Original article

The effects of ethanol seed extract of *Raphia hookeri* (Palmaceae) on exogenous testosterone and estradiol induced benign prostatic hyperplasia in adult male rats

Mbaka, GO.[1]*, Ogbonnia, SO.[2],Olarewaju, OT.[3] and Duru, FI.[4]

[1]Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria
[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Idi - Araba, Lagos, Nigeria
[3]Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Nigeria
[4]Department of Anatomy, College of Medicine of the Unversity of Lagos, Idi - Araba, Lagos, Nigeria
*E-mail: mbaaka@yahoo.com

Abstract

Objective: To evaluate the effect of ethanol seed extract of Raphia hookeri (RH) on exogenous testosterone and estradiol induced benign prostatic hyperplasia in adult male rats. **Methods:** Male rats weighing 200 ± 10 g kg^{-1} had exogenous administration of testosterone and estradiol in staggered doses (three times weekly) for three weeks. The induced animals were divided into five groups. Groups 1 and 2 received extract at 50 and 100 mg kg^{-1} body weight (bw) by gavages for forty five days; group 3, fenasteride (0.1 mg kg^{-1}); group 4, untreated for forty five days; group 5 (negative control), sacrificed after twenty one day of induction. Group 6 received extract (100 mg kg^{-1}) and steroid hormones simultaneously; group 7 was normal control. Each group comprised of 6 rats. **Results:** Progressive weight gain occurred overtime in RH extract/fenasteride treated. Prostate specific antigen (PSA) level significantly $p < 0.05$ decreased in the treated groups compared to the negative control with the extract treated exhibiting more effective decrease with dose. Treatment with the extract/fenasteride led to significant decrease in testosterone to a level comparable to normal. The levels of catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) in the RH treated were comparable to normal while in the negative control showed marked reduction. Similarly, the activity of thiobarbituric acid reactive substances (TBARS) in the extract group was within the normal range while showing significant increase in the untreated. The photomicrograph of prostate of the extract treated showed extensive shrinkage of glandular tissue. In contrast, glandular hyperplasia occurred in the negative control. The extract effectively reduced the size of the enlarged prostate gland exogenously induced. It also exhibited effective anti-oxidative activity. **Conclusion:** The extract attenuated hyperplasia and showed to be good prophylaxis against BPH.

Keywords: benign prostatic hyperplasia, phytotherapy, *Raphia hookeri,* tissue histology.

1 Introduction

Benign prostatic hyperplasia otherwise known as BPH is a clinical condition characterized by lower urinary tract symptoms (LUTS) (SEFTEL, ROSEN, ROSENBERG et al., 2008). The incidence of this disease is observed to be prevalent among the aging males. BPH, more often noticed from the age of 45 and above (FONG, MARIHART, HARIK et al., 2004) has become increasingly worrisome because it interferes with urination, increasing frequency and urge of urinating coupled with other discomforts such as pains (LAWSON, 1993; WILT, ISHANI and MACDONALD, 2002).

The disease has been around for centuries and is considered one of the signs of old age in males but because of the organ it affects (the penis) and the symptoms it presents, most men find it difficult to talk to their doctors about it, so there has being no proper documentation of its history and course of development. In recent times, considerable inroad has been made to understand the demographics and natural history of the disease which is of vital importance in seeking for a preventive approach (FONG, MARIHART, HARIK et al., 2004). Investigations showed that it affects over 40% of men in their fifties and nearly 90% of men in their eighties with the rate of occurrence known to differ widely from one country to the other (BERRY, COFFEY, WALSH et al., 1984). Population-based studies showed a prevalence of 25.3% in the UK (GARRAWAY, COLLINS and LEE, 1991) and a prevalence of 24.94% in Spain (CHICHARRO-MOLERO, BURGOS-RODRIGUEZ, SANCHEZ-CRUZ et al., 1998) while in Nigeria, in the west coast of Africa, 25.4% of prevalence (LAWRENCE, CHUKWUNONSO, ONYECHI et al., 2006) was recorded.

The management of BPH has been influenced by the use of orthodox medications such as alpha adrenergic blocking agents, 5-alpha redutase inhibitors and in some cases a combination of both (MCCONNELL, ROEHRBORN, BAUTISTA et al., 2003; BAUTISTA, KUSEK, NYBERG et al., 2003; de SOUZA, PALUMBO, ALVES et al., 2011). A major set back is that these drugs are known to provide temporal relief as there is a re-occurrence of the symptoms following their discontinuous use (AMERICAN..., 2003). This consequently places a demand for the continual use of the drugs which is likely to increase the risk of systemic side effect like dizziness, erectile dysfunction, decreased libido, and decreased ejaculate

volume and in few cases breast enlargement or tenderness (NASLUND, GILSENAN, MIDKIFF et al., 2007).

The use of phytotherapeutic agent in treating BPH dates back to ancient times. Following the successes recorded overtime their popularity has grown all over the world especially in China, Japan and Europe (ZEGARRA, VAISBERG, LOZA et al., 2007). In most developing economies, particularly in Africa it constitutes the predominant mode of treating BPH because of the perceived therapeutic healing powers of herbs (ODUGBEMI, 2006). As far back as in the 1990s, it was estimated that phytotherapeutic agents constituted approximately 50% of all medicines prescribed for BPH in Italy (Di SILVERIO, FLAMMIA, SCIARRA et al., 1993) while almost 90% in Germany and Austria (BUCK, 1996). The major factor that seemed to influence their popularity is the strong belief that herbal remedy has fewer side effects and less toxic due to their rich natural source and also, because they are readily available and cheap to obtain.

Raphia hookeri (RH) commonly known as Raffia palm is a member of Palmaceae family that grows in the eastern and western parts of Nigeria. It grows in fresh water swamps reaching a height of 9 m and possesses breathing roots thereby adapting it for life/support in water logged soils. The fruit is large, cone-shaped with a single hard nut having an outer layer of rhomboid-triangular and overlapping reddish brown scales. Between this outer layer of scales and the very hard seed is a yellow, mealy, oil-bearing mesocarp or pulp. RH is probably the most diversely useful plant in Nigeria as all it parts have various economic values. It is an important source of forest food species in southern Nigeria (AKACHUKWU, 2001). RH has equally shown to have beneficial therapeutic property as it is used in herbal medicine in the treatment of various illnesses. Its seed extract has been used ethno-botanically along with some other concoctions to treat symptoms similar to that exhibited by patients of BPH. However, no scientific evidence to our knowledge exists to justify their use for the purpose. This study therefore attempted to establish the scientific basis using animal model. Although BPH is a clinical condition noticed mostly in elderly male humans, an analogous form of the disease can be induced in male rats using synthetic testosterone and estradiol (WALSH, MADDEN, HARROD et al., 1974).

2 Materials and Methods

2.1 Plant materials

The roots of *Raphia hookeri* were obtained from swampy farm land at Ikorodu, Lagos State, Nigeria. They were authenticated by a taxonomist, Dr. O. A. Ugbogu, of the Forestry Research Institute of Nigeria (FRIN), Ibadan where voucher specimen has been deposited in the herbarium (FHI/108941).

2.2 Preparation of the aqueous seed extract of RH

The fresh fruits of RH obtained from swampy farm land were spread in the sun for a week to enable for the softening and easy removal of the mesocarp. The seeds obtained were dried before being subjected to size reduction to a coarse powder with electric grinder. The seed powder, 1140 g, was extracted with 95% aqueous ethanol in three cycles using Soxhlet extractor. The crude extract was filtered with Whatman filter paper No. 4 and the filtrate concentrated *in vacuo* 30 °C to obtain 138 g residue weight (12.1% w/w). The residue was stored in an air tight bottle kept in a refrigerator at 4 °C till used.

2.3 Animals

Wistar rats (150 ± 10 g) of either sex obtained from the Animal House of the University of Ibadan, Oyo State, Nigeria, were kept under standard environmental condition of 12/12 hrs light/dark cycle. They were housed in polypropylene cages (6 animals per cage), and were maintained on mouse chow (Livestock Feeds Nigeria Ltd), provided with water *ad libitum*. They were allowed to acclimatize for 9 days to the laboratory conditions before the experiment. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals, in experimental studies (INSTITUTE..., 1996).

2.4 BPH Induction

Adult male rats weighing 200±10 g kg^{-1} were induced with BPH by exogenous administration of testosterone and estradiol in staggered doses (three times a week respectively) for three weeks. The steroid hormones were diluted with corn oil which served as the solvent. 19 mL of Corn oil was added to 1mL (25 mg) of testosterone to form a 20 mL stock solution while 24 mL of corn oil was added to 1mL of estradiol to give a stock solution of 25 mL. From the stock solutions, 200 g rat was injected with 400 µg of testosterone and 80 µg of estradiol separately at the respective thighs (BERNOULLI, 2008) with modification.

2.5 Animal grouping and treatment

The induced animals were divided into five groups each comprised of 6 male rats. Groups 1 and 2 received the extract at 50 and 100 mg kg^{-1} body weight (bw) by gavages for forty five days; group 3 received fenasteride at 0.1 mg kg^{-1}; group 4 was left untreated for forty five days before sacrifice to assess possible reversal of the exogenous induction; group 5 (negative control) was sacrificed immediately after the induction. Group 6 were given the extract (100 mg kg^{-1}) simultaneously as benign hyperplasia was being induced with the steroid hormones while group 7 served as normal control. The animals were weighed prior to the commencement of the experiment and subsequently every five days till the end of the experiment.

2.6 Assay for testosterone and prostate specific antigen (PSA)

Enzyme immunoassay technique was used for the quantitative determination of testosterone concentration and PSA evaluation (MARCUS and DUMFORD, 1985; EKINS, 1990).

2.7 Oxidative activities

The oxidative activity assessment was conducted after overnight fast. The animals were sacrificed and the hepatic tissue harvested were homogenized and used for the assays.

2.8 Superoxide dismutase assay

Superoxide dismutase (SOD) was assayed utilizing the technique of Kakkar, Das and Viswanathan (1984). A single unit of the enzyme was expressed as 50% inhibition of Nitroblue Tetrazolium (NBT) reduction/min/mg protein which was measured spectrophotometrically at 420 nm.

2.9 Catalase assay

Catalase (CAT) was assayed colorimetrically at 620 nm and expressed as µmoles of H_2O_2 consumed/min/mg protein (RUKKUMANI, ARUNA, VARMA et al., 2004). The hepatic tissue was homogenized in isotonic buffer (pH 7.4). The homogenate was centrifuged at 1000 rpm for 10 minutes. The reaction mixture contained 1.0 mL of 0.01M pH 7.0 phosphate buffers which was added 0.1 mL of tissue homogenate and 0.4 mL of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratios).

2.10 Estimation of glutathione

The glutathione (GSH) level was determined by the method of Ellman (RUKKUMANI, ARUNA, VARMA et al., 2004). To the hepatic homogenate was added 10% trichloroacetic acid (TCA) and centrifuged. 10 mL of the supernatant was treated with 0.5 mL of Ellmans' reagent in 100 mL of 0.1% of sodium nitrate and 3.0 mL of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm.

2.11 Estimation of lipid peroxidation

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) were measured by the method of Niehaus and Samuelsson (RUKKUMANI, ARUNA, VARMA et al., 2004) and expressed as nmol/mL. In brief, 0.1 mL of hepatic tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.3%, 0.25N HCL and 15% TCA) and placed in water bath for 10 min at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535 nm.

2.12 Tissue histology

The prostatic tissues harvested from each group were fixed in Bouin's fluid for five days before embedding in paraffin wax. The embedded tissues were sectioned at 5 µm, mounted on a slide and stained with Periodic acid Schiff (PAS). Each section was examined under light microscope at high power magnification for structural changes and photomicrographs were taken.

2.13 Statistical analysis

All values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analyzed by means of Student's t-test. $P < 0.05$ was considered significant.

3 Results

3.1 Body weight

The body weight changes of animals treated and untreated are shown in Figure 1. The animals exhibited significant weight loss and decrease in appetite after three weeks of benign hyperplasia induction. Treatment with RH extract/

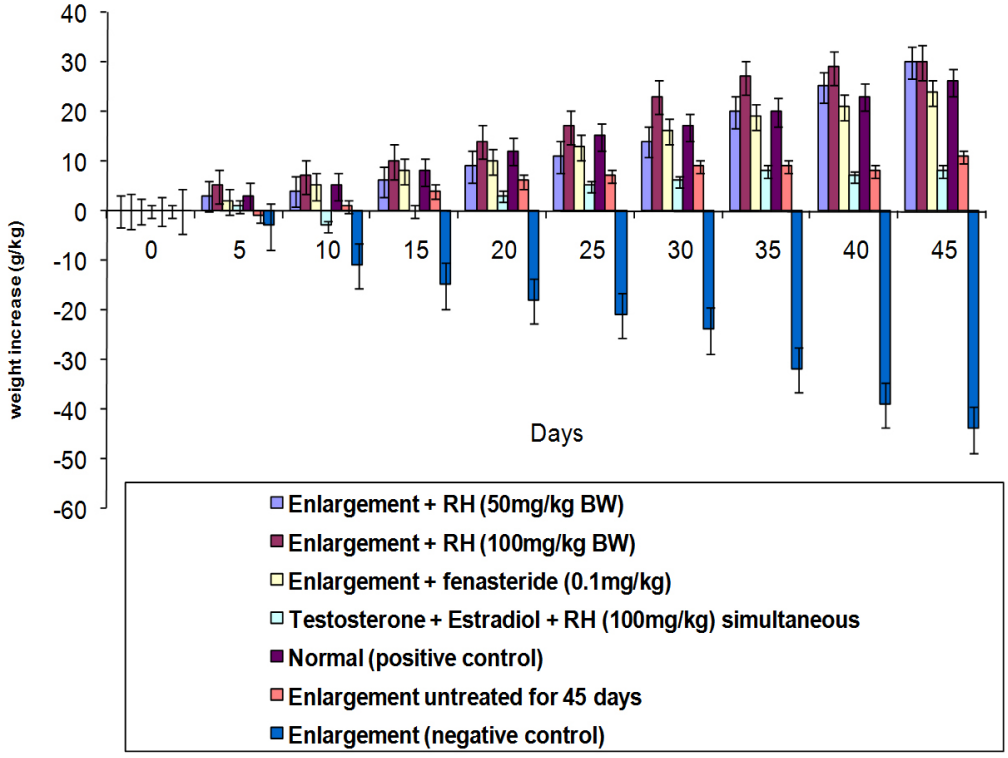

Figure 1. Weight increase in control and treated animals. Values represent mean + - n=6.

fenasteride resulted in progressive weight gain overtime with improvement in appetite. In the untreated however, progressive weight decrease occurred.

3.2 Effect of BPH on PSA level

Figure 2 showed the plasma PSA levels in the treated and controlled groups. There was an elevation of PSA level to 10.9 ng mL^{-1} after induction of prostate enlargement. In the animal groups treated with RH extract/fenasteride, significant $p < 0.05$ decrease in PSA level occurred compared to the negative control. The graded extract doses, 50 and 100 mg kg^{-1} showed reductions of 60.5% and 61.5% respectively, fenasteride, 51.4% while simultaneous extract treatment with induction, 47.7%. The untreated group after forty five days showed a reduction of 19.3%. The extract exhibited dose dependent decrease and also more effective decrease compared to the reference drug.

3.3 Effect of BPH on testosterone level

Figure 3 showed the summary of plasma testosterone levels. In the animals with prostatic hyperplasia (negative control), testosterone level was elevated to 4.0 ng ml^{-1}. The hormone level decreased markedly in the treated groups with the RH extract treated (50 and 100 mg kg^{-1}) showing a decrease of 77.5% and 77.5% respectively, fenasteride treated by 65%; simultaneous extract treatment with induction by 70% and untreated for 45 days by 5%.

3.4 Effects on antioxidant profile

There was oxidative stress in response to BPH induction. The effect of benign hyperplasia resulted to significant decrease in the activity of the enzymes; CAT, SOD and GSH. As shown in Table 1, the activity of the enzymes decreased markedly in the untreated groups. However, in the extract/fenasteride treated, significant ($p < 0.05$) increase in the activity of the enzymes occurred compared to negative control. Improvements in the activity of the enzymes were more pronounced in the RH extract than in fenasteride treated. The levels of the enzymes were however within normal range in the animals treated and simultaneously being induced. TBARS evaluation showed increase in peroxidative activity in the untreated groups while indicating a decrease that was comparable to normal in both the extract and fenasteride treated.

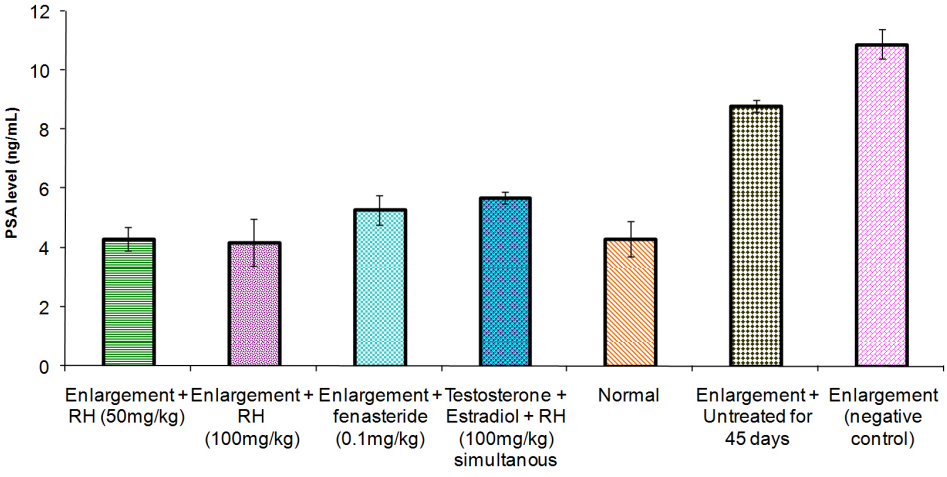

Figure 2. Plasma PSA level post treatment. Values represent mean + - n=6.

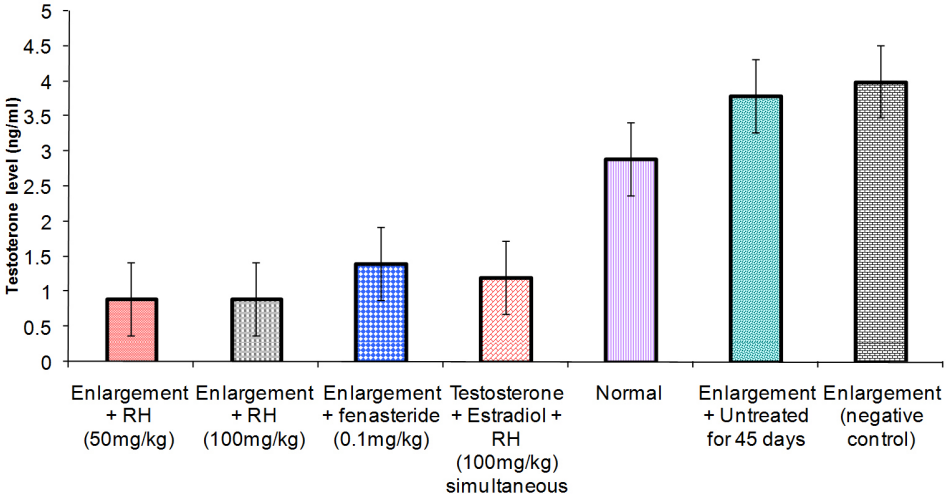

Figure 3. Plasma testosterone level post treatment. Values represent mean + - n=6.

3.5 Histopathology of prostate tissue

A section of normal prostatic tissue histology (Figure 4a) stained with PAS showed glandular tissue with thick glandular epithelial lining with deeply stained epithelial stroma. The fibro-muscular matrix contained smooth muscle fibres and vessels. The photomicrograph of negative control (Figure 4b) showed extensive glandular hyperplasia with thickened glandular epithelium. The fibro-muscular matrix showed a thin outline with few vacoules. The prostatic tissue histology of animals treated with low dose of RH extract (Figure 4c) indicated extensive shrinkage of glandular stroma which formed corpora amylacea with convolution of epithelial lining. The fibro-muscular matrix showed increased density. Figure 4d showed histology of prostatic tissue treated with high dose of RH extract. The section indicated more extensive shrinkage of glandular stroma with thin highly convoluted glandular epithelium. The corpora amylacea appeared diminished in size and having the presence of dark granules. The fibro-muscular matrix showed increased vacuolation with focal areas of infarction. The photomicrograph of prostatic tissue (Figure 4e) treated with fenasteride indicated reduced glandular stroma with also the presence of corpora amylacea. The glandular epithelium appeared thickened with convolution while the fibro-muscular matrix showed coalesced vacuolation. A section of prostate gland induced and simultaneously treated with high dose of RH (Figure 4f) indicated reduced glandular stroma with coalesced intraglandular vacuoles. The epithelial lining appeared thickened with partial convolution while the fibro-muscular matrix appeared more enlarged. The photomicrograph of animals not treated for forty days (Figure 4g) showed extensive glandular hyperplasia with thick glandular epithelium comparable to the negative control.

4 Discussion

Animals experimentally induced with BPH exhibited hyperplasia of prostate gland as well as body weight loss. Treatment with RH extract/fenasteride of prostatic hyperplasia resulted in marked decrease in the size of prostate after forty five days of daily administration while periodic body weight gain was also recorded. 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 steroids, testosterone and estradiol.

The PSA level which was elevated after the animals were induced with benign hyperplasia was observed to have decreased significantly after treatment with RH extract/ fenasteride for forty five days. 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 (McPARTLAND and PRUITT, 2000). This glycoprotein is usually referred to as a biological/tumor marker but its level has also been shown to fluctuate during inflammatory changes like in prostatitis (SMITH, HUMPHREY and CATALONA, 1997). Although there might be a good correlation between prostate volume and serum PSA level (ÖESTERLING, COONER, JACOBSEN et al., 1993), the use of PSA level alone as indicator for prostate enlargement might not be sufficient. More often there is in addition other consideration like manifestation of incomplete urinary bladder emptying and in more severe condition, acute urinary retention (AUR) which is an endpoint progression for BPH (BATES, REYNARD, PETERS et al., 1997; FONG, MARIHART, HARIK et al., 2004).

Testosterone is also an important agent considered in benign hyperplesia because of its involvement in prostate cell proliferation (FELDMAN and FELDMAN, 2001). In this study, it was observed that benign hyperplasia led to increase in testosterone level. However, in animals treated with extract/fenasteride the levels decreased appreciably. The reduction in testosterone level in the treated animals may be attributed to RH 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 (CANALES, ZAPZALKA, ERCOLE et al., 2005; LEVINGER, GORNISH, GAT et al., 2007). 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 (WONG and WANG, 2000; ROEHRBORN, NUCKOLLS, WEI et al., 2007). Testosterone has equally been noted to initiate growth stimulation by binding to androgen receptors (EPSTEIN, 2004).

BPH is known to be intimately linked with increased oxidative stress which increases with age (AYDIN, ARSOVA-SARAFINOVSKA, SAYAL et al., 2006; ARYAL, PANDEYA, GAUTAM et al., 2007). Increased lipid peroxidation and

Table 1. Shows lipid concentration during 45 days of extract/ fenasteride administration or 10mg/kg distilled water (control).

		Plasma lipid levels in control and treated animals (μmol/min/mg protein/100mL)			
	Dose (mg/kg)	**CAT**	**SOD**	**GSH**	**MDA**
Enlargement + RH	50	43.7 ± 3.1*	5.5 ± 1.3*	0.7 ± 0.1*	0.05 ± 0.0*
Enlargement + RH	100	45.3 ± 1.4*	6.3 ± 0.4*	0.7 ± 0.2*	0.04 ± 0.0*
Enlargement + fenasteride	0.1	39.8 ± 1.1*	3.9 ± 0.3*	0.4 ± 1.0*	0.09 ± 0.0*
Testosterone + Estradiol + RH	100	40.0 ± 0.1*	5.2 ± 0.6*	0.6 ± 0.1*	0.07 ± 0.1*
Normal		45.7 ± 2.2	5.0 ± 1.3	0.6 ± 0.2	0.06 ± 1.0
Enlargement + Untreated for 45 days		14.1 ± 2.0	1.4 ± 0.4	0.1 ± 0.0	0.30 ± 0.1
Enlargement		13.2 ± 0.8	1.2 ± 0.4	0.1 ± 0.2	0.30 ± 0.1

Values are Mean ± SEM; n=6, *p<0.05 compared to control (Student's t-test).

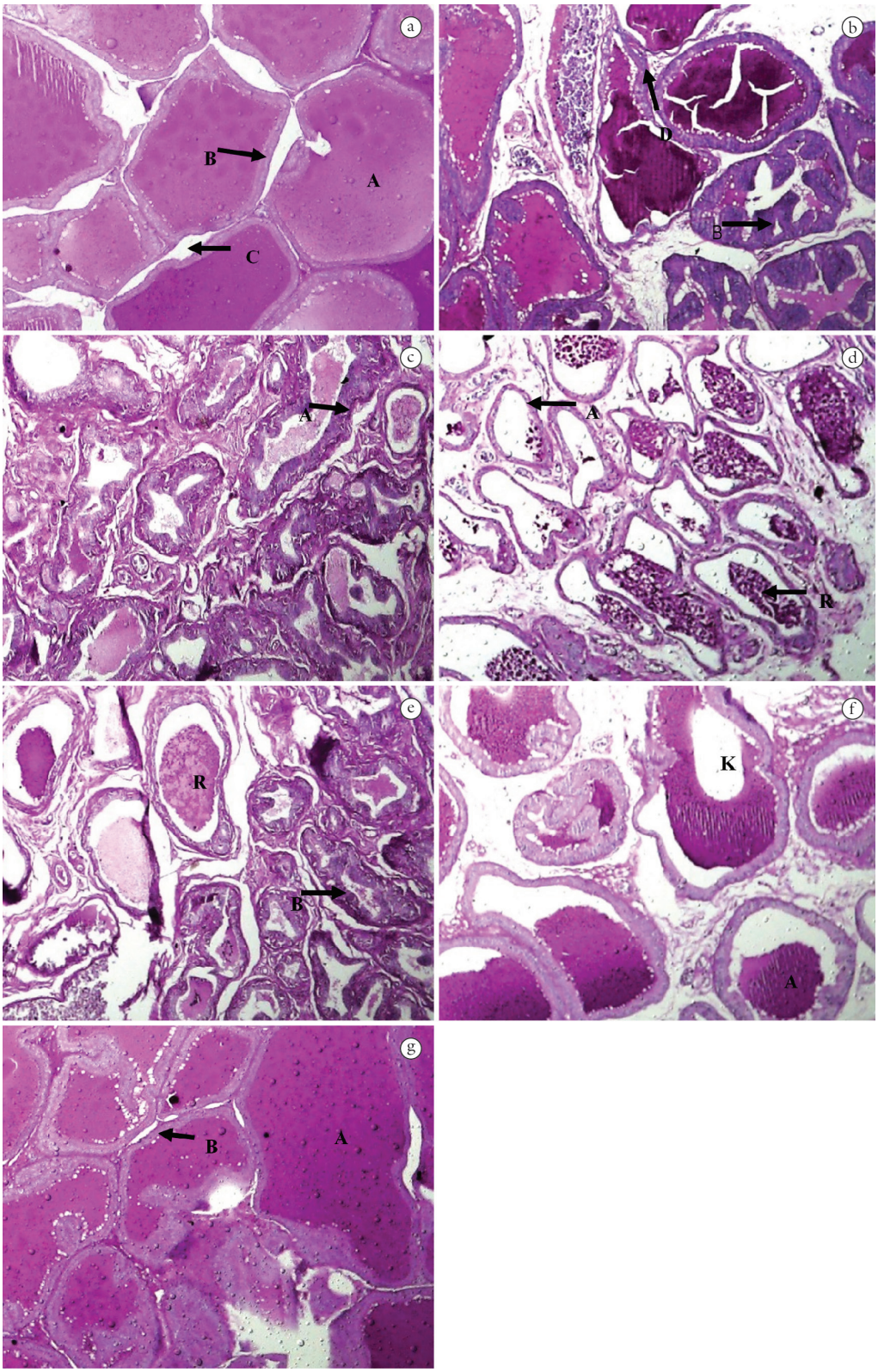

Figure 4. [a-g]: Photomicrographs showing the transverse section of prostate gland. [a] A section of prostate gland of negative control indicating (A) glandular hyperplasia, (B) glandular epithelium, (C) fibromuscular stroma (X 400). [b] A section of normal prostate indicating (D) interglandular smooth muscle, (B) intraglandular epithelia hyperplasia (X400). [c] Treatment with low dose of the extract showing (A) reduced glandular stroma (X400). [d] Treatment with high dose of the extract indicating (A) thin glandular epithelium, (R) corpora amylacea (X400). [e] Treatment with fenasteride indicating (B) thick epithelial convolution, (R) corpora amylacea (X400). [f] Induction with simultaneous treatment with the extract indicating (A) reduced glandular stroma, (K) large intraglandular space (X400). [g] Induced prostate enlargement not treated for 45 days showing (A) glandular hyperplasia, (B) Thick glandular epithelium (X 400).

decreased levels of superoxide dismutase, catalase and antioxidant molecules have been found to be associated with BPH (MERENDINO, SALVO, SAIJA et al., 2003; AYDIN, ARSOVA-SARAFINOVSKA, SAYAL et al., 2006; ALMUSHATAT, TALWAR, MCARDLE et al., 2006; MCHEDLIDZE and SHIOSHRILI, 2006; ARYAL, PANDEYA, GAUTAM et al., 2007). The reduction in the activity of the anti-oxidant enzymes, CAT, SOD and GSH following induced hyperplasia in the animals must have been due to the accumulation of superoxide anion radicals and hydrogen peroxide which accentuates peroxidative activity (SEARLE and WILSON, 1980; HALLIWELL and GUTTERIDGE, 1985). The extract treated animals exhibited significant increase in the activity of these antioxidant enzymes compared to the negative control. The levels of these antioxidant enzymes in post treatment with the extract were observed to be comparable to the normal (control) implying that RH seed extract reactivated the activities of the enzymes through its active principles that enabled for effective scavenging of reactive oxygen species (ROS) and reducing oxidative stress. Although the plant antioxidative status has not been fully determined, the preliminary phytochemical screening showed it to be rich in polyphenol (AKPAN and USOH, 2004) and polyphenols are known for their antioxidant property (SHIRWAIKAR, RAJENDRAN, KUMAR et al., 2004).

The seed extract of *Raphia hookeri* has demonstrated to promote morphological changes in prostatic tissue having caused extensive shrinkage to glandular-stroma and the epithelial cells by inducing necrotic changes, unlike the untreated in which there was glandular proliferation characterized by extensive intra acinar stroma. Similar decrease in prostate mass occurred in fenasteride treated which however exhibited comparably less effect than the herbal agent. The marked necrotic change and shrinkage that occurred in the extract treated was confirmatory that the prostate size reduction could not have been caused by reversal of hyperplasia exogenously induced. In this mode, the extract showed to have inhibited growth factor that causes cell proliferation or glandular dilation. The extract equally showed to be good prophylaxis by inhibiting prostate enlargement in simultaneous extract treatment with induction. Reports of effective use of plant extracts in reducing prostate mass and reversing degenerative changes in the structure of the prostate gland have been documented (SCHWARZ, OBERMULLER-JEVIC, HELLMIS et al., 2008; SKAUDICKAS, KONDROTAS, KEVELAITIS et al., 2009; de SOUZA, PALUMBO, ALVES et al., 2011). Some of these plants have been established to display antioxidant effects and inhibit 5α-reductase activity (BAYNE, GRANT, CHAPMAN et al., 1997).

5 Conclusion

In this investigation, RH extract effectively reduced the size of the enlarged prostate gland exogenously induced. It also exhibited effective anti-oxidative activity and showed to be good prophylaxis which can be explored for preventive purposes. These beneficial effects of RH seed extract justified its use in traditional medical practice in treating BPH and other related cases.

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