

Tissue-Protective Effect Of Prosopis Africana Seed Extract On Testosterone And Estradiol Induced Benign Prostatic Hyperplasia Of Adult Male Rats

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Abstract: Benign prostatic hyperplasia (BPH) is one of the most common diseases affecting aging man. In this study, we investigated the effect of Nigerian indigenous plant, *Prosopis africana* (PA) on BPH. BPH was induced in male rats weighing 250-350g through exogenous administration of testosterone and estradiol by subcutaneous injection. A total of 30 rats were divided into five groups. One group was used as a control and the other groups received subcutaneous injections of the hormones for 3 weeks to induce BPH. Groups 1 and 2 were treated with different doses of PA extracts and group 3 received finasteride, all by gavages for forty-five days, while group 4 was left untreated, group 5 served as normal control. After forty-five days of treatment with PA extract, the rats were anaesthetised by short contact with trichloromethane vapour. Blood was also collected by cardiac puncture and the sera were cautiously centrifuged and used for the determination of biochemical indices. The liver and kidney were also harvested and homogenized and used for the assays of oxidative activities. The levels of CAT, SOD and GSH in the extract treated rats were comparable to normal control while in the BPH control showed a significant reduction. Similarly, the activity of TBARS in the extract treated group was comparable to normal control while showing significant ($P < 0.05$) increase in the BPH control group. Therefore *Prosopis africana* seed can be used to prevent oxidative tissue damage resulting from BPH, by maintaining the integrity of the tissue as well as preventing the progression or complications that may arise from the disease.

Keywords: Catalase, Superoxide dismutase, Thiobarbituric acid reactive substance, Glutathione, dihydrotestosterone and finasteride

I. INTRODUCTION

Benign prostatic hyperplasia (BPH) is an age-related non-malignant enlargement of the prostate gland that results from a neoplastic unregulated growth of the prostate gland (Paglione, 2010). Its etiology is still largely unresolved. However, it seems that the pathoetiologic mechanism is endocrine controlled and involves alterations in the metabolism of androgens and estrogens (Suzuki *et al.*, 1995). Some studies have however linked the metabolic syndrome to the etiology

of BPH (Ejike and Ezeanyika, 2008). Benign prostatic hyperplasia (BPH, benign prostatic hypertrophy), affects almost all men in some degree as they age and can cause a significant disruption of lifestyle due to urinary outflow obstructive and irritative symptoms. An accumulation of estrogen in the aging prostate, along with increased conversion of testosterone to its more active metabolite dihydrotestosterone (DHT) seems to induce this aberrant hyperplasia.

Plant materials are central to tradomedical practices and have remained useful sources of new drugs (O'Brien, 2004). Although, orthodox medical practice is generally acceptable, alternative healthcare is still relied on all over the world (O'Brien, 2004; Leckridge, 2004). In the developing countries of the world, traditional herbal medicine is often used side by side Western medicine with herbal medicine taking the upper hand when the cost of Western medicine is beyond reach (Busia, 2005). Hence, the need to study this plant to substantiate scientifically its' claim in management of benign prostatic hyperplasia.

II. MATERIALS AND METHODS

PLANT MATERIAL

Prosopis seeds were purchased from Ishibori market in Ogoja Local Government of Cross river State, Nigeria. The seeds which weighed 500g was sorted, cleaned and boiled for 5h using a gas cooker and allowed to cool to room temperature. The boiling helped 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 decorticated seeds 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 Achi, (1992). The fermentation was done at room temperature for 72h. The fermented seeds were then sun-dried to a constant weight and milled using hammer mill to produce *Prosopis* seed flour (Yusuf *et al.*, 2008). 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 Medipharma Ltd., 108-Kotlakhpat industrial Est; Lahore, India. Testosterone propionate (T) and estradiol valerate E 2 (puregon depot) were used for the induction of prostate enlargement at a dose of 400µg T and 80µg E2 (Bernoulli *et al.*, 2008). 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 thirty (30) 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 commenced. The rats were exposed to approximately 12-hour light/dark cycles under humid tropical conditions, given tap water and feed *ad libitum*. They were housed in standard plastic cages (six per cage) throughout the 45-day duration of the study. The animal

room was well be ventilated with a temperature range of 27±2°C.

INDUCTION OF BPH

BPH was induced by exogenous administration of testosterone and estradiol in staggered doses (three times a week) for three weeks according to (Bernoulli, 2008) with modification by Mbaka *et al.* (2013).

EXPERIMENTAL DESIGN

The animals were divided into five (5) groups each comprised of six (6) 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. The liver and kidney were harvested and homogenized and used for the assays of oxidative activities.

DETERMINATION OF THIOBARBITURIC ACID REACTIVE SUBSTANCE (TBARS) CONCENTRATION

Thiobarbituric acid-reactive substance (TBARS) in tissues was determined by the procedure of Fraga *et al.* (1988). At low pH 3.5 and high temperature (100°C) Malondi-aldehyde (MDA) binds with thiobarbituric acid (TBA) to produce a pink colour that can be measured at 532nm.

ASSAY FOR CATALASE ACTIVITY

Calatase was assayed according to the method of Machly and Chance (1954). Catalase can act on H₂O₂ to yield H₂O and O₂. The concentration of H₂O₂ in the in absorbance reading after 10min was determined as catalase activity expressed in terms of units/mg protein. The absorbance was measured at 230nm.

DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Superoxide Dismutase activity assay was carried out according to the method described by Martin *et al.*, (1987). Exactly 920 μ L of assay buffer (Phosphate buffer pH 7.8) of 0.05M was added into clean test tube containing 40 μ L of sample; they were mixed and incubated for 2mins at 25°C. 40 μ L of hematoxylin solution was added, mixed quickly and the absorbance was measured at 560nm. Auto-oxidation of hematoxylin is inhibited by SOD at the assay pH, the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range (Martin *et al.*, 1987).

ESTIMATION OF GLUTATHIONE CONCENTRATION

The method of Rukkumani *et al.* (2004) was followed in estimating the level of reduced glutathione (GSH). The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow colour when 5, 5 – dithiobis – (2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman's reagent with the reduced glutathione, 2- nitro-5-thiobenzoic acid possesses a molar absorption at 412nm. Reduced GSH is proportional to the absorbance at 412nm.

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 $P < 0.05$.

III. RESULTS

LIVER AND KIDNEY SUPEROXIDE DISMUTASE (SOD) ACTIVITY

There was a significant ($P < 0.05$) reduction in the expression of superoxide dismutase in the liver and kidney of the BPH control group when compared with the normal control. However, upon treatment all the treated groups showed a significant rise in tissue SOD activity when compared with the BPH control.

CONCENTRATION OF GLUTATHIONE (GSH)

There was oxidative stress due to benign prostatic hyperplasia induction shown in the significant decrease in concentration of the GSH of BPH control compared to normal control ($P < 0.05$). However, there was a significant ($P < 0.05$) rise in the concentration of glutathione (GSH) in the PA extract and finasteride treated groups when compared with the untreated group. The concentration of GSH in 100 mg *Prosopis africana* and standard drug are similar tending towards normal control.

LIVER AND KIDNEY CATALASE (CAT) ACTIVITY

There was significant ($P < 0.05$) reduction in the expression of catalase in the liver and kidney of the BPH control group when compared with normal control. Administration of fermented seed of *Prosopis africana* and the standard drug tended to increase the catalase expression in the liver and kidney of treated groups.

CONCENTRATION OF LIVER AND KIDNEY MALONDIALDEHYDE (MDA)

The assessment of TBARS confirmed amplification in peroxidative expression in the BPH control group while showing a decline that was statistically similar to normal control in the extract treated groups and finasteride treated group. So there was a considerable ($P < 0.05$) rise in malondialdehyde (MDA) in the liver and kidney of BPH control group when compared with the normal control. Treatment with fermented seed of *Prosopis africana* and the standard drug tended to significantly ($P < 0.05$) reduce the MDA concentration in the liver and kidney of treated groups.

Group	SOD Liver (mg Protein)	CAT Liver (mg Protein)	MDA Liver (mg Protein)	GSH (mg/gm)
BPH + 50mg PA	6.13 \pm 0.52 ^b	44.85 \pm 4.55 ^b	21.18 \pm 0.84 ^b	45.08 \pm 2.75 ^b
BPH + 100mg PA	6.79 \pm 0.51 ^{bc}	46.07 \pm 8.73 ^b	20.98 \pm 1.78 ^b	47.60 \pm 1.90 ^{bc}
BPH + Finasteride	8.03 \pm 0.67 ^{cde}	57.46 \pm 1.81 ^{cd}	19.62 \pm 0.57 ^{ab}	55.61 \pm 2.94 ^{ef}
BPH Control	3.13 \pm 0.76 ^a	20.53 \pm 4.88 ^a	31.32 \pm 1.40 ^c	27.76 \pm 1.52 ^a
Normal Control	11.19 \pm 1.10 ^f	68.43 \pm 10.44 ^e	16.49 \pm 4.44 ^a	63.81 \pm 0.67 ^f

Table 1: Effect of PA and Finasteride on SOD, GSH, CAT and MDA

Values are expressed as Mean \pm SD. Benign prostatic hyperplasia (BPH), *Prosopis africana* (PA); superoxide dismutase, (SOD); glutathione (GSH); catalase (CAT) and malondialdehyde (MDA). 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, f, g) means there is significance between the comparing groups at $P < 0.05$.

Group	SOD Kidney (mg Protein)	CAT Kidney (mg Protein)	MDA Kidney (mg Protein)
BPH + 50mg PA	4.45 \pm 1.23 ^b	39.11 \pm 3.23 ^b	18.31 \pm 0.37 ^c
BPH + 100mg PA	5.07 \pm 0.44 ^b	40.90 \pm 6.23 ^b	18.14 \pm 0.23 ^c
BPH + Finasteride	6.75 \pm 1.24 ^{cd}	56.30 \pm 5.71 ^{de}	16.11 \pm 0.35 ^{bc}
BPH Control	2.47 \pm 0.63 ^a	18.17 \pm 1.60 ^a	24.29 \pm 2.45 ^f
Normal Control	9.11 \pm 0.63 ^c	60.63 \pm 5.60 ^e	14.34 \pm 1.65 ^a

Table 2: Effect of PA and Finasteride on SOD, GSH, CAT and MDA

Values are expressed as Mean \pm SD. Benign prostatic hyperplasia (BPH), *Prosopis africana* (PA); superoxide dismutase, (SOD); glutathione (GSH); catalase (CAT) and malondialdehyde (MDA). 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, f) means there is significance between the comparing groups at $P < 0.05$.

IV. DISCUSSION

Benign prostatic hyperplasia (BPH) is known to be intimately linked with increased oxidative stress which increases with age (Aryal *et al.*, 2007). Increased lipid peroxidation and decreased levels of superoxide dismutase, catalase and GSH have been found to be associated with BPH (Ahmad *et al.*, 2012; Eze *et al.*, 2015; Minciullo *et al.*, 2015). Various herbal extracts reduce oxidative stress in induced BPH rats (Lopez *et al.*, 2009; Hevesi *et al.*, 2009). In addition; *Saw palmetto* extract, which is commonly used to treat BPH, has shown antioxidant effects (Belostottskaia *et al.*, 2006). Therefore, antioxidants can be considered good candidates to suppress the development of BPH. The reduction in the activity of the antioxidant enzymes, CAT, SOD and GSH following induced hyperplasia in the untreated animals must have been due to the accumulation of superoxide anion radicals and hydrogen peroxide which accentuates peroxidative activity (Nurozten and Maarten, 2011). The extract treated animals exhibited significant increase in the activity of these antioxidant enzymes compared to the BPH control. The extract might have reactivated the activities of the enzymes through its active principles that enabled for effective scavenging of reactive oxygen species (ROS) and reducing oxidative stress. Previous studies of seed and pod extracts of *Prosopis africana* revealed that it contains phlobatannin, flavonoid, Polyphenols, tannin, saponin, steroid and alkaloid (Adeiza *et al.*, 2009; Ajiboye *et al.*, 2013; Olajide *et al.*, 2013; Mariko *et al.*, 2016).

Flavonoids are plant pigments that are synthesized from phenylalanine (Harborne and Turner, 1984), comprises of a large group of polyphenolic compounds that are characterized by a benzo-y-pyrone structure. They contain conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants *in vitro* or cell free systems by scavenging superoxide anion, singlet oxygen, lipid peroxy-radicals, and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species (Duthie and Dobson, 1999; Birt *et al.*, 2001). Clifford and Cuppett (2000) divided the antioxidant mechanisms of flavonoids into free radical chain breaking, metal chelating, and singlet oxygen quenching (Cook and Samman, 1996; Rice-Evans *et al.*, 1995). The functionality in human health is supported by the ability of the flavonoids to induce human protective enzyme systems, and by a number of epidemiological studies suggesting protective effects against cardiovascular diseases, cancers, and other age-related diseases (Cook and Samman, 1996).

Some mechanisms have been proposed how flavonoids may help prevent steroid hormone-dependent cancers and other health related issues (Rice-Evans *et al.*, 1995). Isoflavonoids, such as phytoestrogens, have a wide range of hormonal and non-hormonal activities in animals or *in vitro* (Cassidy *et al.*, 2000), suggesting potential human health benefits of diets rich in these compounds. Flavonoids may act as antioxidants to inhibit free-radical mediated cytotoxicity and lipid peroxidation, as antiproliferative agents to inhibit tumor, growth or as weak estrogen agonists or antagonists to modulate endogenous hormone activity (Lyons-Wall and Samman, 1997; Birt *et al.*, 1999; Mao *et al.*, 2010).

Free radical injury has been linked with the pathogenesis of prostate cancer and BPH (Savas *et al.*, 2009). Free radicals cause attack on polyunsaturated membrane lipid (lipid peroxidation) generating a product called malondialdehyde (MDA) (Ugwu *et al.*, 2013). Serum levels of malondialdehyde however may be elevated in many prostatic lesions (Mittal and Scrivastava, 2005). Malondialdehyde is produced as a stable product of free radical peroxidation of cellular organelles and used as an index of free radical injury. This study observed higher serum levels of MDA in BPH control groups when compared with extract treated and normal control. These findings are similar to those observed by Mittal and Scrivastava (2005) and Savas *et al.*, (2009). In previous studies total flavonoid and phenolic contents was found to be correlated positively with total antioxidant activity of the plant (Erasto *et al.*, 2007). *Prosopis africana* seed can be suggested to contain antioxidants against aqueous radicals and reactive species ions and can be used in management of benign prostatic hyperplasia.

V. CONCLUSION

It can be inferred from the results obtained that *Prosopis africana* seed has the capacity to scavenge free radicals. Therefore it could be helpful in the management of benign prostatic hyperplasia.

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