPH activity via 5α-reductase inhibition (13). Furthermore, the plant seed was shown to be rich in polyphenols and exhibits substantial antioxidant activity (28). An antioxidant-rich phytotherapeutic agent could contribute to neutralizing the adverse effect of free radicals in the prostate which is capable of accelerating the progression of BPH (7), the usefulness of dietary polyphenols in this regard for management of BPH has been suggested (31). Proper dietary supplements may delay the development of BPH (19). Therefore, this study investigated the effect of ethanol extract of *S. stenocarpa* seeds on BPH experimentally induced

in rats.

MATERIALS AND METHODS

Drug and chemicals

Testosterone propionate (TP) was procured from Sigma-Aldrich Company, Germany. Finasteride (Finstals-5®) was produced by Stallion Laboratories PVT. Limited, Gujarat, India. Testosterone and estradiol enzyme-linked immunosorbent assay (ELISA) test kits were products of Biocheck, Inc. Foster City, USA. PSA and prolactin enzyme-linked immunosorbent assay test kits were products of Elabscience Biotechnology, Inc., Houston, Texas, USA. The assays were done by commercial test kits bought from Randox Laboratories Ltd., UK. Other reagents and chemicals utilized in this work were of analytical grade.

Preparation of the seed extract

S. stenocarpa seeds were bought from the Ogbete main market in Enugu, Enugu State, Nigeria. The plant seed was authenticated at the Department of Agriculture, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria. The neatly selected seeds were washed, sun-dried and then pulverized coarsely with a laboratory mill. The pulverized sample was placed in a Soxhlet extractor and extracted with 300 ml of ethanol for 6 h. A rotary evaporator was used to separate the extract from the solvent.

Determination of the chemical composition of the seed extract

The phytochemical quantification for flavonoids, steroids, alkaloids and glycosides were determined in line with methods of (32) and (33). Vitamins C and E were quantified by the method of Association of Official Analytical Chemists (34). The amount of selenium, zinc, iron and copper were quantified using atomic absorption spectroscopy (AAS). Briefly, the sample (0.5 g) was measured into a 250 ml Erlenmeyer conical flask. Perchloric acid (4 ml), 25 ml concentrated nitric acid and 2 ml concentrated sulphuric acid were added to the conical flask under a fume cupboard to digest the sample. The content was mixed and heated gently with a hot plate (under a perchloric acid fume hood) until white fumes appeared. The mixture was allowed to cool, followed by addition of 50 ml distilled water. The content was thereafter filtered into 100 ml volumetric flask using Whatman no. 42 filter paper. Appropriate hollow cathode lamp, wavelength and slit width for each element were selected accordingly for selenium, zinc, iron and copper. The obtained concentrations were thereafter recorded.

ANIMAL EXPERIMENTATION

Acute toxicity test

Thirteen mice (BALB/c) weighing 20 – 25 g were used for the median lethal dose (LD_{50}) test (35). The mice were procured from TwinVet Resource farm, nearby the University of Nigeria, Nsukka, and fed commercial chow and tap water *ad libitum*. Handling of the mice was according to the Guide for the Care and Use of Laboratory Animals (36). The test was done in two stages; in the first stage, nine mice were randomly divided into three groups comprising three mice each. The animals were administered oral doses of 10, 100 and 1000 mg/kg respectively of the ethanol extract. In the second stage, three mice were also placed in three different groups and were orally administered 1600, 2900 and 5000 mg/kg body weight of the extract while one mouse served as a control. The mice were observed for toxicity and possible death within 24 hours. No toxicity was observed among the mice even up to 5000 mg/kg of the extract; hence 200 and 400 mg/kg body weight doses were selected for the animal study.

BPH study

Twenty-five male Wistar rats of average body weight 190 ± 0.5 g were used for this study. They were purchased from TwinVet Resource farm, close to the University of Nigeria, Nsukka. The rats were housed in standard cages and fed commercial rat chow and tap water *ad libitum*. They were randomly distributed into five different groups of five rats each. The ethical procedure of the study was approved by the Ethics and Biosafety Committee, Faculty of Biological Sciences, University of Nigeria, Nsukka (UNN/FBS/EC/1010). The experimental procedures followed the standard on Guide for

the Care and Use of Laboratory Animals (36).

Experimental BPH was induced in the rats by injecting subcutaneously 3 mg/kg body weight of TP (dissolved in olive oil) in the rats from day 1 to day 28 (12). The extract was concurrently administered orally to the rats once daily for 28 consecutive days (Table 1). On Day 29, the rats were fasted, weighed and sacrificed. Blood samples were collected into plain sample bottles. The blood samples were allowed to clot and the sera were separated by centrifugation at 3000 g for 20 min. Carcasses of the rats were quickly dissected and the prostates excised. The obtained prostates were patted with tissue paper and weighed on a sensitive balance. Their dorsolateral lobes were processed for histology and protein content evaluation.

Table 1: The experimental protocol for the study

Group	Subcutaneous injection	Oral administration
Group 1 (Normal control)	Olive oil 1 ml/kg	Physiological saline 2 ml/kg
Group 2 (Model group)	TP (3 mg/kg)	Physiological saline 2 ml/kg
Group 3 (Positive control)	TP (3 mg/kg)	Finasteride (5 mg/70 kg)
Group 4 (Test group a)	TP (3 mg/kg)	Ethanol extract of *S. stenocarpa* seed (200 mg/kg)
Group 5 (Test group b)	TP (3 mg/kg)	Ethanol extract of *S. stenocarpa* seed (400 mg/kg)

TP = testosterone propionate

Table 2: Chemical composition of *S. stenocarpa* ethanol extract

Test	Concentration
Selenium (mg/l)	0.95 ± 0.05
Zinc (mg/l)	10.34 ± 0.12
Iron (mg/l)	12.30 ± 0.23
Copper (mg/l)	3.51 ± 0.11
Vitamin C (mg/100g)	30.19 ± 4.54
Vitamin E (mg/100g)	5.95 ± 0.30
Steriods (%)	14.60 ± 1.20
Saponins (%)	33.33 ± 0.85
Total flavonoids (%)	53.46 ± 3.60
Glycoside (mg/100g)	74.71 ± 8.09
Tannins (mg/100g)	0.64 ± 0.01
Alkaloids (%)	7.75 ± 0.35

Biochemical Analyzes

The PSA, testosterone, estradiol and prolactin concentrations were estimated using ELISA test kit following the methods of their manufacturers. Total protein content in the supernatant fraction of the prostate homogenate was measured according to the method described by (37).

Lipid peroxidation was determined by calculating the thiobarbituric acid reactive substances expressed in terms of malondialdehyde (MDA) concentration as described by (38). Glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities were assayed according to the methods of 39, 40, 41 and 42 respectively. The method of (43) was used to estimate the reduced glutathione (GSH).

The serum concentrations of urea and creatinine were analyzed using Randox test kits according to the methods of the manufacturer.

Histopathology

The dorsolateral lobes of the prostate glands were fixed for 48 h in 10% phosphate-buffered formalin. The tissues were thereafter dehydrated in four grades of ethanol, cleared in xylene and embedded in molten wax. After solidification, they were sectioned into 5 μm thickness using a rotary microtome and then stained with hematoxylin for 15 minutes before counterstaining with eosin. The prepared slides were examined and photomicrographed with a Motic™ 9.0 megapixels microscope camera at x100 magnification.

Statistical Analyzes

Statistical analyzes of the data were done using the Statistical Package for the Social Sciences (SPSS) software, version 20.0. Results were presented as mean ± standard error of the mean (SEM) and test of the statistical significance was carried out using one-way analysis of variance followed by post hoc multiple comparisons, with the Duncan test to detect significant differences between the groups at $p < 0.05$.

RESULTS

Chemical composition of *S. stenocarpa* seed extract

The ethanol extract of *S. stenocarpa* seed was obtained as a light brown semi-solid (8.44%). The results of selected phytochemicals, vitamins and minerals quantification of the extract are presented in Table 2. It was shown that the seeds were rich in flavonoid, glycosides, vitamin C, zinc and selenium.

Effect of ethanol extract of *S. stenocarpa* seeds on the BPH markers

The subcutaneous injection of testosterone propionate in the rats caused a significant increase ($p < 0.05$) in BPH markers namely PSA level (2.24 ± 0.28 ng/ml), prostate weight (0.96 ± 0.08 g) and prostate protein content (5.04 ± 0.15 g/tissue) of the model group when compared to their respective normal control (1.54 ± 0.01 ng/ml, 0.62 ± 0.05 g, 4.04 ± 0.07 g/tissue), confirming successful induction of experimental BPH. A significant reduction ($p < 0.05$) in the PSA level (1.70 ± 0.05 ng/ml), prostate weight (0.52 ± 0.04 g) and prostate protein content (4.02 ± 0.37 g/tissue) were observed in the test rats that received 400 mg/kg EESS concurrently for 28 days when compared to the model group (Figure 1).

Effect of ethanol extract of *S. stenocarpa* seeds on hormonal profile of the rats

The hormonal profile of the rats is presented in Figure 2. It was shown that the subcutaneous injection of exogenous hormone significantly ($p < 0.05$) increased the concentrations of testosterone (7.72 ± 0.22 ng/ml) and prolactin (36.6 ± 0.11 ng/ml) in the rats that received only the hormone when compared to their respective normal control values (5.08 ± 0.29 ng/ml, 31.64 ± 0.36 ng/ml). There was a significant reduction ($p < 0.05$) in the testosterone and prolactin levels in the rats administered the standard drug and different doses of *S. stenocarpa* seed extract when compared to the model group. The exogenous hormone also increased the estradiol level of the rats in the model group compared to the normal

control group.

Effect of ethanol extract of *S. stenocarpa* seed on the MDA concentration and antioxidant indices in the rat sera

A significant increase ($p < 0.05$) in MDA concentration (5.4 ± 0.06 mg/ml) and a corresponding decrease in the SOD (1.00 ± 0.03 U/L x10), CAT (0.87 ± 0.08 U/L), GPx (33.39 ± 0.61 U/L), GR (20.73 ± 0.23 U/mg protein) activities were observed in the rats that received the hormone alone when compared to their respective normal control (4.5 ± 0.1 mg/ml, 1.14 ± 0.02 U/L x10, 1.53 ± 0.01 U/L, 68.24 ± 2.00 U/L, 22.85 ± 0.38 U/mg protein). Administration of the seed extract (at both doses) significantly reduced ($p < 0.05$) the MDA concentration of the test rats (Figure 3), and as well improved the antioxidant capacity when compar

ed to the model and positive control groups (Figures 4A and B).

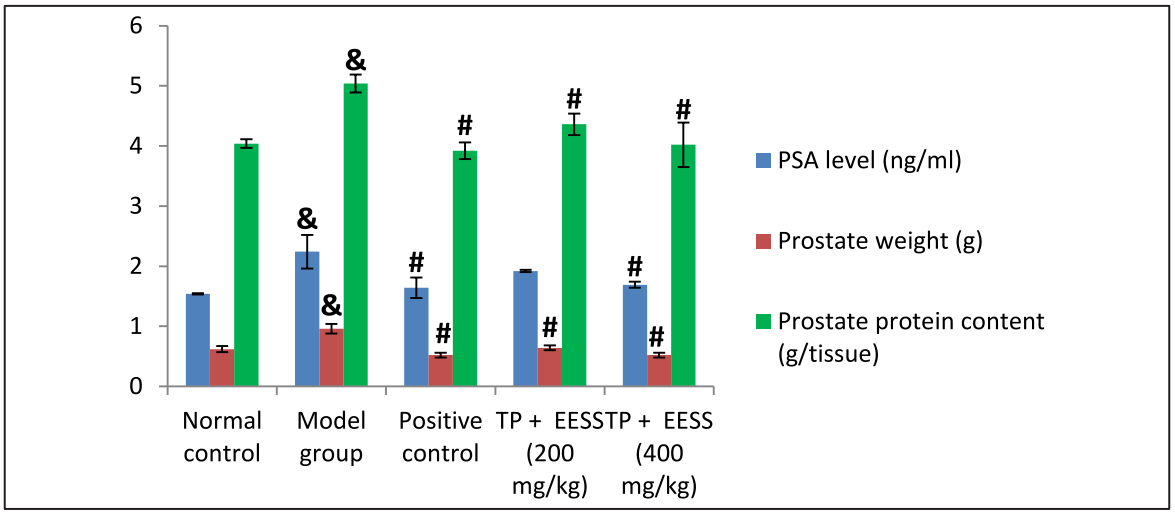


Figure 1: Effect of ethanol extract of *Sphenostylis stenocarpa* seeds (EESS) on prostate-specific antigen (PSA) level, prostate weight and prostate protein content of the rats. [&]Significant when compared to normal control ($p < 0.05$); [#]significant when compared to model group ($p < 0.05$).

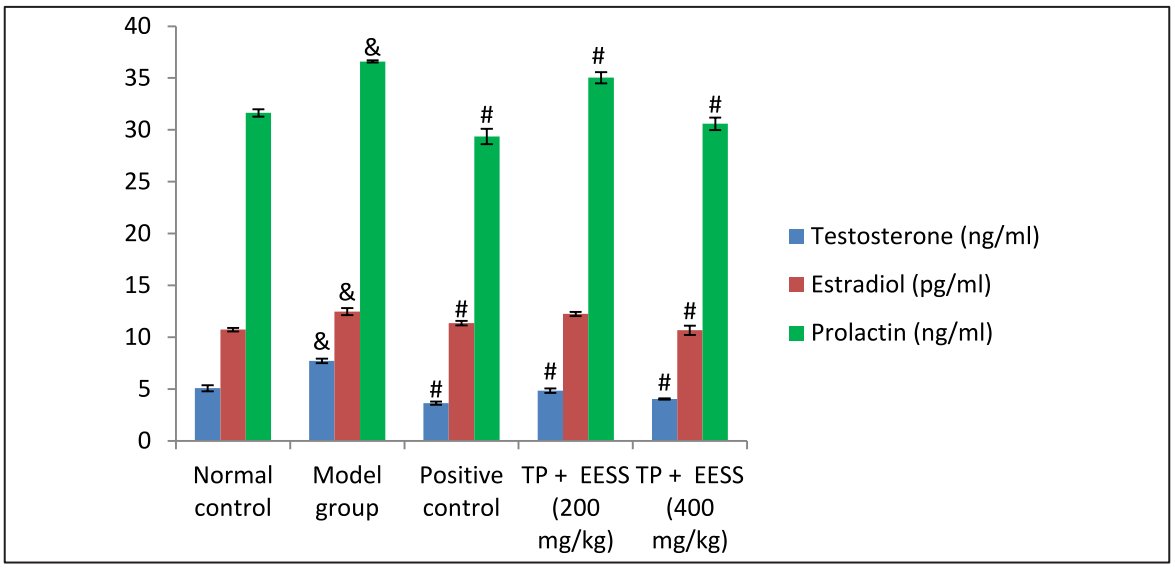


Figure 2: Effect of ethanol extract of *Sphenostylis stenocarpa* seeds (EESS) on hormonal profile of the rats. [&]Significant when compared to normal control ($p < 0.05$); [#]significant when compared to model group ($p < 0.05$).

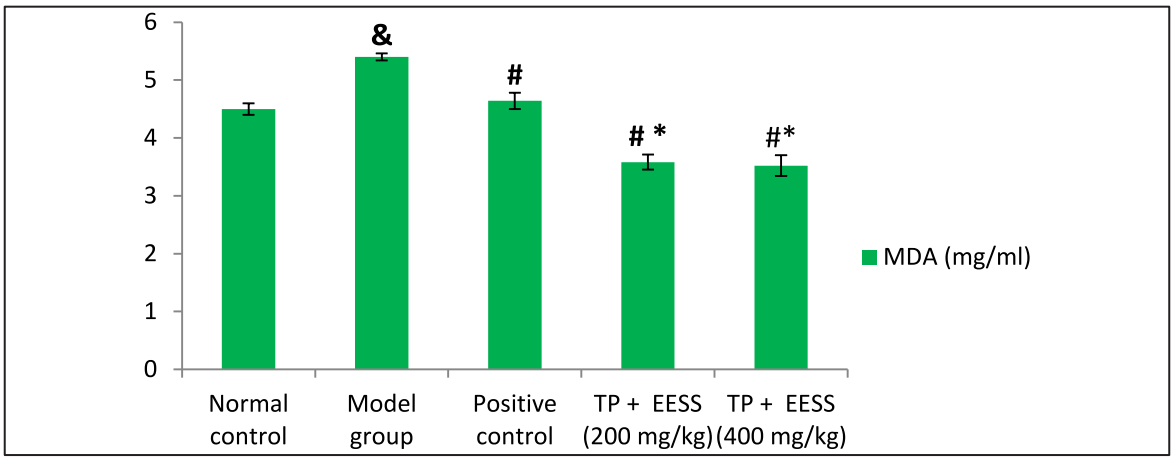
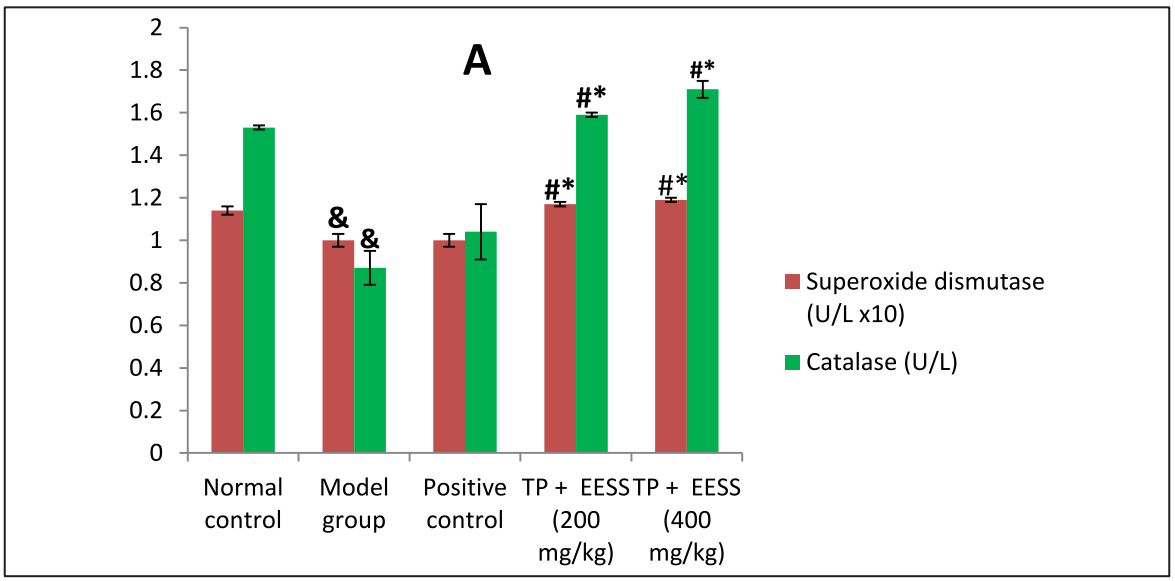


Figure 3: Effect of ethanol extract of *Sphenostylis stenocarpa* seeds (EESS) on malondialdehyde (MDA) concentration of the rats.



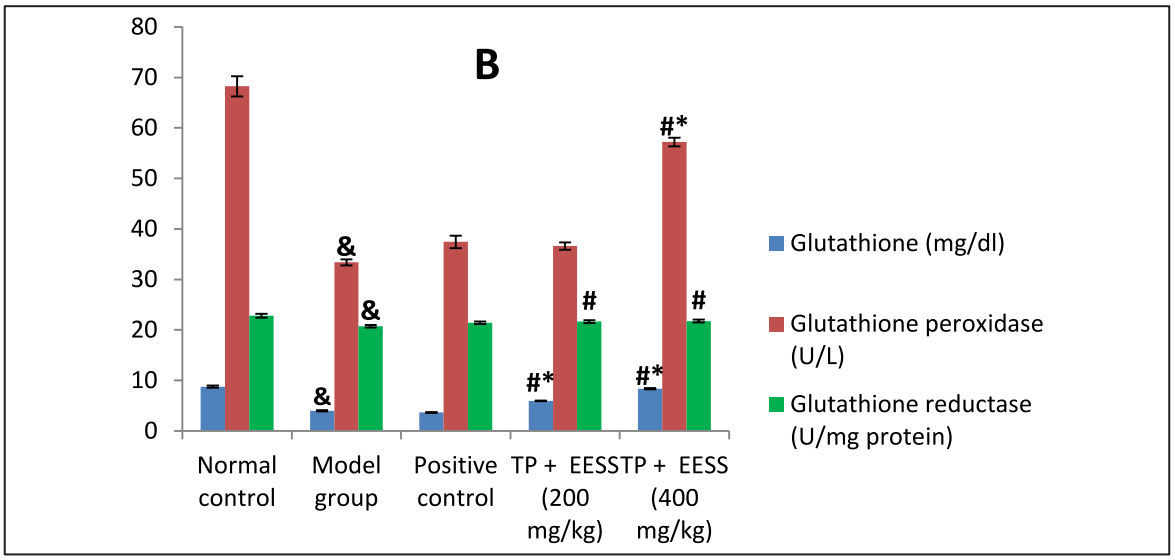
Effect of ethanol extract of *S. stenocarpa* seed on urea and creatinine concentrations in the sera

The values of serum urea (5.12 ± 0.12 mg/dl x10) and creatinine (1.52 ± 0.01 mg/dl) concentrations were significantly elevated in the group that received the testosterone propionate alone when compared to the respective values of the normal control group (4.02 ± 0.02 mg/dl x10, 1.3 ± 0.00 mg/dl). A significant reduction ($p < 0.05$) in the concentrations of urea and creatinine were observed in the rats administered the seed extract or standard drug when compared to the model group (Figure 5).

Histological examination

From the results of histology, rats in the normal control group had a standard prostatic histo-architecture; the epithelium was lined by a single layer of low columnar epithelial cells with infrequent in-folding into the alveolar lumen. However, rats that received the hormone alone showed alveolar epithelium with tall columnar epithelial cells and severe widespread multifocal

areas of hyperplasia. The rats treated with finasteride had mild hyperplasia with decreased in-folding of the epithelium. This pattern was similar to the photomicrograph of prostates of rats that received the seed extract, this suggests recovery from the induced hyperplasia (Plate 1).



Figures 4A and 4B: Effect of ethanol extract of *Sphenostylis stenocarpa* seeds (EESS) on the antioxidant indices of the rats. [&]Significant when compared to normal control ($p < 0.05$); [#]significant when compared to model group ($p < 0.05$); [*]significant when compared to positive control group ($p < 0.05$).

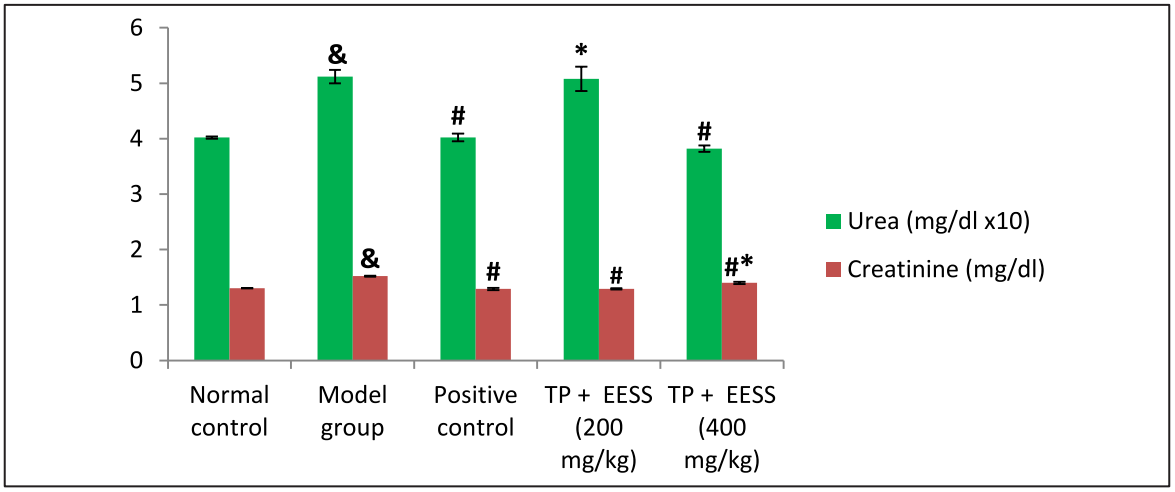


Figure 5: Effect of ethanol extract of *Sphenostylis stenocarpa* seeds (EESS) on urea and creatinine concentrations of the rats. [&]Significant when compared to normal control ($p < 0.05$); [#]significant when compared to model group ($p < 0.05$); [*]significant when compared to positive control group ($p < 0.05$).

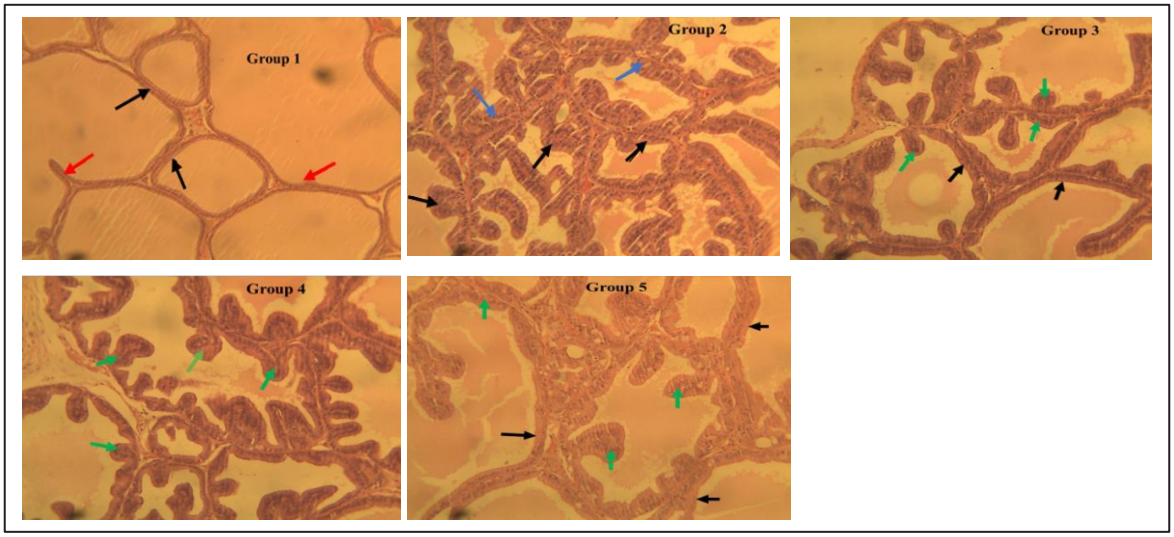


Plate 1: Histological sections of the prostate of the rats in group 1: normal control, Group 2: model Group, Group 3: TP + finasteride (5 mg/70 kg), Group 4: TP + ethanol extract (200 mg/kg), Group 5: TP + ethanol extract (400 mg/kg), (H-E: ×100)

= Alveolus/acini epithelium lined by cuboidal to low columnar epithelial cells

= The folding of the epithelium into the lumen

= Severe multifocal area of hyperplasias

= Moderate hyperplasia of the alveolar epithelial lining cells

DISCUSSION

Prostate hyperplasia manifests with an increase in PSA level, prostate size/weight, and prostate protein content. These biomarkers are indicators of BPH development and complement the morphological changes that take place in the prostate as the disease progresses (10, 44). In this study, a significant increase in PSA, prostate weight, and prostate protein content observed in the rats that received hormone alone indicated that there was an aberrant growth of the prostate, signifying successful induction of experimental BPH in the rats. The significant decrease in values of these BPH markers observed in the test rats administered *S. stenocarpa* seed extract or standard drug, compared to the model group showed that EESS inhibited the development of the disease. This was buttressed by the results of histological examination of the prostate tissues; the groups treated with the seed extract or standard drug showed milder glandular hyperplasia when compared to the model group which revealed stromal proliferation and severe glandular hyperplasia of the prostate. The rats administered 400 mg/kg of EESS had milder glandular hyperplasia compared to that of 200 mg/kg, suggesting a dose-dependent effect of the seed extract on the induced BPH. The seed extract possibly interfered with the androgen action perhaps through inhibition of the action of 5α-reductase, an enzyme responsible for conversion of testosterone to DHT- a potent agent of prostate hyperplasia.

The significant increase in testosterone level observed in the model group compared to the normal rats could be attributed to the supernormal dose of testosterone propionate used for the BPH induction. A similar observation was equally reported (45). Notably, rats treated with *S. stenocarpa* ethanol seed extract showed a significant reduction in the circulating testosterone level compared to the model group. The seed extract possibly modulated the synthesis of this androgenic hormone thereby inhibiting the BPH development. An increase in testosterone

circulation would activate 5α-reductase enzyme to convert testosterone into DHT, which plays a great role in BPH activation and progression (46).

Prolactin and estradiol levels of the rats that received the hormone alone were significantly higher compared to the normal control, and this could be associated with physiological changes during prostate enlargement and/or the effect of the exogenous hormone used for the BPH induction. Prolactin plays an important role in the modulation of stress response (47). An increased prolactin level in experimentally-induced BPH in rats was equally reported by (16). The administration of *S. stenocarpa* ethanol extract reduced the level of these hormones in the test rats compared to the model group.

The mechanisms by which androgen promotes prostatic cellular metabolism are believed to generate enormous free radicals and a great depletion in the antioxidant enzymes (48). This was evident in this study by a significant increase in the concentration of malondialdehyde with a corresponding decrease in antioxidant activities of the rats in the model group compared to other groups. Cai et al. (49) and Sun et al. (50) reported a similar observation for an experimentally induced BPH in rats. Groups that received the seed extract at doses of 200 and 400 mg/kg evidently had enhanced antioxidant enzymes with a corresponding reduction in the concentration of malondialdehyde compared to the model group, indicating that *S. stenocarpa* ethanol extract exhibited a substantial protective effect against oxidative stress. The seed extract, therefore, served as a boost to depleted antioxidants in the BPH rats and could also be a possible mechanism by which the plant extract inhibited the development of BPH.

The quantitative phytochemical composition of the *S. stenocarpa* seed showed that the plant seed contained appreciable amounts of phytoconstituents with a known history for management of BPH. Flavonoids and phytosterols which were abundant in the seed have been shown to exhibit anti-BPH effects through various mechanisms including anti-androgenic and antioxidant effects (31, 51). Also, notable from the preliminary analysis of the plant seed were appreciable quantities of dietary vitamin C, zinc and selenium which are known to alleviate urinary tract symptoms and prevent oxidative destruction of prostate epithelial cells, thereby ensuring normal functioning of the renal system (18, 19, 52).

The significantly elevated urea and creatinine concentrations observed in the model group compared to normal control suggest a possible impairment of the renal system of rats that received the hormone alone. However, this effect was reversed in the groups that were administered *S. stenocarpa* seed extract or finasteride. Hyperplasia of the prostate gland is known to constrict the urethra and obstruct the flow of urine from the bladder, leading to lower urinary tract symptoms and associated renal failure (27, 53). This may result in the retention of urea and creatinine with a corresponding rise in their concentration in the blood (16, 53).

CONCLUSION

This study indicated that ethanol extract of *S. stenocarpa* seed exhibited an inhibitory effect against testosterone propionate-induced BPH in rats. Increased antioxidant activity could be among the possible mechanism through which the extract exerted its preventive role on BPH development. This suggests that *S. stenocarpa* seeds could be useful in the management of BPH For further work, we recommend that isoflavones and phytosterols present in the plant seed be fully characterized.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests

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