

Effect of Ethanol Extract of *Abrus precatorious* Seed on Testosterone-Induced Benign

Prostatic Hyperplasia in Adult Male Wistar Rats

Abstract

Abrus precatorious is a plant which is used as male contraceptive. Its seed has been reported to reduce serum testosterone levels. The effect of *Abrus precatorious* seed on testosterone-induced benign prostatic hyperplasia in rats was examined. Thirty male rats were divided into six groups (n=5) A, B, C, D, E and F. Group A served as control and groups C and D received 40 and 60 mg/kg of the seed extract orally for 28 days respectively. Benign prostatic hyperplasia was induced in groups B, E and F via subcutaneous injection of 1.2 mL/kg b.w of testosterone. Groups E and F received concurrently 40 and 60 mg/kg of the extract orally for 28 days respectively. The rats were sacrificed on the 28th day. Serum testosterone, estrogen and prostate dihydrotestosterone (DHT) levels were measured. Epididymis, testis and prostate weight were determined. Caudal epididymal sperm was collected for analysis and histological analysis of the testis and prostate were carried out. Mean $\pm$ SEM were calculated and compared for significant differences using ANOVA and Duncan's multiple range test at $P < 0.05$. Prostate weight significantly decreased in groups C and D when compared to group A; and in groups C and E when compared to group B. Groups C and D had lower sperm motility and viability compared with group A and sperm count of group D decreased significantly. The serum estrogen level of groups E and F compared to group B; C and D compared to group A decreased significantly. There were significant increases in DHT level of groups C and D compared to group A, and in groups E and F compared to group B. Histologically, sections of testes in groups C and D

showed depletion of germinal epithelium and there was severe cystic prostate hyperplasia in group B and only prostate hyperplasia in groups E and F.

The results show that *Abrus precatorious* seed has the potential to reduce severity of testosterone-induced BPH.

Keywords: *Abrus precatorious*, Testosterone, Benign Prostatic Hyperplasia, Male rats

1.0 Introduction

The prostate is a part of the male reproductive system which contributes to the formation of semen by producing alkaline fluid that maintains and nourishes sperm. Benign prostatic hyperplasia (BPH) is an increase in the size of the prostate and is one of the most common chronic diseases of adult males (Ejike and Eeanyika, 2008; Parson *et al.*, 2013). Benign prostatic hyperplasia affects the quality of life of patients adversely and alteration in the size of the prostate seen in BPH affects the bladder or constricts the urethra, resulting in lower urinary tract symptoms (Jacobsen *et al.*, 1997; Eaton, 2003). These symptoms develop as a result of direct obstruction of urinary flow due to enlarged prostate and indirect obstruction of urinary flow secondary to contraction of the smooth muscles of the prostate, urethra and bladder neck.

The precise molecular etiology of BPH is complicated and it involves hyperplasia of prostatic stroma and epithelial cells, resulting in the formation of large, fairly discrete nodules in the transition zone of the prostate (Cunningham and Kadmon, 2013). Proposed aetiological theories of BPH include embryonic reawakening, aging, androgens, estrogens, oxidoreductase and inflammation as aetiologies of BPH (Bostwick, 2005; Ho *et al.*, 2008). However, all of the proposed theories require the presence of androgens as androgens appear to play permissive roles in the development of BPH (Ho and Habib, 2011). The role of androgens such as testosterone in

the development of BPH has been ascribed to its conversion to a more potent form called dihydrotestosterone (DHT) by the enzyme 5α - reductase in prostate stromal cells. Dihydrotestosterone plays an inductive role in BPH development by acting in an autocrine manner to promote stromal cell proliferation and paracrine manner to increase secretory function of prostate epithelial cells (Bringati *et al.*, 2009). Prevention of DHT synthesis via 5α - reductase inhibitions has a remarkable effect on BPH (Hudak *et al.*, 2006). Estrogens are reproductive hormone produced from testosterone through action of aromatase enzyme. They have gonadal and extra-gonadal effects in males and females. Estrogens contribute to male fertility and increase sperm concentration by promoting net outflux of fluid from the efferent ductules (Hess *et al.*, 1997). The prostate is an estrogen target tissue and estrogens have the potential to initiate changes in the prostate (Risbridger *et al.*, 2003; Ho *et al.* 2008). The role of estrogens in the development and maintenance of BPH is a complex one that is particularly reliant on local signaling mechanisms in the prostate (Tristan and William, 2011). Estrogens exert their effects on target cells and tissues through interaction with estrogen receptors notably estrogen receptor alpha and estrogen receptor beta. These receptors play significantly different roles in the prostate growth with estrogen receptor alpha mediates the negative-proliferative roles of estrogen in the prostate (Risbridger *et al.*, 2001) and the beneficial-anti-proliferative role of estrogen in prostate growth being mediated by estrogen receptor beta (McPherson *et al.*, 2007).

Alpha adrenergic receptors are G protein-coupled receptors that are targets of catecholamine especially adrenaline and nor-adrenaline. Alpha 1 and 2 adrenoceptors are present in the human prostate tissue (Shapiro and Lepor, 1986) and studies suggested that alpha 1 adrenoceptor subtype mediate prostate muscle contraction (Lepor *et al.*, 1988). As a form symptomatic treatment, alpha1-adrenergic blocking agents are commonly used to block alpha1-androgen

receptors which are predominantly present in the bladder neck or prostate. The use of herbal medicine in the management of BPH is well documented. *Abrus precatorius* is a woody twinning plant with characteristic toxic red seeds (Mensah *et al.*, 2011). The Leaves, roots and seeds of the plant are used for medicinal purposes and the seed is reported to have male contraceptive property (Ratnasooriya *et al.*, 1991; Bhatt *et al.*, 2007). *Abrus precatorius* seed extract causes male infertility by reducing serum testosterone levels (Bhatt *et al.*, 2007; Talukder *et al.*, 2011) and helps in the management of androgenic alopecia by inhibiting the activity of 5 α - reductase enzyme (Surkiti *et al.*, 2011). Most management options of BPH are instituted to safely improve quality of life by providing symptom relief and as well as reducing disease progression (Rigatti, 2006). However, combination therapy seems to be the most effective in the management of BPH as studies have indicated that they provide fast relief and reduce the need for BPH-related surgery (Marberger, 2000)

Most often, BPH managements (except in combination therapy) are targeted towards blocking one of the patho-aetiologic pathways of BPH and therefore provide little relief to BPH patients with complex underlying patho-aetiology. There is paucity of information on comprehensive effects of *Abrus precatorious* on BPH management and this study was carried out to assess the effects of *Abrus precatorious* seed on testosterone-induced benign prostatic hyperplasia.

2.0 Materials and Methods

2.1 *Abrus precatorious* (Licorice) Seed Extract Preparation

Fresh seeds of *Abrus precatorious* were purchased from commercial market in Ibadan, Nigeria and were verified at the Department of Botany, University of Ibadan, Nigeria. The seeds were grinded into powdery form and the ethanol extraction of the seeds was done as described by Prasad *et al.* (2016)

2.2 Experimental Animal Model

Thirty adult male Wistar rats weighing 180-250 g were used for the study. They were obtained from the Central Animal House, College of Medicine, University of Ibadan, Oyo state, Nigeria. They were allowed to acclimatize for two weeks before the commencement of the study. They were housed in clean and well ventilated cages. The animals were given water and feed (Top feeds, Ibadan) *ad libitum*.

2.3 Experimental Design

The animals were randomly divided into six groups (A to F) each consisted of five rats. Control group, C (Ap40mg/kg) and D (Ap40mg/kg) groups were given 1 ml/kg of distilled water, 40 mg/kg of the seed extract and 60 mg/kg of the seed extract respectively via oral gavage. BPH was induced by subcutaneous administration of testosterone (3mg/kg) in groups B (Testosterone), E (T+Ap40mg/kg) and F (T+Ap60mg/kg) daily for twenty eight (28) days

(Naslund and Coffey, 1986; Guo *et al.*, 2004). These groups were simultaneously given 1 ml/kg of distilled water, 40 mg/kg and 60 mg/kg of the seed extract orally for 28 days respectively.

2.4 Animal Sacrifice, Blood and Prostate Tissue Collection

The rats were sacrificed on the 28th day via intraperitoneal injection of 120 mg/kg of sodium thiopentone (Kawiya *et al.*, 2012). Blood sample for serum hormonal assay was collected from each animal via cardiac puncture into plain serum bottle and was allowed to clot. The serum was obtained as described by Mellisa *et al.* (2009). Serum concentrations of testosterone and estrogen were assayed using the Enzyme-linked immunosorbent assay (ELIZA) kits (Calbiotech, USA). One of the two lobes of prostate gland was harvested from each rat into an organ bottle containing 1 mL of phosphate buffer solution and homogenized. Homogenized prostate tissues were cold centrifuged at 10000 rpm for 15 minutes, supernatant was collected and DHT was assayed using an ELIZA (Elabscience, China).

2.5 Organ Collection and Histology of Tissues

The testes, prostate gland and epididymis were harvested from the rats and weighed with an electric weighing balance. The testis and prostates were then fixed in Bouin's fluid and 10% formalin respectively before being dehydrated in descending grades of alcohol, cleared in chloroform and impregnated in paraffin. Then 5-6 μ m sections were placed into the grease free slides, de-paraffinized in xylene and stained with haematoxyline and eosin (Luna, 1968).

2.6 Sperm Analysis

The epididymis was carefully removed from the testis and its content was released unto slides. Sperm vitality was assessed by staining of sperm smear with eosin-nigrosin staining technique (WHO, 2010). Sperm count was done by using the method described by Seed *et al.* (1996).

2.7 Statistical Analysis

Data were analyzed with GraphPad Prism (Version 5.04). Differences between the means were tested with analysis of variance. The results from each group were expressed as mean $\pm$ standard error of mean (S.E.M) and means were compared for significant differences at $P < 0.05$.

3.0 Results

3.1 Reproductive Organ weights

There was a significant increase in the mean weight of prostate gland in group B when compared with control group. The mean weights of prostate gland in groups C and D were significantly lower ($p < 0.05$) when compared with the control group. There was significant decrease in the mean prostate weight of groups E and F when compared with group B.

No significant difference was recorded in epididymis weight for group C and control group. Epididymis weight in group D was significantly lower when compared with the control group. There was no significant difference in the weight of epididymis for groups E and F when compared with group B.

The testicular weight of groups C and D when compared with the control showed no significant difference. Testicular weights recorded for groups E and F were not significantly different when compared with group B.

TABLE 1. Reproductive Organ weights

Groups	Prostate Weight (x 10⁻⁴ g)	Epididymis Weight (x 10⁻⁴ g)	Testicular Weight (x 10⁻⁴ g)
A (Control)	7.30 ± 0.58	25.00 ± 2.70	51.00 ± 4.50
B (Testosterone)	14.40 ± 0.16	27.00 ± 1.90	53.00 ± 0.21
C (Ap40mg/kg)	2.20 ± 0.25 [*]	20.00 ± 0.20	41.00 ± 3.00
D (Ap60mg/kg)	1.60 ± 0.29 [*]	17.00 ± 0.81 [*]	42.00 ± 3.80
E (T+Ap40mg/kg)	8.00 ± 0.84 [#]	27.00 ± 1.70	53.00 ± 2.70
F (T +Ap60mg/kg)	9.60 ± 0.75 [#]	26.00 ± 1.20	59.00 ± 8.50

Key: * indicates values that were significantly different when compared with the control group

indicates values that were significantly different when compared with group B

3.2 Sperm Characteristics

Group C showed no significant difference when compared with the control group. There was significant decrease ($p < 0.05$) in sperm count of group D when compared with the control group. Sperm counts in groups E and F were not significantly different when compared with group B. The sperm motility in groups C and D decreased significantly when compared with the control group. No significant difference was observed in sperm motility of groups E and F when compared with group B. There were significant decreases in sperm viability of groups C and D when compared with the control group. The sperm viability of groups E and F were not significantly different when compared with group B.

TABLE 2. Sperm characteristics

Groups	Sperm Count (10⁶/mm³)	Sperm Motility (%)	Sperm Viability (%)
A (Control)	14.84 ± 0.81	78.00 ± 4.64	84.00 ± 6.20
B (Testosterone)	21.20 ± 1.84	80.00 ± 5.48	77.00 ± 7.35
C (Ap40mg/kg)	12.96 ± 1.00	38.00 ± 5.83*	46.00 ± 5.10*
D (Ap60mg/kg)	5.00 ± 0.30*	56.00 ± 8.72*	40.00 ± 8.94*
E (T+40mg/kg)	18.00 ± 2.31	73.00 ± 7.68	72.00 ± 9.17
F (T+60mg/kg)	18.76 ± 1.52	89.00 ± 2.45	85.00 ± 2.24

Key: * indicates values that were significantly different when compared with the control group

3.3 Hormone levels

The serum testosterone and estrogen level in groups C and D decreased significantly when compared with group A. Groups E and F had significantly lower serum testosterone and estrogen level when compared with group B. Furthermore, prostate DHT level in groups C and D increased significantly when compared with control group. Also, groups E and F had significantly higher prostate DHT when compared with group B.

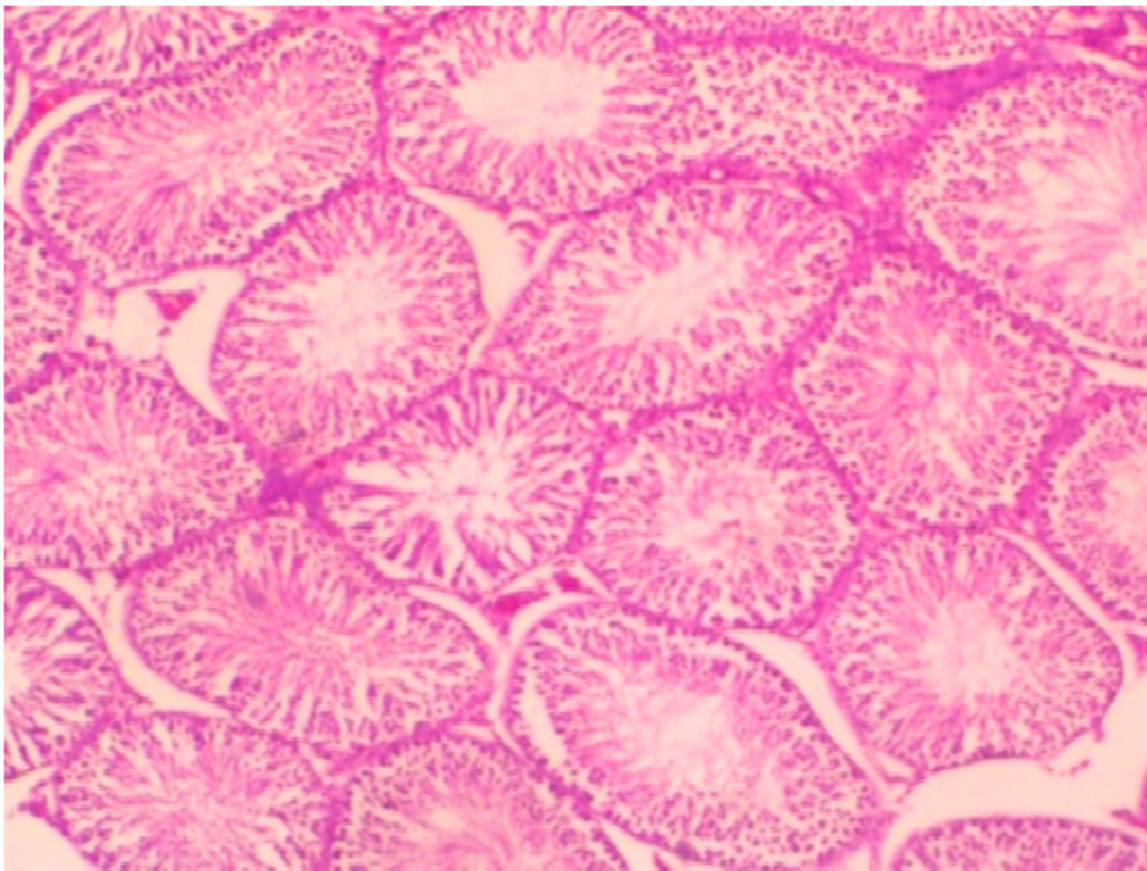
TABLE 3. Hormone levels

Groups	Serum Testosterone level (ng/ml)	Serum Estrogen level (ng/ml)	Prostate DHT level (Pg/ml)
A (Control)	5.52 ± 0.90	15.20± 0.58	20.00 ± 5.48
B (Testosterone)	49.38 ± 1.25	19.40 ± 1.33	110.00 ±18.97
C (Ap40mg/kg)	1.10 ± 0.11 [*]	11.80 ± 0.66 [*]	40.00 ±14.05 [*]
D (Ap60mg/kg)	1.10 ± 0.07 [*]	11.60 ± 0.51 [*]	73.00 ±4.06 [*]
E (T +Ap40mg/kg)	44.56 ±1.34	16.20 ± 0.80 [#]	138.00 ±9.57 [#]
F (T+Ap60mg/kg)	40.56 ± 2.16 [#]	16.40 ± 0.51 [#]	166.00 ±16.54 [#]

Key: * indicates values that were significantly different when compared with the control group

indicates values that were significantly different when compared with group B

3.4 Histology of Testis (Photomicrograph Sections of Group A to F)



Photomicrograph Section of Testis (Control): Normal maturation of germinal epithelium in seminiferous tubules ($\times 400$ H&E stain)



Photomicrograph Section of Testis (B- Testosterone Group): Moderate and complete depletion of germinal epithelium in some of the seminiferous tubules (black arrows) ($\times 400$ H&E stain)



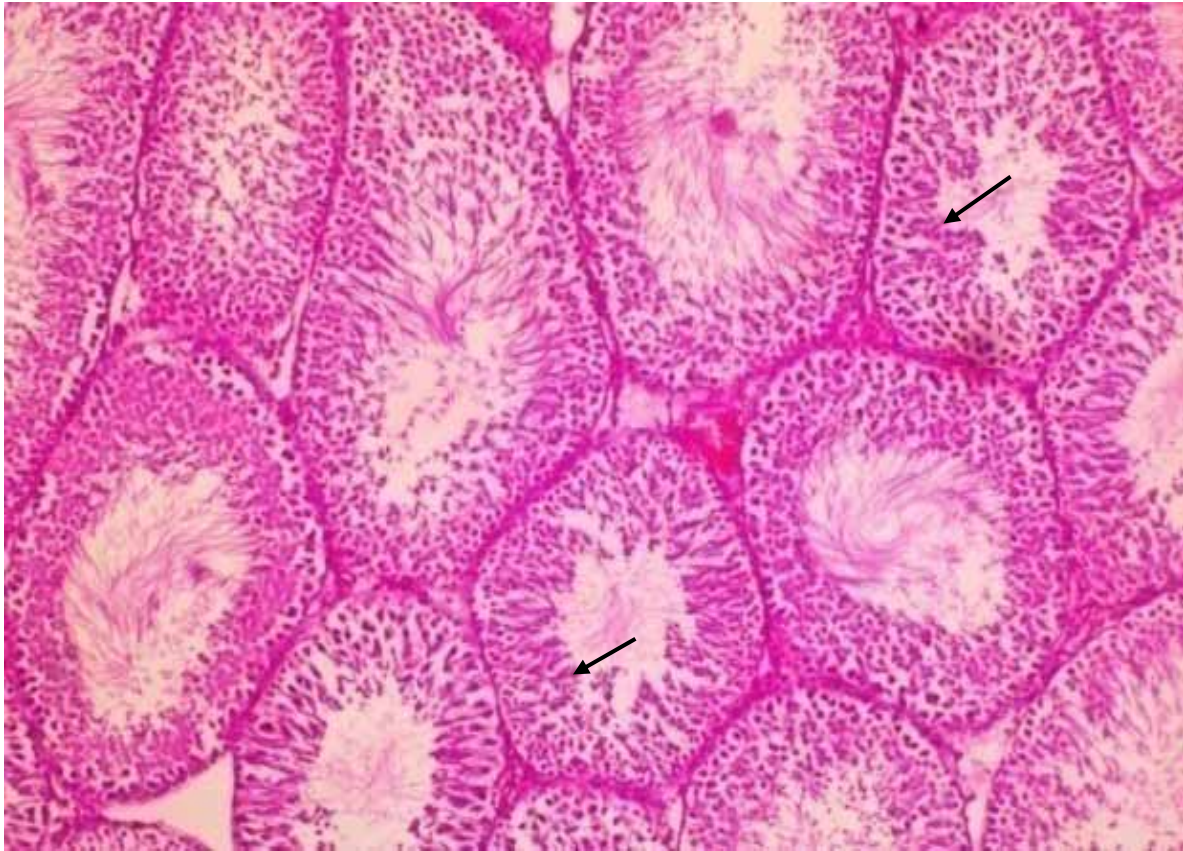
Photomicrograph Section of Testis (C- Ap40mg/kg Group): Moderate depletion of germinal epithelium in few seminiferous tubules (black arrow) ($\times 400$ H&E stain)



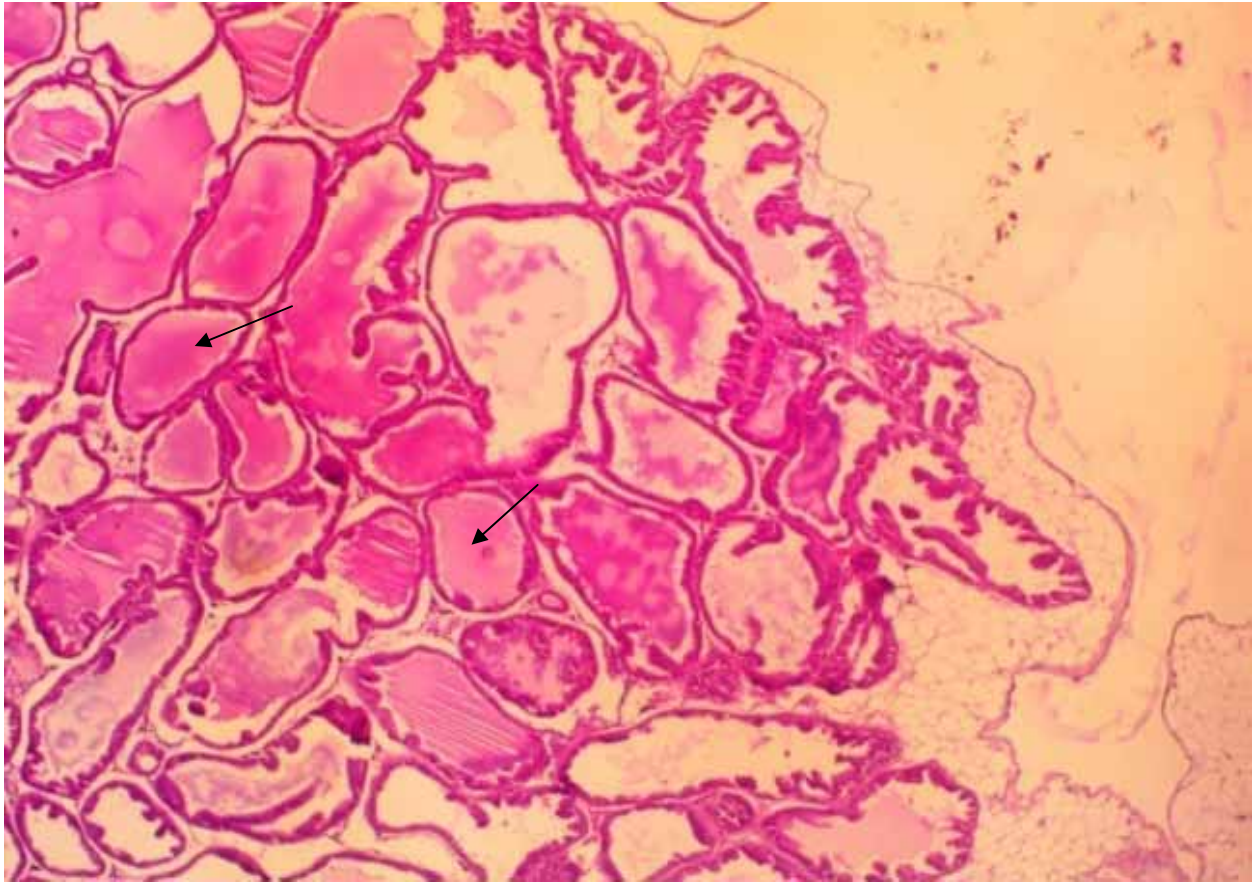
Photomicrograph Sections of Testis (D- Ap60mg/kg): Severe germinal epithelium depletion; irregular shape of seminiferous tubule (black arrows) and severe interstitial congestion (white arrows) ($\times 400$ H&E stain)



Photomicrograph Section of Testis (E- T+Ap40mg/kg Group): Normal maturation of germinal epithelium in the seminiferous tubules ($\times 400$ H&E stain)



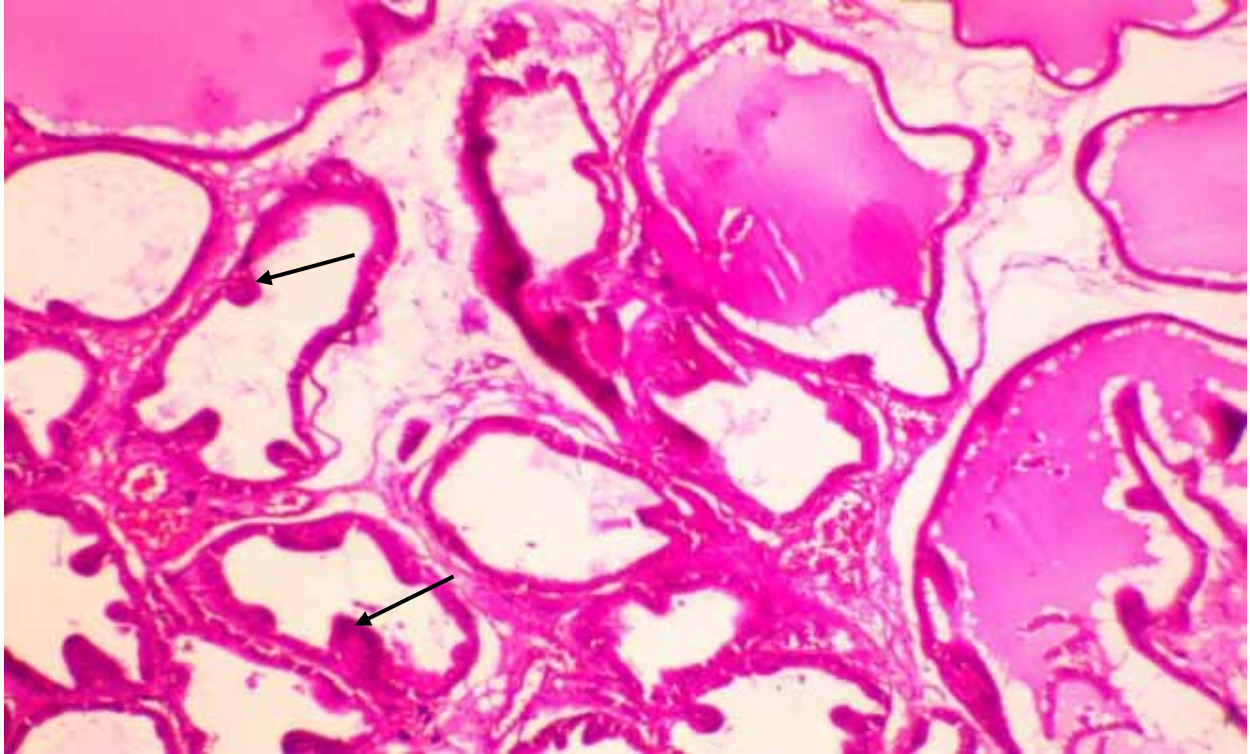
Photomicrograph Section of Testis (F- T+Ap60mg/kg): Moderate depletion of germinal epithelium in seminiferous tubules (black arrows) ($\times 400$ H&E stain)



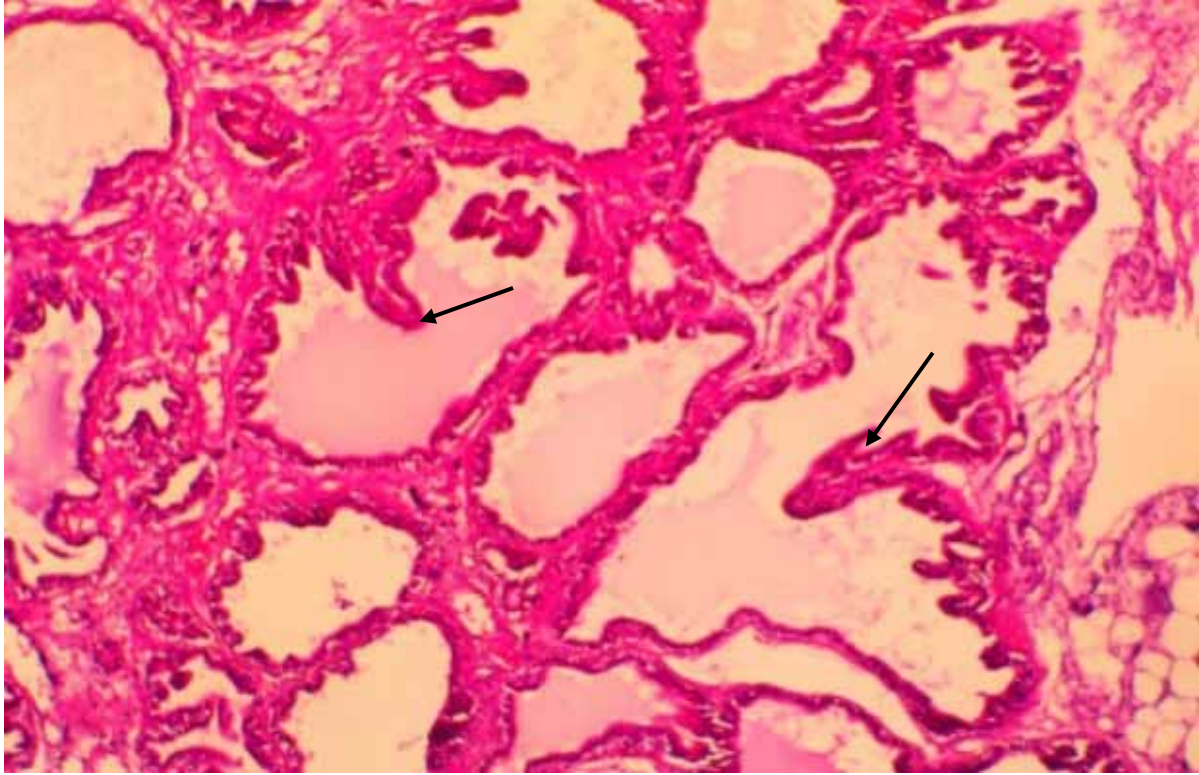
Photomicrograph Section of Testis (Control): Mild prostate hyperplasia with cystic hyperplasia (black arrow) ($\times 400$ H&E stain)



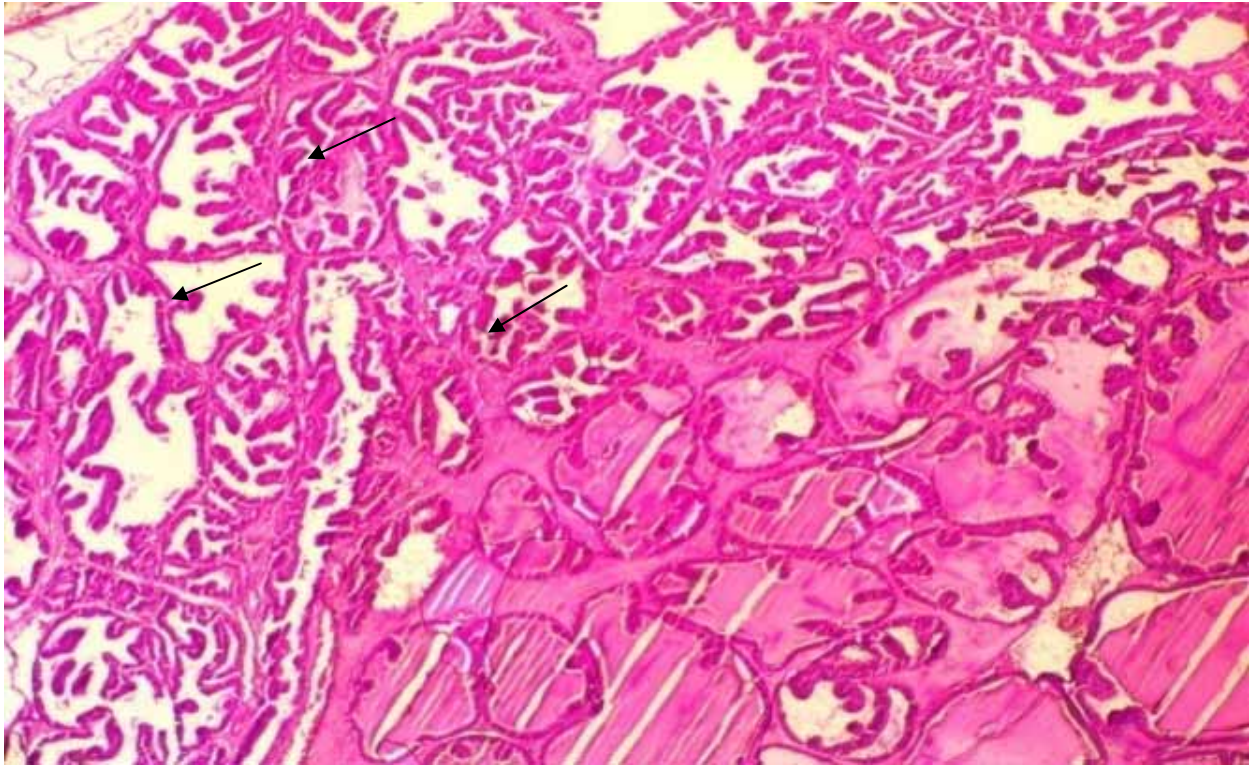
Photomicrograph Section of Testis (B- Testosterone group): Severe hyperplasia with protusion of epithelium into the lumen (white arrows) and moderate cystic hyperplasia (black arrows) of the prostate ($\times 400$ H&E stain)



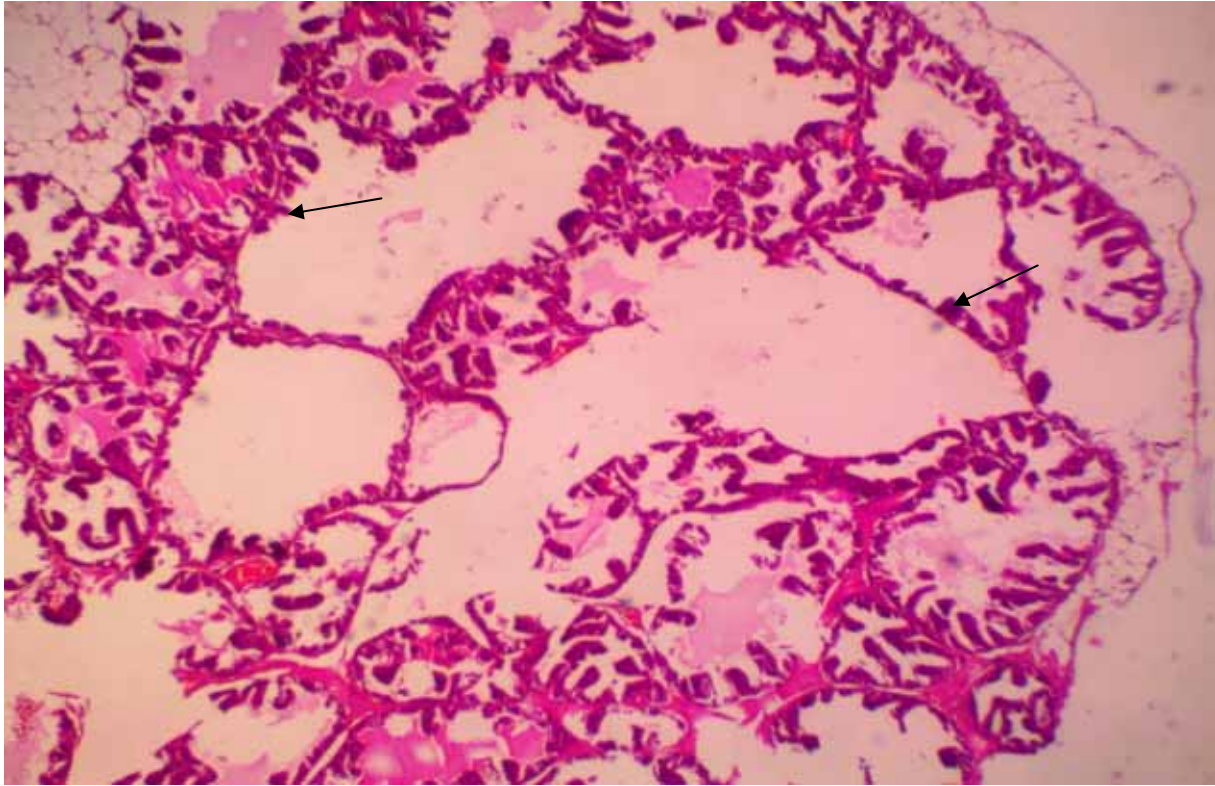
Photomicrograph Section of Testis (C- Ap40mg/kg): Moderate hyperplasia of prostate gland (black arrow) ($\times 400$ H&E stain)



Photomicrograph Section of Testis (D- Ap60mg/kg): Prostate hyperplasia with epithelium extending into the lumen ($\times 400$ H&E stain)



Photomicrograph Section of Testis (E- T+Ap40mg/kg): Marked prostate hyperplasia with epithelial ingrowth into the lumen (black arrows) ($\times 400$ H&E stain)



Photomicrograph Section of Testis (F- T+Ap60mg/kg): Mild hyperplasia of the prostate (black arrows) ($\times 400$ H&E stain)

4.0 Discussion

BPH is a common proliferative disorder and age-associated disease affecting 70% of men aged seventy years or over (McVary, 2006). The role of androgens to the normal physiological functioning cannot be over emphasized and the contribution of estrogens on the prostate development is complex and well reported (McPherson *et al.*, 2008; Su *et al.*, 2015). Androgens and estrogen significantly influence the development of BPH and the relative prostate weight is used as one of the important markers of BPH development (Arruzazabala *et al.*, 2006; Veeresh Babu *et al.*, 2010).

In this study, a significantly higher relative prostate weight observed in testosterone group as compared with the control group indicated the effectiveness of testosterone in BPH induction and this is in consistence with reports on the contribution of testosterone to prostate function independently of DHT (Khera *et al.*, 2014). The significantly lower prostate weight observed in Ap40mg/kg and Ap60mg/kg groups as compared with the control group and T+Ap40mg/kg and T+Ap60mg/kg groups in comparism with group B is plausible since histopathology results showed the presence of prostatic cysts only in the control and testosterone groups . Although prostatic hyperplasia is present in all the groups, but cysts observed in the prostate of control group and group B could have contributed to their higher weight. Prostatic cysts are encapsulated cysts containing either clear or cloudy fluid within the glandular tissue (White *et al.*, 1987), and contribute to the serious health consequences of BPH by causing irritative or obstructive lower urinary tracts symptoms (Issa *et al.*, 1999; Bayram *et al.*, 2011). Their presence in the control and testosterone groups of this study can be associated with high serum estrogen level observed in the groups which could have caused proliferation of prostate fibromuscular stroma cells with attendant dilation of glandular acini sequel to obstruction of ductuli. Previous studies revealed

the role of hyperestrogenization in the ethiopathogenesis of the prostatic fluid accumulation within the excretory ducts (Ursula et al., 1986; Dawn, 2010) and abundance of estrogen receptor beta mRNA, aromatase enzyme in the prostate stromal cells (Ho *et al.*, 2008).

The significant reduction observed in the weight of epididymis in Ap40mg/kg group can be linked with reduction in sperm count due to low testosterone observed in the group. Sperm presence in the epididymis contributes to epididymal weight (Robaire et al., 1977) and studies involving hypophysectomy, castration, and androgen replacement therapy suggest that androgens are essential for physiological maturation and survival of the spermatozoa in the epididymis (Kastner and Apfelbach, 1987). Studies suggest that seed causes reduction in sperm counts, degeneration of spermatozoa and vacoulation of epididymis (Sinha and Mathur, 1990; Talukder *et al.*, 2011).

Significantly lower serum testosterone and estrogen level in groups Ap40mg/kg, Ap60mg/kg, T+Ap40mg/kg and T+Ap60mg/kg may have occurred due to the inhibitory effect of high prostate DHT level in the groups on hypothalamic-pituitary-gonadal axis hence suppressed the endogenous production of testosterone with attendant and estrogen. Study show that the seed extract caused decrease in serum FSH, LH and testosterone levels (Talukder *et al.*, 2011) and had anti-estrogenic activity (Johri *et al.*, 2009). However, local prostatic DHT production has an influence on systemic serum DHT levels (Olsson et al., 2011) and high serum DHT levels suppresses of pituitary gonadotropin production (Mara and Stephanie, 2011).

Contrary to high prostate DHT levels observed in groups C, D, E and F of this study is the report which showed low DHT level in the prostate of rats treated with the seed extract (Surkiti *et al.*, 2011). However, it seems *Abrus precatorious* seed has pro- DHT activity as the high prostate DHT level observed in this study cannot be attributed to testosterone injection since the rats in

the groups treated with the seed extract (C and D) alone similarly demonstrated significantly high prostate DHT level.

In conclusion, the results of this study showed that BPH as typified by protusion of prostate epithelium into the lumen and cyst formation at advanced stage is milder in rats treated with the seed extract and testosterone plus the seed extract (groups E and F). *Abrus precatorious* seed therefore has potential to reduce severity of testosterone-induced BPH.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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