

Effect of Ethanol Extract of *Abrus precatorious* Seed on Testosterone-Induced Benign Prostatic Hyperplasia in Adult Male Wistar Rats

Abstract

Aim: To assess the effects of *Abrus precatorious* seed extract on prostate weight and hormonal levels (dihydrotestosterone, testosterone and estrogen) of male Wistar rats.

Study design: The study was divided into six groups A, B, C, D, E and F.

Place and Duration of Study: The study was conducted at Department of Physiology, College of Medicine, University of Ibadan between February and March, 2016.

Methodology: Thirty adult male rats were divided into six groups (n=5). Group A received water orally and groups C and D received 40 and 60 mg/kg of extract orally for 28 days respectively. Benign prostatic hyperplasia was induced in groups B, E and F via subcutaneous injection of 3mg/kg of testosterone. Groups E and F received concurrently 40 and 60 mg/kg of extract orally for 28 days respectively. Hormonal levels, epididymis, testis and prostate weight were determined. Sperm analysis and histological analysis of the testis and prostate were conducted.

Results: Prostate weight decreased ($P = .05$) in groups C (2.20 ± 0.25) and D (1.60 ± 0.29) when compared to group A (7.30 ± 0.58), and in groups E (8.00 ± 0.84) and F (9.60 ± 0.75) when compared to group B (14.40 ± 0.16). The serum estrogen level decreased in groups E (16.20 ± 0.80) and F (16.40 ± 0.51) as compared to group B (19.40 ± 1.33), and also in groups C (11.80 ± 0.66) and D (11.60 ± 0.51) when compared to group A (15.20 ± 0.58). There were increases in

prostate dihydrotestosterone levels of groups C and D compared to group A, and in groups E and F compared to group B. There was severe cystic prostate hyperplasia in group B.

Conclusion: *A. precatorious* seed has potential to reduce severity of testosterone-induced BPH.

Keywords: *Abrus precatorious*, Testosterone, Estrogen, 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 [1, 2]. 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 [3, 4]. 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 [5]. Proposed aetiological theories of BPH include embryonic reawakening, aging, androgens, estrogens, oxidoreductase and inflammation as aetiologies of BPH [6, 7]. However, all of the proposed theories require the presence of androgens as it appears that androgen play permissive roles in the development of BPH [8]. 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 5 α - 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 [9]. Prevention of DHT synthesis via 5 α - reductase inhibitions has a remarkable effect on BPH pathogenesis [10]. 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 outfluxes of fluid from the efferent ductules [11]. The prostate is an estrogen target tissue and estrogens have the potential to initiate changes in the prostate [12, 7]. 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 [13]. 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 mediating the negative-proliferative roles of estrogen in the prostate [14] and anti-proliferative role of estrogen in prostate growth being mediated by estrogen receptor beta [12].

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 [15] and studies suggested that alpha 1 adrenoceptor subtype mediate prostate muscle contraction [16]. As a form symptomatic treatment, alpha1-adrenergic blocking agents are commonly used to block alpha 1-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 [17]. The Leaves, roots and seeds of the plant are used for medicinal purposes and the seed is

reported to have male contraceptive property [18, 19]. *Abrus precatorius* seed extract causes male infertility by reducing serum testosterone levels [19, 20] and helps in the management of androgenic alopecia by inhibiting the activity of 5 α - reductase enzyme [21]. Most management options of BPH are instituted to safely improve quality of life by providing symptom relief and as well as reducing disease progression [22]. 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 [23]

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 [24]

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

88 were housed in clean and well ventilated cages. The animals were given water and feed (Top
89 feeds, Ibadan) *ad libitum*.

90 **2.3 Experimental Design**

91 The animals were randomly divided into six groups (A to F) each consisted of five rats. Control
92 group, group C (Ap40mg/kg) and group D (Ap40mg/kg) were given 1 ml/kg of distilled water,
93 40 mg/kg of the seed extract and 60 mg/kg of the seed extract respectively via oral gavage. BPH
94 was induced by subcutaneous administration of testosterone (3mg/kg) in groups B
95 (Testosterone), E (T+Ap40mg/kg) and F (T+Ap60mg/kg) daily for twenty eight (28) days [25,
96 26]. These groups were simultaneously given 1 ml/kg of distilled water, 40 mg/kg and 60 mg/kg
97 of the seed extract orally for 28 days respectively.

98 **2.4 Animal Sacrifice, Blood and Prostate Tissue Collection**

99 The rats were sacrificed on the 28th day via intraperitoneal injection of 120 mg/kg of sodium
100 thiopentone [27]. Blood sample for serum hormonal assay was collected from each animal via
101 cardiac puncture into plain serum bottle and was allowed to clot. The serum was obtained [28].
102 Serum concentrations of testosterone and estrogen were assayed using the Enzyme-linked
103 immunosorbent assay (ELIZA) kits (Calbiotech, USA). One of the two lobes of prostate gland
104 was harvested from each rat into an organ bottle containing 1 mL of phosphate buffer solution
105 and homogenized. Homogenized prostate tissues were cold centrifuged at 10000 rpm for 15
106 minutes, supernatant was collected and DHT was assayed using an ELIZA (Elabscience, China).

107 **2.5 Organ Collection and Histology of Tissues**

108 The testes, prostate gland and epididymis were harvested from the rats and weighed with an
109 electric weighing balance. The testis and prostates were then fixed in Bouin's fluid and 10%
110 formalin respectively before being dehydrated in descending grades of alcohol, cleared in
111 chloroform and impregnated in paraffin. Then 5-6 μ m sections were placed into the grease free
112 slides, de-paraffinized in xylene and stained with haematoxyline and eosin [29].

113

114 **2.6 Sperm Analysis**

115 The epididymis was carefully removed from the testis and its content was released unto slides.
116 Sperm vitality was assessed by staining of sperm smear with eosin-nigrosin staining technique
117 (WHO, 2010). Sperm count was done using established method [30]

118 **2.7 Statistical Analysis**

119 Data were analyzed with GraphPad Prism (Version 5.04). Differences between the means were
120 tested with analysis of variance. The results from each group were expressed as mean $\pm$ standard
121 error of mean (S.E.M) and means were compared for significant differences at $P = .05$.

122 **3.0 Results**

123 **3.1 Reproductive Organ weights**

124 There was a significant increase in the mean weight of prostate gland in group B when compared
125 with control group. The mean weights of prostate gland in groups C and D were significantly
126 lower ($P = .05$) when compared with the control group. There was significant decrease in the
127 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 ($P = .05$) 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 ($10^6/\text{mm}^3$)	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	$38.00 \pm 5.83^*$	$46.00 \pm 5.10^*$
D (Ap60mg/kg)	$5.00 \pm 0.30^*$	$56.00 \pm 8.72^*$	$40.00 \pm 8.94^*$
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

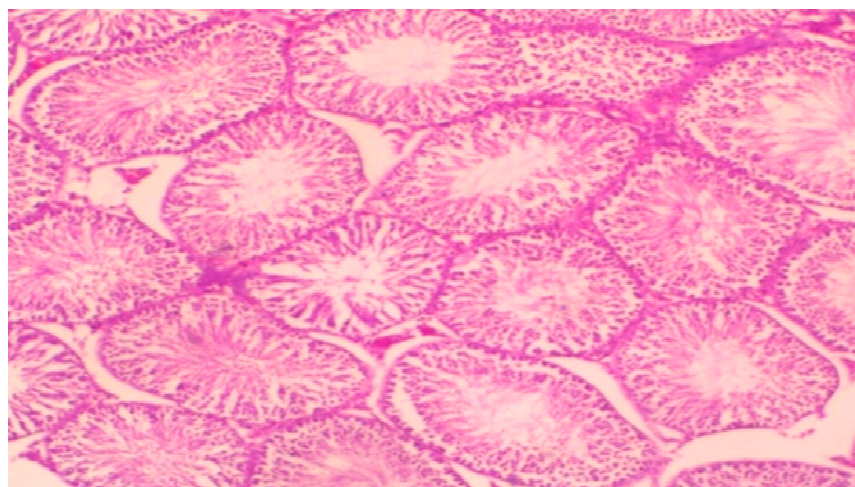
Groups	Serum Testosterone level (ng/ml)	Serum Estrogen level (ng/ml)	Prostate DHT level (Pg/ml)
A (Control)	5.52 ± 0.90	15.20± 0.58	20.00 ± 5.48
B (Testosterone)	49.38 ± 1.25	19.40 ± 1.33	110.00 ±18.97
C (Ap40mg/kg)	1.10 ± 0.11 [*]	11.80 ± 0.66 [*]	40.00 ±14.05 [*]
D (Ap60mg/kg)	1.10 ± 0.07 [*]	11.60 ± 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 ($P = .05$) when compared with the control group

[#] indicates values that were significantly different ($P = .05$) when compared with group B

202 3.4 Histology of Testis

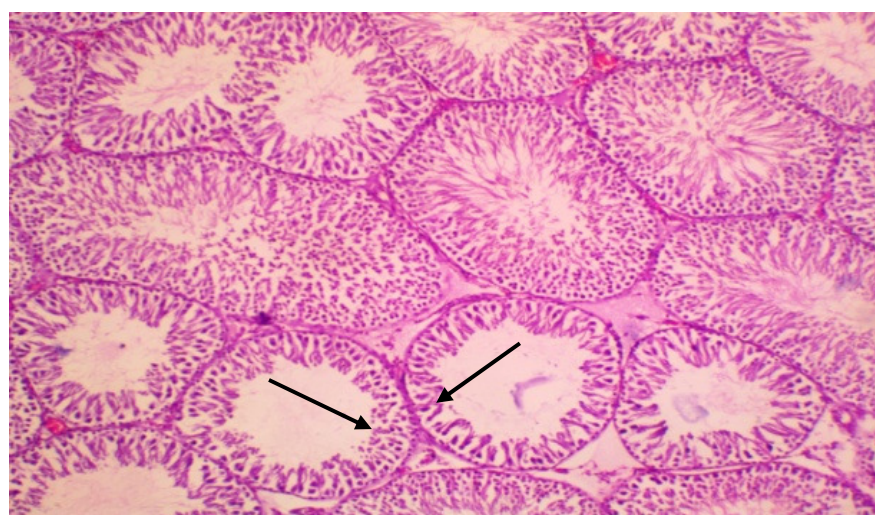
203 There was normal maturation of germinal epithelium in seminiferous tubules of testes in control
204 group



(× 400 H&E stain)

206 **Figure 1:** Photomicrograph Section of Testis (Control)

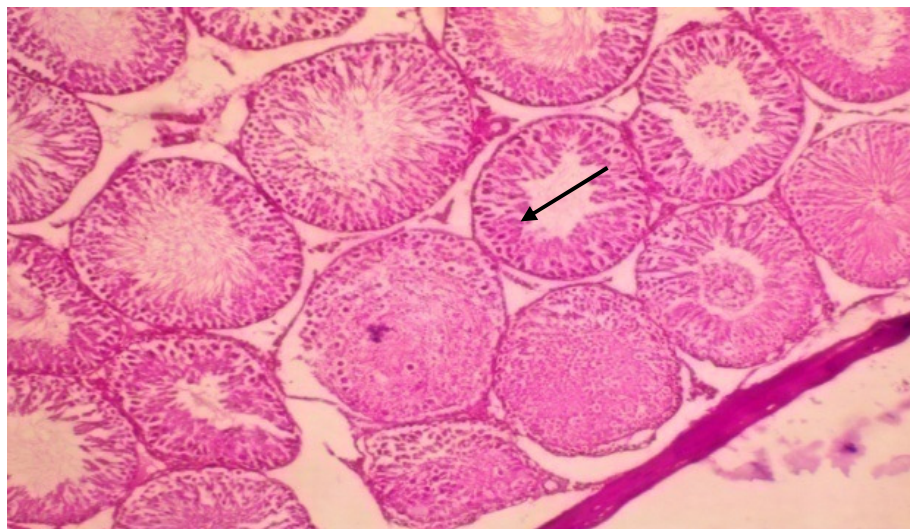
207 There was moderate and complete depletion of germinal epithelium in some of the seminiferous
208 tubules (black arrows) of testes in group B



(× 400 H&E stain)

210 **Figure 2:** Photomicrograph Section of Testis (B- Testosterone Group)

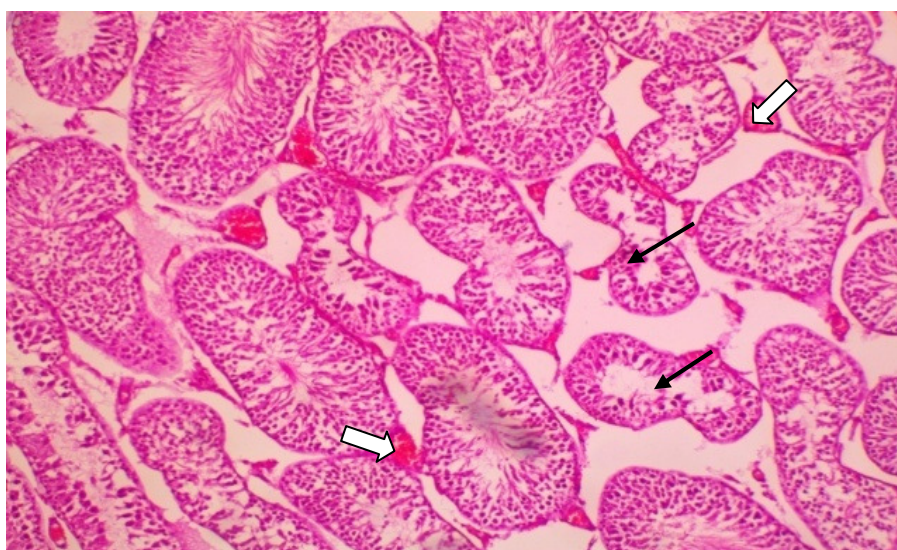
211 There was moderate depletion of germinal epithelium in few seminiferous tubules (black arrow)
 212 of testes in group C



(× 400 H&E stain)

214 **Figure 3:** Photomicrograph Section of Testis (C- Ap40mg/kg Group))

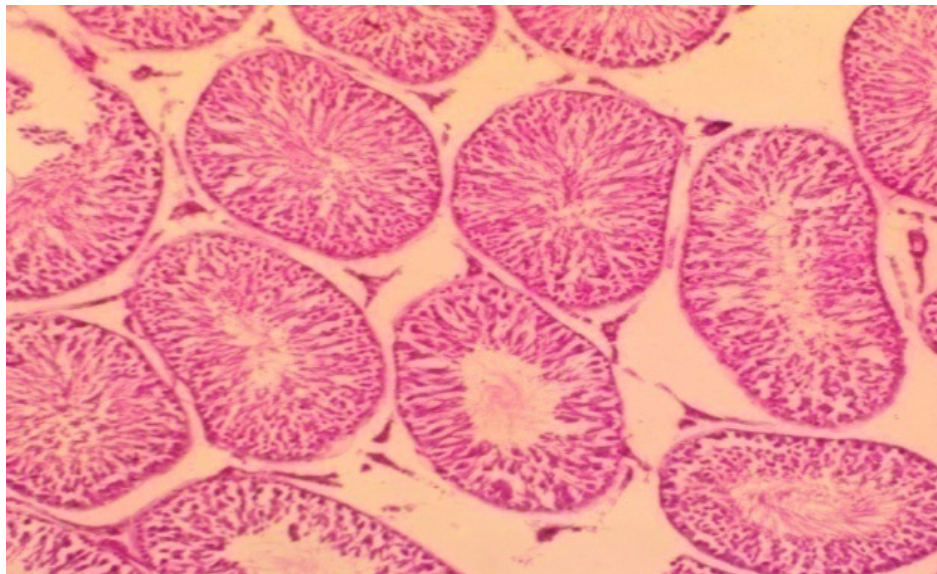
215 There was severe germinal epithelium depletion, irregular shape of seminiferous tubule (black
 216 arrows) and severe interstitial congestion (white arrows) in testes of group D



(× 400 H&E stain)

218 **Figure 4:** Photomicrograph Sections of Testis (D- Ap60mg/kg)

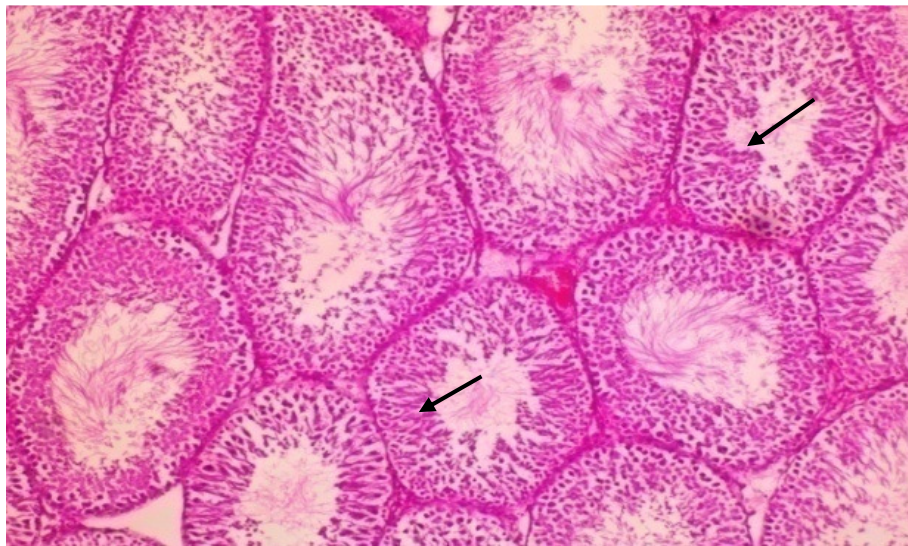
219 There was normal maturation of germinal epithelium in the seminiferous tubules of testes in
220 group E



(× 400 H&E stain)

222 **Figure 5:** Photomicrograph Section of Testis (E- T+Ap40mg/kg Group)

223 There was moderate depletion of germinal epithelium in seminiferous tubules (black arrows) of
224 testes in group F

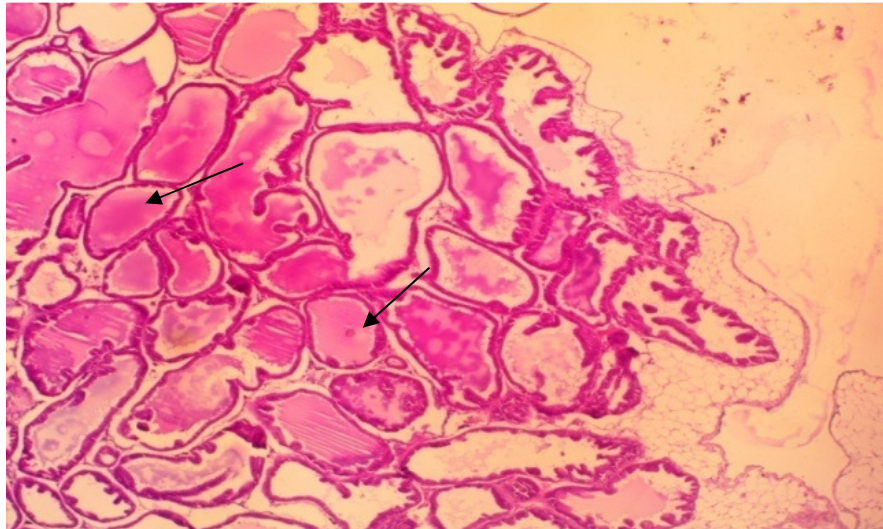


(× 400 H&E stain)

226 **Figure 6:** Photomicrograph Section of Testis (F- T+Ap60mg/kg)

227 **Histology of Prostate gland**

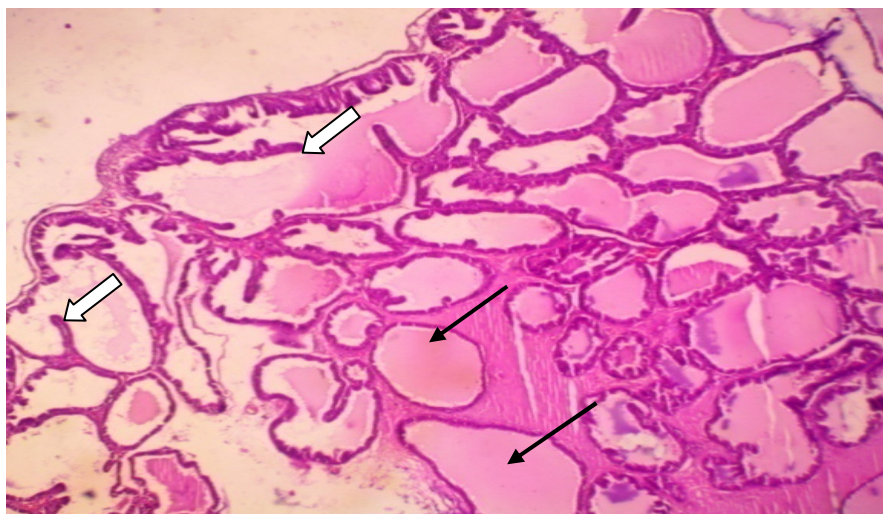
228 There was mild prostate hyperplasia with cystic hyperplasia (black arrow) in group A



229 (× 400 H&E stain)

230 **Figure 7: Photomicrograph Section of Testis (Control)**

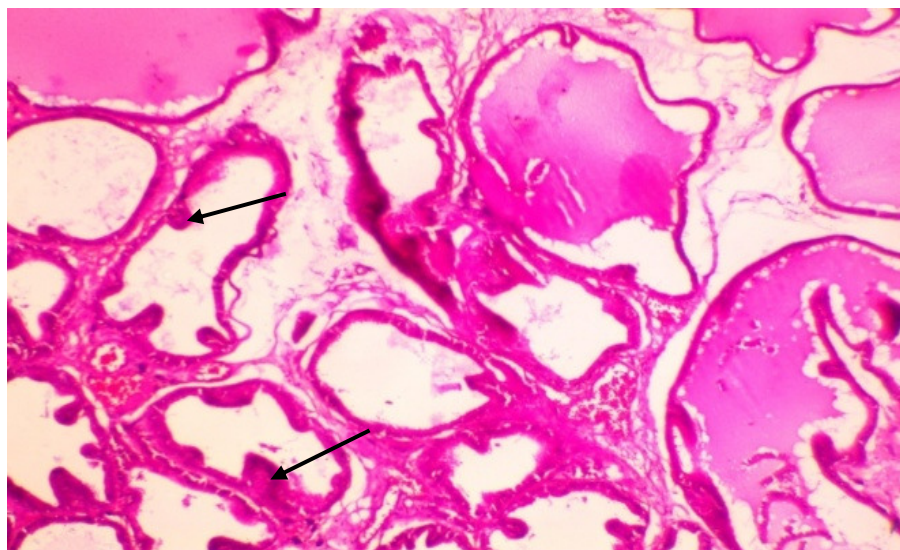
231 There was severe hyperplasia with protusion of epithelium into the lumen (white arrows) and
 232 moderate cystic hyperplasia (black arrows) of the prostate in group B



233 (× 400 H&E stain)

234 **Figure 8: Photomicrograph Section of Testis (B- Testosterone group)**

235 There was moderate hyperplasia of prostate gland (black arrow) in group C

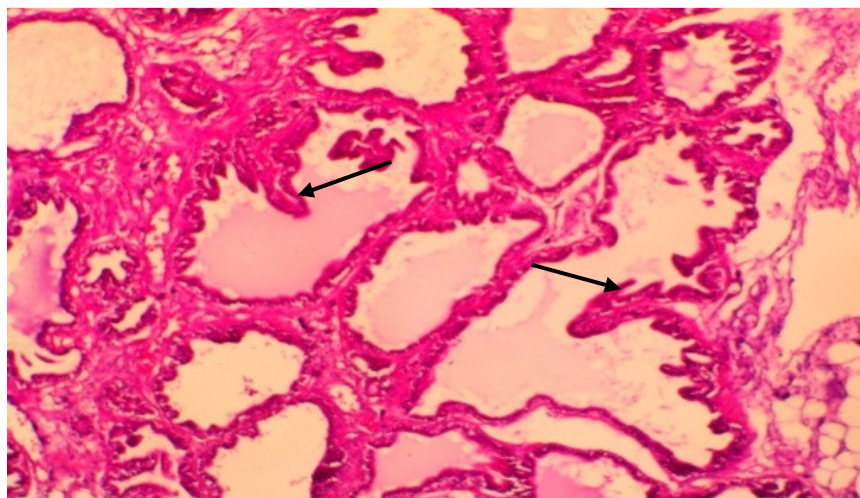


236 (× 400 H&E stain)

237 **Figure 9:** Photomicrograph Section of Testis (C- Ap40mg/kg)

238 There was prostate hyperplasia with epithelium extending into the lumen (black arrows) in group

239 D

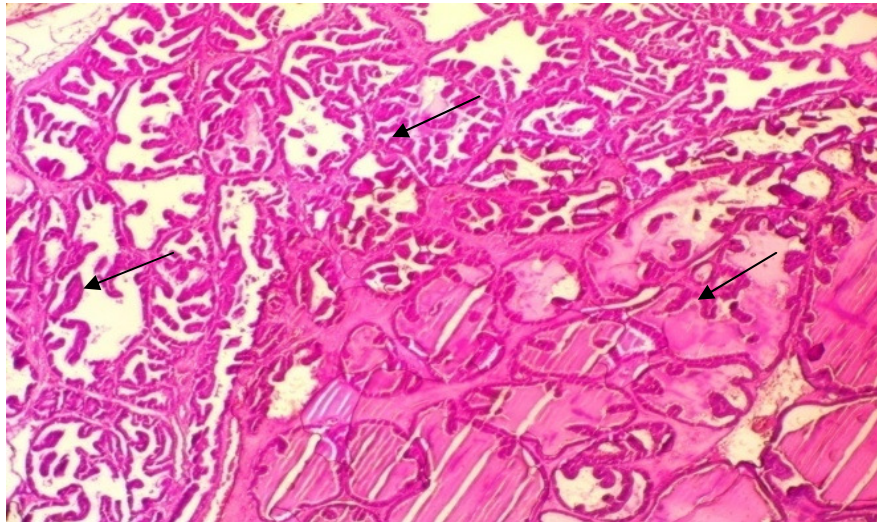


240 (× 400 H&E stain)

241 **Figure 10:** Photomicrograph Section of Testis (D- Ap60mg/kg)

242

243 There was marked prostate hyperplasia with epithelial ingrowth into the lumen (black arrows) in
 244 group E

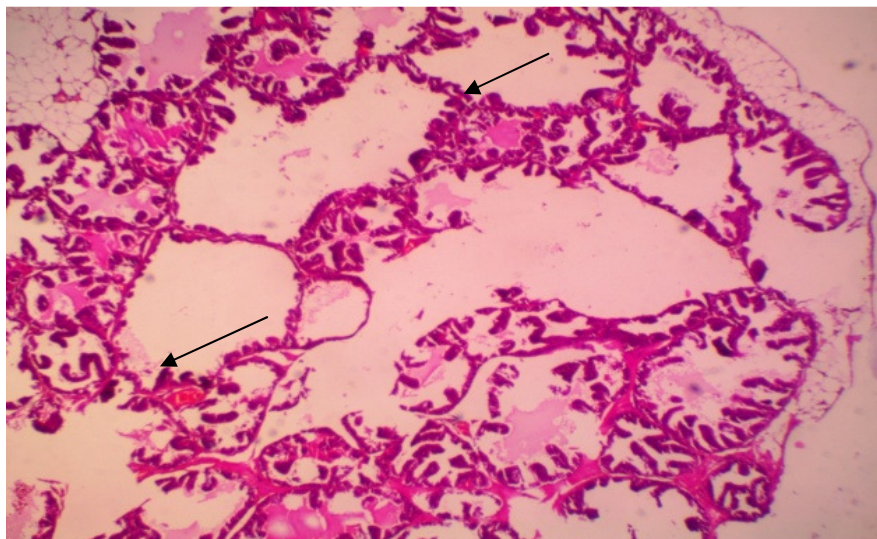


245 (× 400 H&E stain)

246 **Figure 11:** Photomicrograph Section of Testis (E- T+Ap40mg/kg)

247

248 There was mild hyperplasia of the prostate (black arrows) in group F



249 (× 400 H&E stain)

250 **Figure 12:** Photomicrograph Section of Testis (F- T+Ap60mg/kg)

251 4.0 Discussion

252 BPH is a common proliferative disorder and age-associated disease affecting 70% of men aged
 253 seventy years or over [31]. The role of androgens to the normal physiological functioning cannot
 254 be over emphasized and the contribution of estrogens on the prostate development is complex
 255 and well reported [14, 32]. Androgens and estrogen significantly influence the development of
 256 BPH and the relative prostate weight is used as one of the important markers of BPH
 257 development [33, 34]

258 In this study, a significantly higher relative prostate weight observed in testosterone group as
 259 compared with the control group indicated the effectiveness of testosterone in BPH induction
 260 and this is in consistence with reports on the contribution of testosterone to prostate function
 261 independently of DHT [35]. Although prostatic hyperplasia is present in all the groups, but
 262 significantly lower prostate weight observed in Ap40mg/kg and Ap60mg/kg groups as compared
 263 with the control group, and T+Ap40mg/kg and T+Ap60mg/kg groups in comparison with group
 264 B (testosterone group) is plausible, as histopathology results showed the presence of prostatic
 265 cysts only in the control and testosterone groups. Prostatic cysts are encapsulated cysts [36], and
 266 contribute to the serious health consequences of BPH by causing irritative or obstructive lower
 267 urinary tracts symptoms [37, 38]. The presence of cysts in the control and testosterone groups of
 268 this study can be associated with high serum estrogen level observed in the groups. Previous
 269 studies revealed the role of hyperestrogenization in the ethiopathogenesis of the prostatic fluid
 270 accumulation within the excretory ducts [39, 40] and abundance of estrogen receptor beta which
 271 has anti-proliferative effect in the prostate [7].

272 The significant reduction observed in the weight of epididymis in Ap40mg/kg group can be
 273 linked with reduction in sperm count due to low testosterone observed in the group. Sperm

presence in the epididymis contributes to epididymal weight [41] 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 [42]. Studies suggest that the seed extract causes reduction in sperm counts, degeneration of spermatozoa and vacuolation of epididymis [43, 20].

Significantly lower serum testosterone and estrogen level in groups Ap40mg/kg, Ap60mg/kg, T+Ap40mg/kg and T+Ap60mg/kg resulted from the inhibitory effect of high prostate DHT level in the groups on hypothalamic-pituitary-gonadal axis hence suppressed the endogenous production of testosterone and estrogen. Study has shown that the seed extract caused decrease in serum FSH, LH and testosterone levels [20] and had anti-estrogenic activity [44]. Local prostatic DHT production has been reported to have an influence on systemic serum DHT levels [45] and high serum DHT levels suppress pituitary gonadotropin production [46].

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 [21]. 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.

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