



Effect of *Prosopis africana* Seed Extract on Lipid Profile of Experimentally Induced Prostatic Hyperplasia Animal Model

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Abstract Benign prostatic hyperplasia (BPH) is an age-related disease, as the prostate enlarges, the layer of tissue surrounding it stops it from expanding, causing the gland to press against the urethra. The symptoms of BPH vary, but the most common is problems with urination which makes life unbearable. 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 weighed. Treatment with extract caused a significant decrease in the size of the enlarged prostate in a dose related manner ($p < 0.05$) when compared with the BPH control group. Progressive weight gain occurred in extract and finasteride treated groups. The level of cholesterol and LDL-C were significantly reduced when compared to the BPH control. There was no significant change in HDL concentration in all groups. The significant reduction of cholesterol and LDL supported by reduced prostate weight suggest that the extract hold potentials for reversal and reduction of induced BPH in rats model.

Keywords Testosterone, estradiol, cholesterol, TG, HDL, LDL-C

Introduction

Benign prostatic hyperplasia (BPH) is characterized by a non-malignant hypertrophy of the prostate which is caused by hormonal processes and/or imbalances within the glandular tissue. Hyperplasia begins in the periurethral region and includes the stromal, epithelial, and smooth muscle tissues of the gland. The fibrous capsule surrounding the gland forces most of the growth inward, compressing the urethra and causing the typical urinary symptoms characteristic of this disease [1]. Decreased force and caliber of the urine stream, urinary hesitancy, urgency, frequency, post-void dribbling, a sensation as if the bladder is not completely emptying, dysuria, and nocturia are all common, and are related to a blockage of urinary outflow and inflammation of the urethra as it passes through the prostate. Incontinence and hydronephrosis are also possible complications of advanced BPH. When BPH is found in its earlier stages, there is a lower risk of developing such complications.



Local conversion of testosterone to its metabolite dihydrotestosterone (DHT), catalyzed by the enzyme steroid 5- α -reductase (5-AR), is implicated as a causative factor in BPH. DHT exerts its effects by binding to androgen receptors in the nucleus of prostate cells, stimulating cellular growth and division [2]. It is interesting to note that DHT binds many times stronger than testosterone to these androgen receptors [3] and thus it is more likely that DHT is the preferred substrate for these receptors.

Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of either herbal preparations or conventional drugs of plant origin. *Prosopis africana* has been reported to possess anti-nociceptive property. It has the ability to reduce pain as in the same way as the aspirin [4]. Pain and inflammation are associated with pathology of various clinical conditions including BPH and prostate cancer [5]. This plant has been used traditional in the treatment of various types of ailments including prostate disorders and curing of male sterility [6-11]. The endocarp gum of *Prosopis africana* seed contains high content of galactose and mannose. Galactose is a special type of natural sugar that gives sustained energy for a longer time compared to other sugar. Mannose is important for treatment of urinary tract infections [12].

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 [13]. The fermentation was done at room temperature ($\approx 25^{\circ}\text{C}$) for 72h. The fermented seeds were then sun-dried to a constant weight and milled using hammer mill to produce *prosopis* seed flour [14]. The flour was kept in a refrigerator at 4°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 $400\mu\text{g}$ T and $80\mu\text{g}$ E2 [15]. 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. All Chemicals used in this study were of analytical grade and were obtained from reputable companies.

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 ventilated with a temperature range of $27\text{-}29^{\circ}\text{C}$. The Institutional Animal Ethics Committee approved the study before the experiment and certified all experimental protocols.

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 [15] with modification [16].

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 per group were freed of external fascias, washed in cold normal saline, blotted with filter paper and weighed on a sensitive balance.

Determination of total cholesterol, High density lipoprotein (HDL-cholesterol) and Triacylglycerol determination

Determination of total cholesterol, high density lipoprotein (CHOD-PAP method) as described by [17] while Triacylglycerol (GPO-PAP method) as described by [18]. Low density lipoprotein was determined as described by [19]. All were done using Randox assay kits.

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 \pm SD and statistical significance was accepted at $P < 0.05$.

Results

Feed and fluid intake of the different experimental groups

The effect of oral administration of extract of *Prosopis africana* and finasteride (standard drug) on feed and fluid intakes of BPH induced rats for 45 days is presented in Table 1. The results proved that there was significant ($P < 0.05$) rise in feed and fluid intakes in treated groups when compared to BPH control group. Administration of extract or standard drug (finasteride) improved the feed and water intakes close to normal when compared with normal control.

Body weight

The effect of oral administration of extract and standard drug (finasteride) on body weight is presented in Table 2. 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 compared 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 when compared with normal control group (Table 2). Therefore, BPH control group showed a significant ($P < 0.05$) 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.

Effect of extract on serum cholesterol concentration of BPH-induced rats

Serum cholesterol concentrations (in mg/dl) were 180.92 ± 21.81 for BPH control, 136.88 ± 5.89 for normal control, 163.67 ± 6.00 for 50mg PA, 162.94 ± 4.38 for 100mg PA, and 160.00 ± 7.93 for finasteride. There was a significant ($P < 0.05$) rise in the serum cholesterol level in BPH control group when compared with the treated groups. In all the treated groups there was an improved reduction of serum cholesterol levels when compared to the BPH control group.



Effect of extract on serum triacylglycerol (TG) concentrations of BPH-induced rats

Serum TG concentrations (in mg/dl) were 82.13±5.42 for BPH control group, 67.38±1.62 for normal control, 74.38±5.14 for finasteride, 81.50±2.98 for 50mg PA and 81.25±2.96 for 100mg PA. The results indicate that there was no significant reduction in the TG concentrations in the PA treated groups.

Effect of extract on serum high density lipoprotein (HDL-c) concentrations of BPH induced rats

Serum HDL-c concentrations (in mg/dl) were 49.80±1.76 for BPH control, 53.33±1.64 for normal control, 49.11±5.53 for 50mg PA, 51.43±4.07 for 100mg PA and 50.75±1.57 for finasteride. All groups treated were statistically similar with normal control.

Effect of extract on serum low density lipoprotein (LDL-c) concentrations of BPH induced rats

Serum LDL-c concentrations (in mg/dl) were 22.27±12.35 for BPH control, 7.35±2.69 for normal control, 15.03±2.04 for 50mg PA, 13.75±3.81 for 100mg PA and 15.85±3.59 for finasteride. There was a significant ($P < 0.05$) increase in LDL-C level of BPH control group when compared with the treated groups. The administration of extract showed a significant decline in the levels of LDL concentrations when compared to the BPH control.

Table 1: Effect of extract of PA and finasteride on feed and fluid intake

Group	Feed intake (g)	Fluid intake (ml)
BPH + 50mg PA	98.01±19.26 ^{bc}	94.19±3.89 ^{bc}
BPH + 100mg PA	96.42±15.79 ^c	93.37±5.04 ^{bc}
BPH + Finasteride	98.30±19.86 ^{bc}	97.02±6.72 ^b
BPH Control	69.63±5.42 ^a	68.02±5.28 ^a
Normal Control	116.21±21.29 ^b	98.63±6.59 ^b

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) means there is significance between the comparing groups at $P < 0.05$.

Table 2: 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 3: Effect of extracts of PA and finasteride on serum lipid profile

Group	Cholesterol (mg/dl)	Triacyl-glycerol (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
BPH + 50mg PA	163.67±6.00 ^d	81.50±2.98 ^c	49.11±5.53 ^a	15.03±2.04 ^{abc}
BPH + 100mg PA	162.94±4.38 ^d	81.25±2.96 ^c	51.43±4.07 ^a	13.75±3.81 ^{ab}
BPH + Finasteride	160.00±7.93 ^{cd}	74.38±5.14 ^b	50.75±1.57 ^a	15.85±3.59 ^{bc}
BPH Control	180.92±21.81 ^e	82.13±5.42 ^c	49.80±1.76 ^a	22.27±12.35 ^c
Normal Control	136.88±5.89 ^a	67.38±1.62 ^a	53.33±1.64 ^a	7.35±2.69 ^a

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), High density lipoprotein (HDL), Low density lipoprotein (LDL), *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, e) means there is significance between the comparing groups at $P < 0.05$.



Discussion

The recent shift from orthodox medicine to plants with therapeutic potentials is a major contribution to a decline in the prevalence of atherosclerosis and other cardiovascular diseases [20-21]. The evaluation of lipid profile showed that the extract was able to reduce the concentrations of cholesterol, triacylglycerol, low density lipoprotein and very low density lipoprotein in the treated animals this was in line with previous report by [22] which showed similar progression. Some researchers have established that total-cholesterol and low-density lipoprotein were considerably elevated in BPH patients compared to normal individual [23]. Following the report by [24], the quantity of body fat found in men increases as the individual gets older. Moreover, obesity has been found to possess the capacity to stimulate actions of sympathetic nerves and such actions have an effect on the development and progression of prostate hyperplasia [24].

Hence, in addition to testosterone, which is one of the risk factor for BPH, the increased quantity of body fat brought about by increased age can combine to increase the attack rates of BPH. The reduction of the body fat is imperative to enhance the treatment of BPH. HDL-Cholesterol is known to have a protective effect against cardiovascular disease, since it removes excess cholesterol from circulation and carries it back to the liver where it is degraded or converted into bile acid [25]. Also, HDL-C is considered to have anti atherogenic properties. It has also been shown that an increase in HDL-Cholesterol correlates inversely to coronary heart disease [26-27]. It can be suggested that *Prosopis africana* can be used in management of benign prostatic hyperplasia which is an age-related disease. In the elderly there is the tendency of deposition of fat in the adipose tissue. This investigation shows that *Prosopis africana* has the ability to influence the metabolism of lipid and this can facilitate the management of this disease condition.

Conclusion

The quantity of body fat has been established to increase as a man gets older; this will result to deposition of more fats on the arteries narrowing it. Furthermore, obesity has been reported to possess the capacity to stimulate actions of sympathetic nerves and such actions have an effect on the development and progression of prostate hyperplasia. Therefore logically it can be suggested that reduction of lipid component of the body will go a long way in management of benign prostatic hyperplasia.

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