

MANAGEMENT OF EXPERIMENTAL BENIGN PROSTATIC HYPERPLASIA IN RATS USING A FOOD-BASED THERAPY CONTAINING *TELFAIRIA OCCIDENTALIS* SEEDSChukwunonso E C C Ejike^{1,2} and Lawrence U S Ezeanyika^{*2}¹Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Nigeria, ²Department of Biochemistry, University of Nigeria, Nsukka – Nigeria^{*}E-mail: nonsoejkeccc@yahoo.com; ejike.nonso@mouau.edu.ng**Abstract**

The usefulness of diet containing *Telfairia occidentalis* seeds, in managing benign prostatic hyperplasia (BPH) in rats was studied. Twenty male Wistar rats were divided into four equal groups. BPH was induced by sub-cutaneous injection of dihydrotestosterone (DHT) and estradiol valerate (ratio, 10:1) every other day for 28 days. Rats in the test group were placed on the test diet for 7 days following disease induction. One control group (DC) was fed on a normal diet for 7 days following disease induction. Two other control groups, HC and HDC, were given sub-cutaneous olive oil (vehicle) for the same duration, and placed on the test diet and normal diet, respectively. Markers of BPH, and hormone profile were determined using standard methods. The results show that relative prostate weight and protein content of the prostates were lower [albeit not significantly ($p>0.05$)] in the test group, relative to the DC group. Serum prostatic acid phosphatase concentrations (U/L) decreased significantly ($p<0.05$) from 2.9 ± 0.2 in the DC group to 2.1 ± 0.7 in the test group. Histological findings corroborate these data. The testosterone: estradiol ratio ($\times 10^3$) was increased from 4.0 ± 0.2 in the DC group to 4.6 ± 0.2 in the test group. The test diet reduced the mass and secretory activity of the enlarged prostate and may act by increasing the testosterone: estradiol ratio.

Key words: benign prostatic hyperplasia, fluted pumpkin, induction, management

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 patho-etiological 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).

Treatment options for BPH target either a reduction in the mass of the prostate gland (static component of BPH) or a relaxation of the prostatic muscle tone (dynamic component of BPH). They include watchful waiting, medical therapy with α -blockers or 5 α -reductase inhibitors, hormone therapy, surgery and phytotherapy (Watson *et al.*, 2004; Kaplan, 2006). The use of complementary and alternative medicine (CAM) for the treatment of BPH is becoming popular. It is estimated that 30% of men diagnosed with prostate disease in North America use some CAM products (Nickel *et al.*, 2008), while such products constitute approximately 50% of all medicines prescribed for BPH in Italy (Di Silverio *et al.*, 1993) and almost 60% of such prescriptions in Germany and Austria (Buck, 1996).

This study investigated the usefulness of the seeds of fluted pumpkin (*Telfairia occidentalis* Hook f.) – a creeping tropical vine, belonging to the family Curcubitaceae – as a principal agent in a food-based therapy for the management of the static component of experimental (hormone-induced) BPH in Wistar rats. The results will contribute to the search for locally available food-based phytotherapeutic agents that can help manage this debilitating disease especially in resource-poor settings.

Materials and Methods**Diet Formulation**

Mature fluted pumpkin seeds were purchased from a local market in Nigeria. The fruits were sliced open and the seeds harvested, cleaned and shelled manually. Good seeds were carefully selected and boiled for 1 hour in tap water. The boiled seeds were thereafter dried in an oven at 40°C to a constant weight, and then milled in a laboratory mill. A diet containing 15% of the boiled, oven-dried and milled seeds and 75% normal rat chow was prepared, homogenized thoroughly and pelletized manually. The pellets were dried to a constant weight in an oven at 40°C and thereafter cooled and stored in air-tight containers until used as test diet. Normal manually-pelletized and oven-dried rat chow was used as control diets in the relevant groups.

Rats

Twenty 4-week old male Wistar rats weighing approximately 111 g each were obtained from the Animal Breeding Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. On arrival, the rats were acclimatized to the animal house for one week before being randomly assigned to four cages/groups. Housed in standard plastic cages (5 per cage), the rats were exposed to approximately 12 hour light/dark cycles under humid tropical conditions, and given tap water and feed *ad libitum* throughout the 35-day duration of the study. The protocol below was followed in treating the rats in the four groups.

Table 1: Design of study

Group	Hormonal Treatment (sub-cutaneous injections)	Diet (0-28 th day)	Diet (29 th -35 th day)
Test	9mg/kg body weight DHT + 0.9mg/kg body weight estradiol valerate every other day	Normal	15% TO incorporated
Diet Control (DC)	9mg/kg body weight. DHT + 0.9mg/kg body weight estradiol valerate every other day	Normal	Normal
Hormone Control (HC)	Olive oil (vehicle) every other day	Normal	15% TO incorporated
Hormone/Diet Control (HDC)	Olive oil (vehicle) every other day	Normal	Normal

DHT and estradiol valerate were procured from Sigma-Aldrich Laboratories GmbH, Germany and Schering AG, Berlin, Germany, respectively. TO stands for *Telfairia occidentalis* seeds.

After 35 days, the rats were fasted for 12 hours, anaesthetized by a brief exposure to trichloromethane vapor, and bled exhaustively by cardiac puncture. The sera were carefully separated and used for the 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. Subsequently, they were homogenized in ice-cold normal saline and the homogenates used for the determination of the protein content of the prostates.

Assays and Determinations

Prostatic Acid Phosphatase (PAP) Assay

PAP levels in serum were measured using the method of Fishman and Lerhner (1953). The assay is based on the hydrolysis of p-nitrophenyl phosphate by acid phosphatases present in serum, in an acid pH medium, to give p-nitrophenol. Since tartaric acid is an inhibitor of acid phosphatases of prostatic origin, the tartarate-sensitive fraction of the acid phosphatases in serum (PAP) is measured by the difference in activity observed when the assay is carried out in the absence and presence of tartaric acid.

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 Babel *et al.*, (1990). The testosterone protocol is based on the method of Turkes *et al.*, (1979) 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 Bouve *et al.*, (1992).

Determination of Protein Content of the Prostate

A 200 mg portion of prostate tissue was homogenized in 10 ml of normal saline. An aliquot (0.02 ml) of the homogenate was used to determine the protein content using the Biuret method as described by Layne (1957). The method is based on the principle that proteins form a stable violet-colored complex with copper II ions at alkaline pH. The depth of the color is a measure of the number of peptide bonds present in the sample. The protein content of the prostate was determined by multiplying the amount of protein per aliquot by the dilution factor, and thereafter adjusting for the total volume of tissue homogenate and tissue weight.

Histology

The dorso-lateral lobes of the prostates were fixed in Bouin's fixative for 24 h. They were then dehydrated in grades of ethanol, cleared in xylene, then infiltrated with, and embedded in paraffin. Each was sectioned at 5 µm and stained with hematoxylin and eosin. The sections were subsequently viewed and photomicrographs taken.

Data Analysis

Descriptive statistics was done and the results presented as means \pm standard deviations. Differences between means were separated by One-Way ANOVA, followed by *post hoc* multiple comparisons, with the least significant threshold employed at $p \leq 0.05$.

Table 2: Markers of prostatic hyperplasia in rats in the test and control groups

	TEST	DC	HC	HDC
Prostate Wt (g)	0.55 \pm 0.14	0.60 \pm 0.04	0.50 \pm 0.07	0.50 \pm 0.07
<i>P</i>		0.321	0.158	0.158
Rel. Prostate Wt ($\times 1000$)	2.8 \pm 0.8	3.0 \pm 0.9	2.5 \pm 0.8	2.4 \pm 0.9
<i>P</i>		0.750	0.048	0.041
Prostate Wt/Testes Wt	0.88 \pm 0.18	1.13 \pm 0.18	0.23 \pm 0.03	0.23 \pm 0.06
<i>P</i>		0.125	0.007	0.008
Protein Content (mg/tissue)	60.0 \pm 3.3	65.2 \pm 1.9	33.3 \pm 2.0	35.7 \pm 0.8
<i>P</i>		0.074	<0.001	<0.001
PAP (U/L)	2.1 \pm 0.7	2.9 \pm 0.2	1.6 \pm 0.4	1.8 \pm 0.5
<i>P</i>		0.038	0.135	0.333
PAP/Prostate Wt (U/L/g)	5.4 \pm 1.0	5.6 \pm 0.6	3.2 \pm 1.2	3.7 \pm 0.5
<i>P</i>		0.776	0.066	0.129

P values are for comparisons between the test group and the different control groups

Table 3: Hormonal profile of rats in the test and control groups

	TEST	DC	HC	HDC
Prolactin (ng/ml)	6.4 \pm 0.1	5.6 \pm 0.4	5.7 \pm 0.7	5.6 \pm 0.7
<i>P</i>		0.073	0.112	0.087
Estradiol (ng/ml)	883.1 \pm 11.0	902.4 \pm 5.6	796.1 \pm 73.2	692.5 \pm 22.3
<i>P</i>		0.558	0.025	<0.001
Testosterone (ng/ml)	4.1 \pm 0.2	3.6 \pm 0.2	12.0 \pm 0.6	12.9 \pm 0.2
<i>P</i>		0.163	<0.001	<0.001
Testosterone/Estradiol ($\times 1000$)	4.6 \pm 0.2	4.0 \pm 0.2	15.2 \pm 1.7	20.6 \pm 0.7
<i>P</i>		0.463	<0.01	<0.01

P values are for comparisons between the test group and the different control groups

Results

The mean wet weight of the prostates was highest in the DC group and lowest in the HC and HDC groups. However, there was no statistically significant ($p > 0.05$) difference between the mean weight of the prostates of rats in the test group and those in any of the three control groups. Mean relative prostate weights were significantly ($p < 0.05$) lower in the HC and HDC groups, relative to the test group. The value for the test group, though less than that of the DC group, was statistically similar ($p > 0.05$) to it. The prostate weight relative to testes weight values followed a pattern that is similar to that of the relative prostate weight. The mean protein content of the rats' prostates was highest in the DC group and lowest in the HC group. The value for the test group was not significantly ($p > 0.05$) different from that of the DC group, though it was clearly smaller.

Akin to protein content of the prostates, mean serum PAP was highest in the DC group and lowest in the HC group. The mean PAP values of the DC group was significantly ($p < 0.05$) higher than that of the test group which was statistically comparable ($p > 0.05$) to those of the HC and HDC groups. Mean PAP relative to prostate weight values were not significantly ($p > 0.05$) different between the test group and any of the three control groups.

Figures 1 and 2 show extensive branching of the glandular ducts and the massive columnar epithelial in-folding lining the ducts. A decrease in the branching of the glandular ducts and epithelial convolutions in the test group (Fig. 1) relative to the DC group (Fig. 2) is seen. Also seen are the secretions in the lumens (Figs 1 and 2). Figures 3 and 4 show that glandular duct branching is not extensive, and the absence of in-folding of the epithelial cells in the HC group (Fig. 3) and the HDC group (Fig. 4). Epithelial cells in the HC group appear small and disjointed while those in the HDC appear more closely packed and bigger in size. Luminal secretions are also seen in both figures.

The mean concentrations of prolactin in the sera of the rats were statistically similar ($p > 0.05$) between the test group and each of the controls. Mean serum estradiol concentrations were significantly ($p < 0.05$) lower in the HC and HDC groups relative to the test group. However, the test group had values that were not significantly ($p > 0.05$) different from that of the DC group. Conversely, mean serum testosterone values were significantly ($p < 0.05$) higher in the DC and HDC groups, relative to the test group which had (insignificantly) higher mean values compared to the DC group. The testosterone to estradiol ratios followed a similar pattern.

Discussion

A recent study had shown that the seeds of *T. occidentalis* when incorporated into the diets of rats, supported growth and maintenance of body weight, and showed no signs of toxicity (Ejike *et al.*, 2010a). However, beyond a 15% level of dietary incorporation of the seeds, significant hyperglycemia resulted (Ejike *et al.*, 2010b). Since it was important to incorporate a quantity of the seeds large enough to have a therapeutic effect, without resulting in metabolic derangements, the 15% dietary incorporation was chosen for this study.

Eunuchs or hypogonadal individuals, and men who were castrated prior to puberty are known not to suffer from BPH. Also, in the rare disorders of androgen resistance like testicular feminization and 5 α -reductase deficiency, only a remnant prostate is present (Wilson and Roehrborn, 1999). For these reasons, intact rats were chosen for this study such that proper comparison with BPH in men could be made.

The test diet clearly reduced both the mean prostate weight and mean relative weights of the prostates of rats in the test group, relative to the DC group, though not (statistically) significantly so. The mean relative weights of the prostates of the groups that did not receive the exogenous hormones were only marginally significantly lower than values got for the test group. Again, the test diet also resulted in a reduction in the mean ratio of prostate weight to testes weight of the test group, relative to the DC group. In the same vein, the mean protein content of the rat prostates was lower in the test group, compared to the DC group, though the difference was not statistically significant.

Increase in cell number (hyperplasia) of the prostate would rationally come with a collateral increase in its weight (especially its relative weight, since it corrects for differences in whole body weight). It should also come with an increased prostate weight to testes weight ratio, since testosterone (produced by the Leydig cells of the testes) affects the growth of the prostate and organ mass is often related to secretory activity. Furthermore, increase in cell number in a tissue also goes with a collateral increase in the protein content of the tissue, and for the prostate, occurs only when DHT is 12-17% of the normal physiological values (Wright *et al.*, 1999). Though the test diets reduced the increase in cell number in the prostates of rats in the test group (as assessed by the measured parameters) relative to the DC group, the differences between the means of both groups were not statistically significant. However, 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 (Barry, 2001). Symptom severity in BPH is known to correlate with overall health status (Eaton, 2003) such that any agent that can reduce the symptoms of BPH (in this case by reducing the mass of the prostate), no matter how slightly it does it, is usually useful. The test diet therefore could be useful in the management of BPH.

Testosterone (in a dose-dependent fashion) is known to stimulate the secretory capacity of the prostate (Banerjee *et al.*, 1998). PAP is one of the prostatic secretions used as a marker of prostatic diseases and as an indicator of treatment progress (Bauer, 1988). Elevations in PAP levels have been reported in animals treated with DHT and estradiol and may be due to increased lysosomal activity (Jeyaraj *et al.*, 2000). The higher values obtained for PAP in the groups that received the hormones corroborate the increase in secretory activity (due to hyperplasia) especially in the DC group. The fact that the test diet resulted in the test group having a mean PAP value that is statistically similar to those of the HC and HDC groups buttresses its effectiveness in managing aberrant growth of the prostate.

These data are supported by the decrease in the thickening of the epithelial cells and their convolutions as seen in the histological sections of rats in the test group, relative to the DC group. The fact that both the test group and the DC group had higher indices for the markers of hyperplasia assessed in this study, relative to the HC and HDC groups, clearly shows that hyperplasia was successfully induced. Also, the fact that all such assessed indices were lower in the test group, relative to the DC group shows that the test diet contains active ingredients that clearly reversed aberrant growth of prostate cells. The short duration of treatment with the test diet (one week), may have been the reason for the absence of a statistically significant reversal of hyperplasia in the test group as was the case in the study of the ability of the same test diet to inhibit the induction of hyperplasia (where the diet was fed alongside hormonal challenge for 28 days) (Ejike, 2010). However it was important not to exceed one week, as such an elongation of the time of treatment could generate false positive results as the prostates of rats in the test and DC groups would have naturally regressed as a result of the withdrawal of hormone treatment.

Bearing in mind that hormonal induction of BPH in rats has been shown not to result in derangements in serum macromolecular metabolism (Ejike and Ezeanyika, 2010), the serum concentrations of prolactin, estradiol and testosterone were studied, in order to appreciate the possible mechanism(s) through which the test diet achieved the lowering of cell proliferation in the prostates of rats in the test group.

Prolactin provides an additional growth regulatory mechanism for the prostate (Nevalainen *et al.*, 1997) and its over-expression can result in prostatic enlargement (Wennbo *et al.*, 1997). However, estradiol modulates the secretory activity of the pituitary, and thus may in turn moderate the level of prolactin in the system (Kaufman and Vermeulen, 2005). The exogenous estradiol administered to the rats in the test and DC groups may have ensured that their levels of prolactin remained statistically similar to those of the other groups, despite the induction of BPH. Prolactin may therefore not have been responsible for the induction or management of prostate cell proliferation observed in this study.

Estrogen sufficiency typically results in a decrease in testosterone concentration in serum. This is because estrogens exert a negative feed back control on pituitary secretion (McPherson *et al.*, 2001). Though mean testosterone levels were lower in the test and DC groups, relative to the HC and HDC groups, the test group however had higher mean values compared to the DC group. The low mean value for testosterone found in the DC group is not surprising, for reasons adduced above. It appears that the test diet resulted in a slight increase in the serum testosterone concentration of rats in the test group, relative to the DC group.

Serum estradiol levels were expectedly higher in the test and DC groups, due to the supra-normal doses of estradiol valerate which they received. Jeyaraj *et al.* (2000) had reported a similar high level of estradiol in animals treated with DHT and estradiol, and concluded that the induction of hyperplastic changes in the prostate of the animals may be an estrogen related effect. The data from this study support this view, as it appears that the test diet reduced (albeit slightly) the serum concentrations of estradiol in rats in the test group, relative to the DC group.

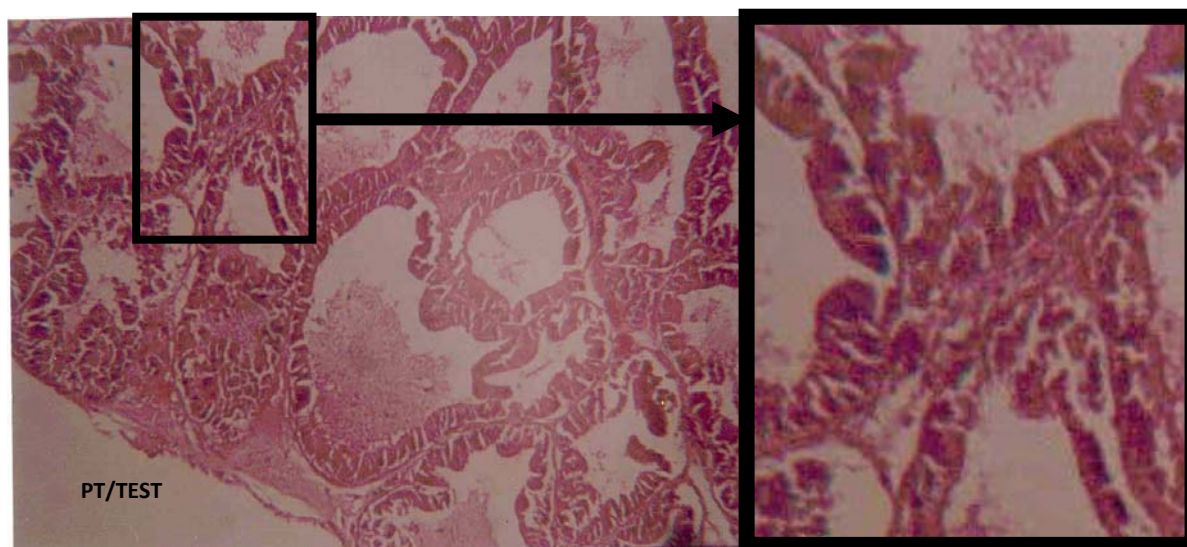


Figure 1: Histological section of prostate tissue from the test group (Magnification: $\times 100$)

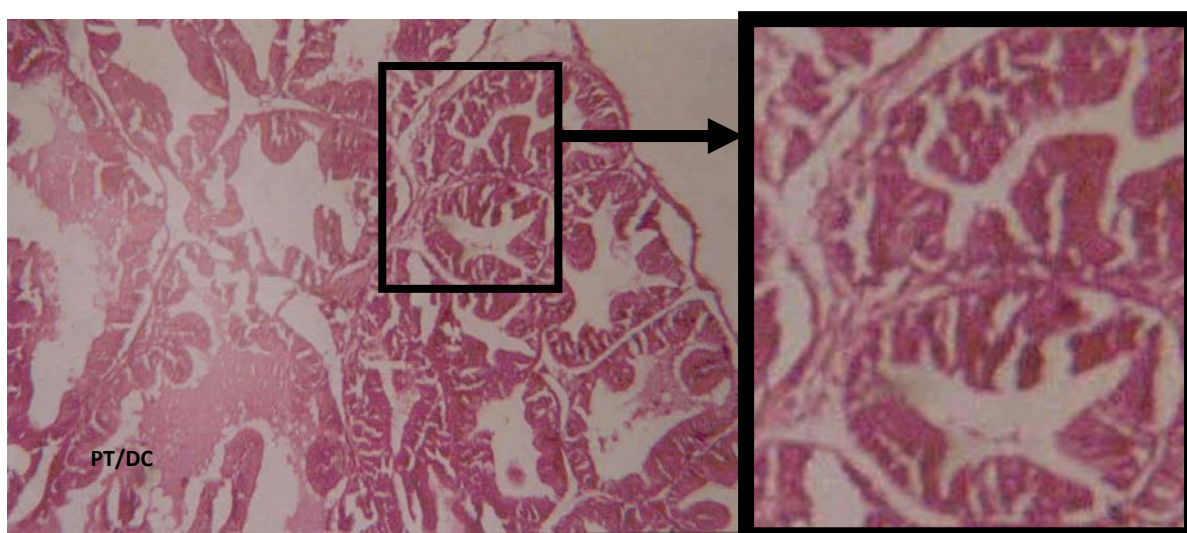


Figure 2: Histological section of prostate tissue from the DC group (Magnification: $\times 100$)

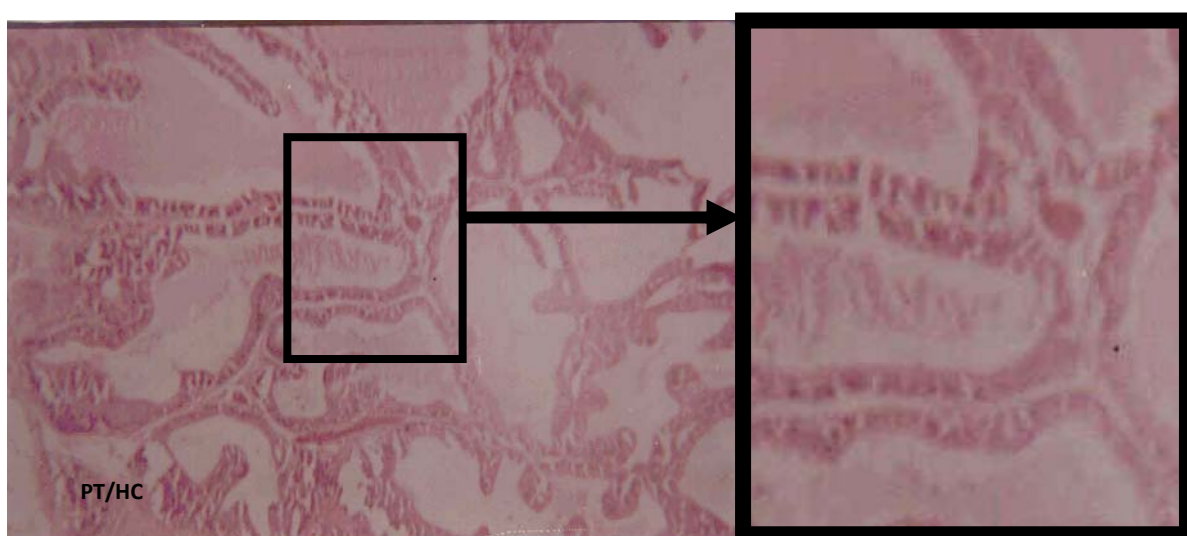


Figure 3: Histological section of prostate tissue in the HC group (Magnification: $\times 100$)

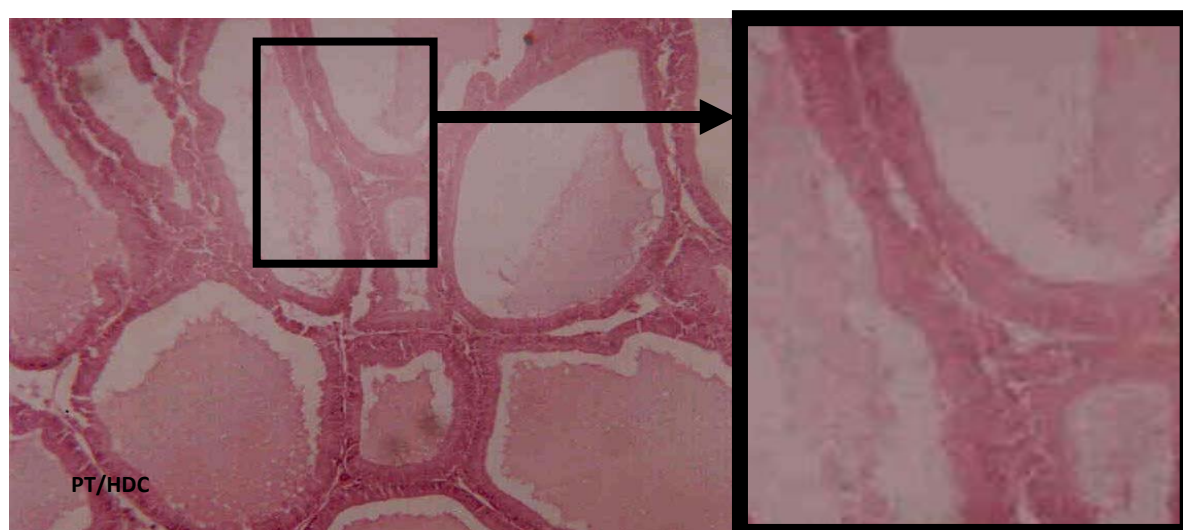


Figure 4: Histological section of prostate tissue from the HDC group (Magnification: $\times 100$)

BPH is associated with a reduced androgen: estrogen ratio (Suzuki *et al.*, 1995). With a reduced androgen: estrogen ratio, epithelial cells secrete transforming growth factor beta (TGF- β), a pleiotropic factor that induces smooth muscle differentiation and increases the extracellular matrix in the surrounding stromal cells (Zavidil and Bottinger, 2005), events that are typical of BPH. Interestingly, the test diet resulted in the testosterone to estradiol ratio of the test group being higher than that of the DC group (though significantly lower than those of the HC and HDC groups). Clearly, the test diet increased the androgen: estrogen ratio of the test group relative to the DC group, and possibly, through that mechanism reversed the hyperplasia induced by the administration of exogenous hormones.

It is important to note that there are as much inter species differences as there are similarities in nature, such that these findings should be cautiously extrapolated to humans. A comparison of the *T. occidentalis* seeds incorporated diet and a standard drug or phytotherapeutic agent of proven efficiency in managing BPH is needed, while further studies to clearly elucidate the mechanism of action of the test diet are imperative.

In conclusion, results from this study show that a diet incorporating 15% *T. occidentalis* seeds was useful in managing experimental BPH in Wistar rats. It possibly achieves this by increasing the level of serum testosterone while concomitantly lowering the level of serum estradiol.

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