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Effect of *Prosopis africana* Seed Extract on Histology and Biochemical Indices of Prostate Functions in Testosterone and Estradiol induced Enlarged Prostate in Adult Rats

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Abstract Benign prostatic hyperplasia (BPH) is characterized as a slow, progressive enlargement of the prostate gland, which eventually causes obstruction and subsequent problems with urination.. We investigated seed extract of Nigerian indigenous plant *Prosopis africana* (PA) effect on BPH. BPH was induced in male rats weighing 250-350g through exogenous administration of testosterone and estradiol. A total of 25 rats were divided into five groups. One group was used as a control and the other groups received subcutaneous injections of the two hormones. Groups 1 to 2 were treated orally with different doses of extracts and group 3 received finasteride, group 4 was left untreated and group 5 served as normal control. After forty-five days of treatment with the extract the animals were sacrificed blood collected through cardiac puncture for biochemical analysis. The prostate were harvested and processed for paraffin embedding and stained with H and E. Treatment with the extract and finasteride resulted to significant ($P < 0.05$) decrease in prostate specific antigen (PSA), estradiol and prolactin and testosterone when compared to BPH control. Also there was a significant increase in the protein content of the prostate when compared to BPH control. Prostate weight was significantly ($P < 0.05$) reduced in treated groups compared to BPH control. This was supported by the histological examination of the prostate. Therefore, *Prosopis africana* was effective in reducing PSA, prolactin, testosterone, estradiol and prostate weight induced BPH in a rat model, and may be useful for the clinical treatment of patients with BPH.

Keywords PSA, prolactin, testosterone and estradiol and prostate

Introduction

Prostate is the tubuloaveolar exocrine gland of the male reproduction system. It is about 3cm long, weight about 20g and is located in the pelvis, under the urinary bladder and in front of the rectum [1]. The prostate gland wraps around the urethra, the tube that carries urine from the bladder out of the tip of the penis. Prostate enlargement is a natural part of aging process [2]. The delicate balance between two hormones (androgens and estrogens) is thought to be the cause of some of the diseases of the prostate. The main diseases of prostate gland are prostate cancer (PCa), benign prostatic hyperplasia (BPH) and prostatitis. Androgen (mainly testosterone) is necessary for the growth of the prostate and also for development of prostate diseases when there is a kind of delicate balance between androgen and estrogen [2].

Benign prostate hyperplasia is the most common benign neoplasm in men, and has a high prevalence that increases with age. BPH involves hyperplasia of prostatic stromal and epithelia cells, resulting in the formation of large, fairly discrete nodules in the pre-urethral region of the prostate. When sufficiently large, the nodules compress the urethral canal to cause partial or sometimes virtually complete, obstruction of the urethra, which interferes with the normal flow of urine [3]. It leads to symptoms of urinary hesitancy, frequent urination, increased risk of urinary tract infections, urinary retention, or contributes to or cause insomnia. Prostate specific antigens (PSA) levels may be elevated in these patients because of increased organ volume and inflammation due to urinary tract infections.

Phytotherapuetic preparations have had a long tradition of use in the medical treatment of BPH in Europe and still commonly used for this purpose [4]. Various plant extracts such as those from *Serenoa repens, Sabalis serrulate, Urtica dioica, Pygenum africanum, Hypoxis rooperi* and the rye pollen extract known as "Cernitin" are marketed for the treatment for BPH and are reported to have 5α reductase inhibitory activity, aromatase inhibitory activity or the ability to modulate the binding of sex hormone-binding globulin to its receptor on membrane [5-6]. This Nigerian indigenous plant *Prosopis africana* has a wide claim to be effective in management of prostate disorders hence the need to scientifically validate it.

Materials and Methods

Plant Material

Prosopis seeds were purchased from Shibori market in Ogoja Local Government of Cross river State, Nigeria. The seeds (500g) was sorted, cleaned and was boiled for 5h using a gas cooker and allowed to cool to room temperature. The boiling helps to soften the hulls for easy removal and separation of the cotyledons. After it was dehulled and decorticated. The dehulled and boiled seeds were washed again with clean water. The processing of decortications was done by hand squeezing the seeds and washing with clean water. The wet decorticated seeds were kept in a large polythene sacks to exclude air and was fermented for three days according to the method described by [7]. The fermentation was done at room temperature (≈25^{0}C) for 72h. The fermented seeds were then sun-dried to a constant weight and milled using hammer mill to produce *prosopis* seed flour [8]. The flour was kept in a refrigerator at 4^o C prior to use.

Hormones

Testosterone propionate Brand name: Ricostrone; a product of Greenfield pharma, Jiangsu Co Ltd., China. Estradiol valerate (by Medipharm Ltd., 108-Kotlakhpat industrial Est; Lahore, India. Testosterone propionate (T) and estradiol valerate E 2 (puregynon depot) were used for the induction of prostate enlargement at a dose of 400µg T and 80µg E2 [9]. This was administered to the rats for three weeks subcutaneously in the inguinal region after which a few rats were sacrificed and inspected for gross examination of prostate enlargement.

Animals

A total of twenty-five (25) Wistar rats weighing between 250-350g were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. The rats were used for the experiment. The rats were acclimatized for two weeks before the experiment commences. The rats were exposed to approximately 12-hour light/dark cycles under humid tropical conditions, given tap water and feed *ad libitum*, and were housed in standard plastic cages (five per cage) throughout the 45-day duration of the study. The animal room was well be ventilated with a temperature range of 27-29 ^{0}C.

Induction of BPH

BPH was induced by exogenous administration of testosterone and estradiol in staggered doses (three times a week respectively) for three weeks according to [9] with modification [10].

Animal grouping and treatment

The animals were divided into nine (5) groups each comprised of five (5) male rats. Four groups were induced with BPH which were grouped as group 1 to group 4). Groups 1 and 2 received 50 and 100mg kg^{-1} body weight (bw) of *Prosopis africana* extract; group 3 received finasteride (orthodox drug) at 0.1mg kg^{-1}; all by gavages for forty five days, group 4 was left untreated for forty five days before sacrifice to assess possible reversal of the exogenous induction and group 5 served as normal control. The animals were weighed prior to the commencement of the

experiment and subsequently every week till the end of the experiment. The fluid and water intake was taken daily till the end of the experiment.

Determinations of Biochemical Parameters

After 45 days, the rats were anaesthetized by a brief exposure to trichloromethane vapour and bled by cardiac puncture. The sera were carefully separated and used for the determination of various biochemical analyses. Each rat's carcass was promptly dissected and the prostates were carefully excised. Two prostates per group were randomly selected and their dorso-lateral lobes were dissected out and immediately processed for histology. The other three prostates pre group were freed of external fascias, washed in cold normal saline, blotted with filter paper and weighed on a sensitive balance. Subsequently, they were homogenized in ice-cold normal saline and the homogenates was used for the determination of the protein content of the prostate.

Determination of PSA

Serum PSA was determined using ready to use Enzyme Immunoassay commercial manufactured kit by Teco Diagostic Laboratory, USA. The ELISA test is based on the principle of solid phase enzyme linked immunosorbent assay, where the antibody to be measured is incubated with specific antigen coupled to a solid phase [11]. PSA molecule was sandwiched between solid phase (rabbit anti-PSA antibody) and enzyme linked antibodies (monoclonal anti-PSA conjugated to Horse raddish peroxidase). After removing the unbound-labelled antibodies, TMB was added as substrate for the conjugated enzyme to digest resulting into colour complex that is proportional to the concentration of PSA in the serum [12].

Determination of Serum Prolactin, Testosterone and Estradiol Concentrations

A solid phase enzyme immunoassay (EIA) quantitative method was employed for the determination of the concentration of each hormone in the serum. The prolactin protocol utilizes 2 antibodies directed against distinct antigenic determinants of the prolactin molecule as described by [13]. The testosterone protocol was based on the method of [14] and involves the competition of testosterone in serum and enzyme-labeled testosterone for binding with anti-testosterone antibody immobilized on the microwell surface. The estradiol protocol also utilizes the competitive binding principle as described by [15].

Determination of protein content of the prostate

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formationof coloured complex. The protein content of the prostate was determined using the modified Biuret method of [16]. Briefly, 3.9ml of deionized water and 4.0 ml of Biuret reagent were added to 0.1ml of the aliquot and allowed for 30 minutes at room temperature to develop. A standard and blank were also prepared by adding 4.0ml of Biuret reagent and 3.9ml of deionized water to 0.1ml of standard albumin and water respectively. Subsequently, the absorbance of the test and standard were read against the blank at 540nm using a UV/VIS spectrophotometer.

Statistical Analysis

The experimental data were analysed for statistical significance by one-way analysis of variance and post hoc comparison using the SPSS version. All data were reported as mean ± SD and statistical significance was accepted at $P < 0.05$.

Results

Weekly body weight

The effect of oral administration of extract and standard drug (finasteride) on body weight is presented in Table 1. The BPH-control group exhibited a decline in body weight when compared with normal control. The animals showed significant weight loss and declined appetite after three weeks of BPH induction. The extract and standard drug treated groups showed an increase in body weight when compared with the BPH control group. Administration of extract or standard drug (finasteride) improved the body weight near normal level when matched with normal control. In the untreated group, weight decrease occurred.

Prostate weight

The average weight of the prostates was at the maximum in the BPH control group matched with normal control group (Table 1). Therefore, BPH control group showed a momentous ($P< 0.05$) increase in prostate weight when

compared to normal control. The extract and standard drug treated groups showed a decrease in prostate weight when compared with the BPH-control group. Administration of extract or standard drug (finasteride) reduced the prostate weight to near normal.

Protein content of the prostate

The content of protein in the rats' prostate gland was at peak in BPH control group and lowest in the normal control group (Table 2). There was significant (P<0.05) rise in protein content of the prostate in BPH-control group when compared with the value obtained for normal control. Treatment of BPH induced groups with extract and standard drug brought a decrease in protein content of the prostate in different groups. Protein content of the prostate of all the treated group was statistically similar to the normal control group.

Effect of extract on PSA concentration of BPH-induced rats

Table 2 showed the plasma PSA concentration in the treated (extract and finasteride) and controlled groups. There was a significant (P< 0.05) elevation of PSA concentration in the BPH control group when compared with the treated groups and normal control while there were significant decrease in PSA concentrations in the treated groups.

Effect of extract on testosterone concentration of BPH-induced rats

Table 2 showed the plasma testosterone concentrations in the treated (extract and finasteride) BPH induced rats relative to the control groups. In the BPH control group the level of testosterone was significantly (P< 0.05) higher when compared with the normal group. However, the hormone level decreased significantly (P< 0.05) in the treated groups when compared with the BPH control.

Effect of extract on estradiol concentration of BPH-induced rats

Table 2 showed the plasma estradiol concentrations in the treated and controlled groups. In the BPH control group the concentration of estradiol was significantly (P < 0.05) higher than the normal control. The hormone level decreased significantly in the treated groups when compared with the BPH control (P< 0.05).

Effect of extracts on prolactin concentration of BPH-induced rats

Table 2 showed the plasma prolactin concentrations in the treated and controlled groups. In the BPH control group the concentration of prolactin was significantly higher than the normal control. The concentrations of prolactin decreased significantly (P< 0.05) in the all the treated groups when compared with the BPH control. The mean concentrations of prolactin was statistically related (P< 0.05) between the normal group and each of the treated group.

Table 1: Effect of extract of PA and finasteride body weight and prostate weight

Group	Body weight (g)	Prostate weight (g)
BPH + 50mg PA	286.80 ± 8.41^{b}	0.97 ± 0.33^{b}
BPH + 100mg PA	311.20 ± 5.45^{c}	0.85 ± 0.41^{ab}
BPH + Finasteride	320.40 ± 8.99^{d}	0.63 ± 0.23^{ab}
BPH Control	270.40 ± 8.93^{a}	2.21 ± 0.28^{c}
Normal Control	322.20 ± 13.99^{d}	0.41 ± 0.071^{a}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), *Prosopis africana* (PA). Identical superscript (i.e. a) means there is no significant difference between the comparing group P>0.05. Non- identical superscripts (i.e. a, b, c, d) means there is significance between the comparing groups at P < 0.05.

Table 2: Effect of extract of PA and finasteride PSA, testosterone, estradiol, prolactin and protein content

Group	PSA (ng/ml)	Testosterone (ng/ml)	Estradiol (ng/ml)	Prolactin (ng/ml)	Protein content of prostate (g/dl)
BPH + 50mg PA	4.31 ± 0.33^{d}	4.70 ± 0.20^{cd}	527.22 ± 4.22^{c}	6.16 ± 0.14^{a}	$6.90\pm.41^{c}$
BPH + 100mg PA	4.17 ± 0.38^{d}	4.68 ± 0.31^{cd}	527.60 ± 8.44^{c}	6.12 ± 0.34^{a}	6.72 ± 0.77^{c}
BPH + Finasteride	2.54 ± 0.39^{a}	3.86 ± 0.34^{ab}	510.27 ± 4.96^{ab}	5.79 ± 0.55^{a}	4.89 ± 0.39^{b}
BPH Control	9.20 ± 0.69^{e}	5.18 ± 0.29^{d}	663.72 ± 22.34^{d}	7.40 ± 0.40^{b}	8.61 ± 0.46^{a}
Normal Control	3.79 ± 0.15^{bcd}	3.66 ± 0.56^{a}	499.27 ± 11.06^{a}	5.77 ± 0.10^{a}	4.24 ± 0.29^{d}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), *Prosopis africana* (PA). Identical superscript (i.e. a) means there is no significant difference between the comparing group $P>0.05$. Non- identical superscripts (i.e. a, b, c, d) means there is significance between the comparing groups at $P < 0.05$.

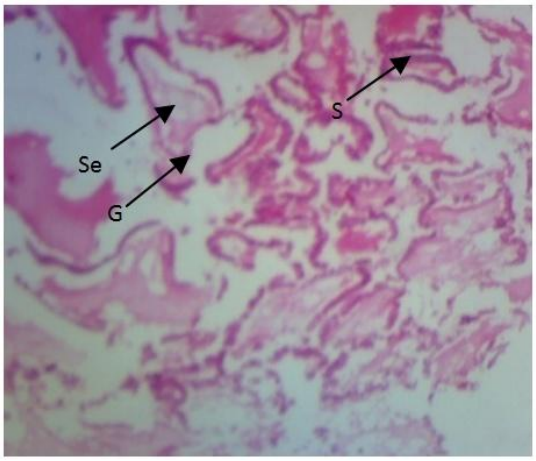

PLATE 1: Photomicrograph of prostate of rat induced with BPH and treated with 50mg PA (mag. x200). G = gland, S = stroma, Se = secretion

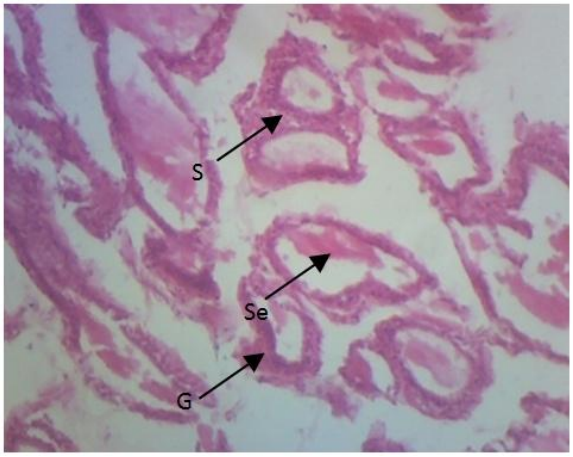

PLATE 2: Photomicrograph of prostate of rat induced with BPH and treated with 100mg PA (mag. x200). G = gland, S = stroma, Se = secretion

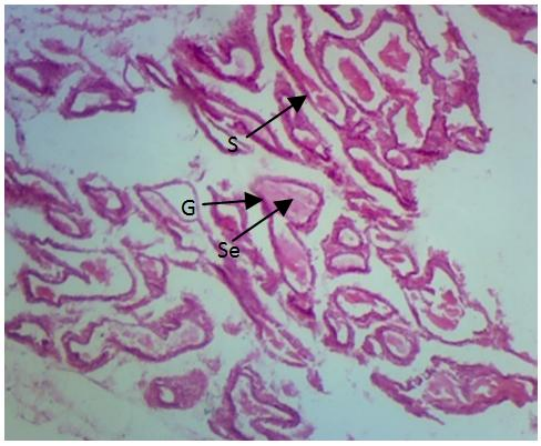

PLATE 3: Photomicrograph of prostate of rat induced with BPH and treated with Finasteride (mag. x200). G = gland, S = stroma, Se = secretion.

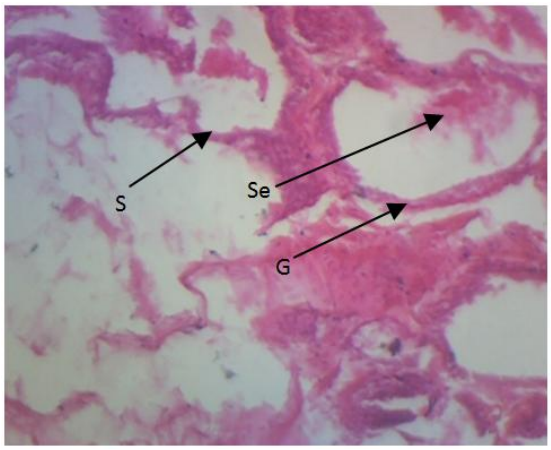

PLATE 4: Group 8: Photomicrograph of prostate of rat induced with BPH and untreated (mag. x200).G = gland, S = stroma, Se = secretion.

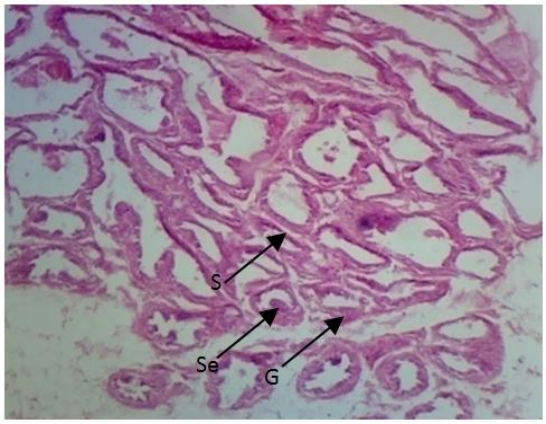

PLATE 5: Photomicrograph of prostate of rat without induction of BPH and no treatment (Normal Control). G = gland, S = stroma, Se = secretion.

Discussion

Benign prostatic hyperplasia (BPH) is one of the most common conditions in elderly males, with an incidence of approximately 50-60% of males age 40-60, and greater than 90% of men over 80 [17]. However, evidence from autopsy studies of young men reveal that this disease process may start as early as the late 20's, and may have an incidence rate of 10% at that age [17].

Treatment with the extract for 45 days significantly inhibited the development of induced prostatic hyperplasia, which was evident in the reduction in the elevated prostate weight, reduced prostatic specific antigen (PSA), testosterone, estradiol and prolactin levels in the serum. The increased prostate weight is used as one of crucial markers of BPH according to previous study [18-19]. BPH is characterized by stromal and epithelial cells hyperplasia, resulting in prostate enlargement. In previous studies, animals with BPH had a significant increase in prostate weight compared with normal control animals, whereas those of animals treated with finasteride or others herbal remedies for the management of BPH had significantly reduced the weight compared with BPH animals [18, 20]. Increase in cell number (hyperplasia) of the prostate would come with a collateral increase in its weight. Also increase in cell number in a tissue also goes with a collateral increase in the protein content of the tissue [21]. This might explain the observed elevation of protein content in the prostate tissue which reduced with the treatment.

Animals experimentally induced with BPH exhibited hyperplasia of prostate gland as well as body weight loss. In the present study, the animals with BPH showed an increased relative prostate weight compare to normal group ($P<0.05$). In contrast, the animals treated with extract showed a reduction in relative prostate weight compared to BPH group ($P<0.05$). Treatment with the extract and finasteride resulted in marked decrease in the size of prostate after 45 days of daily administration while periodic body weight gain was also recorded. These results indicate that the extract attenuated the prostatic enlargement.

The extract seemed to have stimulated increase in appetite for eating and water consumption which appeared to have been suppressed during enlargement process with the testosterone and estradiol which resulted to the observed weight gain. The extract reduced the increase in cell number in the prostates of rats in the treated groups (as assessed by the measured parameters) relative to the BPH control group. In previous studies finasteride or other agents used to treat BPH decrease the relative prostate weight [22]. It is note-worthy that any reduction in the mass of the prostate would translate to a reduction in the irritative symptoms of BPH which are usually the most bothersome symptoms [23]. Symptom severity in BPH is known to correlate with overall health status [24] such that any agent that can reduce the symptoms of BPH (in this case by reducing the mass of the prostate) is usually useful.

The PSA level which was elevated after the animals were induced with benign hyperplasia was observed to have decreased significantly after treatment with the extract and finasteride. PSA is a glycoprotein produced in low quantities by cells of the prostate gland and present in serum which could be used as semi-quantitative indicator or marker for BPH and prostatic cancer [25].

Testosterone is also an important agent considered in benign hyperplesia because of its involvement in prostate cell proliferation [26]. In this study, it was observed that benign hyperplasia led to increase in testosterone level. However, in animals treated with extract/finasteride the levels decreased significantly. The reduction in testosterone level in the treated animals may be attributed to extract activity which enhanced the decrease in the level of circulating testosterone that could constitute a risk factor for hyperplasia of prostate gland. Although the causes of BPH remains incompletely understood, studies showed that high level of free (active) testosterone promote the proliferation of prostate cells [27-28]. The mode of action is most often postulated to be through the activity of 5α-reductase found mainly within the stromal cells which leads to dihydrotestosterone (DHT) production, the major mediator of prostatic growth [29-30]. Testosterone has equally been noted to initiate growth stimulation by binding to androgen receptors [31].

As men age, estrogen (eg, estrone and estradiol) levels appear to increase. Aromatase, an enzyme which converts testosterone into estrogen, also increases with age in men [32]. BPH risk also increases with age and studies have identified high concentrations of estradiol in cells from hyperplastic prostates [33]. Further investigations into the action of estrogen receptors in prostate cells led one group of researches to conclude that *estrogen may contribute at*

some level to the etiology of the most prevalent prostatic diseases including BPH [34]. In this study there is elevated level of estradiol in the BPH control group and decreased level of it in the extract treated groups.

Prolactin (PRL) has classically been considered as a pituitary-derived peptide hormone but over the last decade expression of the PRL gene has also been demonstrated in several extra pituitary tissues [35]. Prolactin (PRL) regulates prostate development, growth, and differentiation [36]. Based on a wide literature in experimental models, hyperprolactinemia should induce prostate hypertrophy [37-38]. PRL is expressed in rat and human prostate epithelium [39], and thus the prostate, in analogy with other tissues, can directly process PRL by posttranslational glycosylation, phosphorylation, or proteolytic cleavage [35] into molecular derivatives, with different cellular targets and biological activities. The level of locally produced prostatic PRL was demonstrated to be regulated by androgens [39]. There is observed significant decrease in level of prolactin in the treated groups when compared to the BPH control which shows significant increase when compared to the normal control and extract treated groups. These findings were supported by histological examination of the prostate tissue. It can be suggested *Prosopis africana* can be very useful in management of BPH because of its capacity to influence the production of these hormones.

Conclusion

Treatment of prostate cells with the fermented seed of *Prosopis africana* might have induced distinct morphologic changes, including polarisation of the nucleus which might have resulted to the reduction in the size of the prostate as observed in this study and subsequent reduction of the secretions by this gland. Hence the preparation can be used in management of induced BPH.

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