

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

Abstract

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

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: Relative prostate weight decreased ($P = .05$) in groups C (0.022 ± 0.0025) and D (0.016 ± 0.0029) when compared to group A (0.0730 ± 0.0058), and in groups E (0.080 ± 0.0084) and F (0.096 ± 0.0075) when compared to group B (0.14 ± 0.0016). 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).

Prostate dihydrotestosterone levels increased in 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 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 and study showed the usefulness of *Gonodermalucidum* extract in the management of testosterone-induced BPH [17]. Previous reports on phytotherapeutic management of BPH

demonstrated inhibitory effect of *Lepidiummeyeni* extract [18] and protective effect of *Echinopsechinatus* extract [19] on testosterone-induced BPH. *BenincasahispidaCongn.*, *Sphaeranthusindicus* and *Urticadioica* Linn. were reported to inhibit 5-alpha reductase enzyme activity [20] and these plants have demonstrated ameliorative effect on testosterone-induced prostate hyperplasia by reducing relative prostate weight in treated animals [21-23]. *Seronoarepens* also known as saw palmetto contains sterols and its inhibitory effect on 5-alpha reductase activity with attendant decrease in DHT production has been reported to be useful in the management of BPH [24]. *Abrusprecatorius* is a woody twinning plant with characteristic toxic red seeds [25]. *Abrusprecatorious* seed contains amino acids, poisonous protein, a fat-splitting enzyme, aglucosideabruassic acid, haemagglutinin, albuminous substance named abrin [26]. The Leaves, roots and seeds of the plant are used for medicinal purposes and the seed is reported to have male contraceptive property [27, 28] due to its ability to cause reversible male infertility [28]. This antifertility property of the seed is associated to its ability to reduce serum testosterone level [29] and its abrin content which inactivates rRNA thus resulting in inhibition of protein synthesis in sertoli and leydig cells [30]. The steroids fraction of the seed also inhibits spermatogenesis by replacing the natural steroids that is, LH, FSH and testosterone [31] and this shows the anti-gonadotropin property of the seed [32]. Findings also revealed the usefulness of the seed in the management of androgenic alopecia due to its inhibitory effect on the activity of 5 α - reductase enzyme [33] and this suggests its potential benefit in the management of BPH.

Most management options of BPH are instituted to safely improve quality of life by providing symptom relief and as well as reducing disease progression [34]. 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 [35]

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 *Abrusprecatorious* on BPH management and this study was carried out to assess the effects of *Abrusprecatorious* seed on testosterone-induced benign prostatic hyperplasia.

2.0 Materials and Methods

2.1 *Abrusprecatorious* (Licorice) Seed Extract Preparation

One and half kilogram (1.5 kg) of fresh seeds of *Abrusprecatorious* 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 [36]. The powdery form of the seed was poured in clean glass jar and soaked with six litres of ethanol for 72 hours with the solution stirred at every 24 hours. The solution was decanted and then filtered using Number 1 Whatman filter paper. The filtrate was evaporated using rotatory evaporator (RE52-2 Search Tech Instrument) at 40 °C and the extract yield was 13.4g

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, group C (Ap40mg/kg) and group D (Ap40mg/kg) 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 [37, 38]. 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 sodiumthiopentone [39]. 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 [40]. 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 relative organ weight of the each animal was then determined as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute weight of organ (g)} \times 100}{\text{Body weight of rat on sacrifice (g)}}$$

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 μm sections were placed into the grease free slides, de-paraffinized in xylene and stained with haematoxyline and eosin [41].

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 using established method [42]

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 $P = .05$.

3.0 Results

3.1 Relative Reproductive Organ weights

There was a significant increase in the relative mean weight of prostate gland in group B when compared with control group. The relative mean weights of prostate gland in groups C and D were significantly lower ($P = .05$) when compared with the control group. There was significant decrease in the relative mean prostate weight of groups E and F when compared with group B.

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

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

TABLE 1.Relative Reproductive Organ Weights

Groups	Relative Prostate	Relative Epididymis	Relative Testicular
Weight (%)	Weight(%)	Weight(%)	
A (Control)	0.073 ± 0.0058	0.25 ± 0.027	0.51 ± 0.045
B(Testosterone)	0.14 ± 0.0016	0.27 ± 0.019	0.53 ± 0.0021
C (Ap40mg/kg)	0.022 ± 0.0025*	0.20 ± 0.0020	0.41 ± 0.030
D(Ap60mg/kg)	0.016 ± 0.0029*	0.17 ± 0.0081*	0.42 ± 0.038
E(T+Ap40mg/kg)	0.080 ± 0.0084#	0.27 ± 0.017	0.53 ± 0.027
F(T +Ap60mg/kg)	0.096 ± 0.0075#	0.26 ± 0.012	0.59 ± 0.085

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 (10 ⁶ /mm ³)	Sperm Count (%) (%)	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.

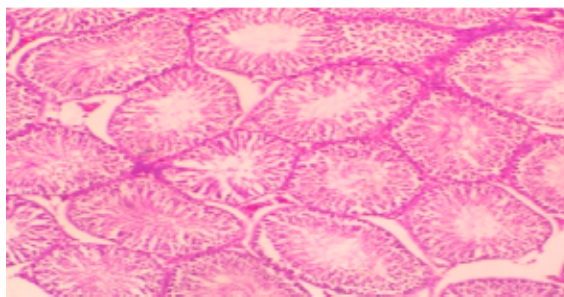
TABLE 3. Hormone levels

Groups	Serum Testosterone	Serum Estrogen	Prostate DHT
level (ng/ml)	level (ng/ml)	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

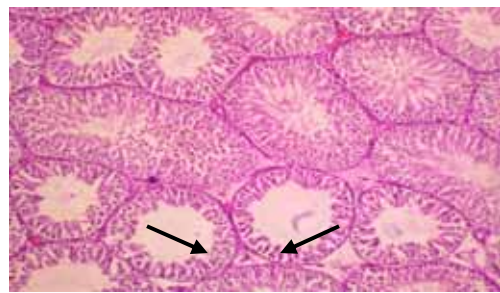
indicates values that were significantly different ($P = .05$) when compared with group B

3.4 Histology of Testis



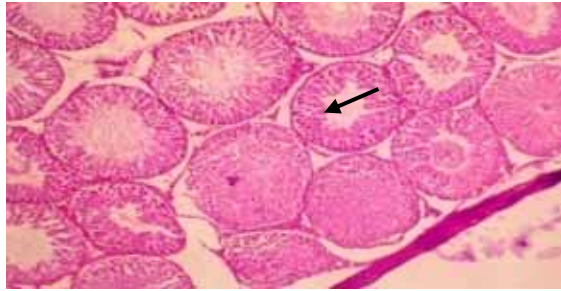
× 400

Control: Normal maturation of seminiferous tubules (H&E)

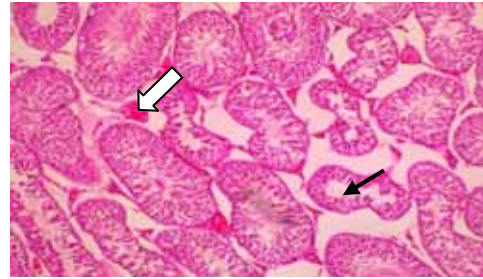


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Group B: Severe depletion of germinal epithelium in few seminiferous tubules (black arrows) (H&E)

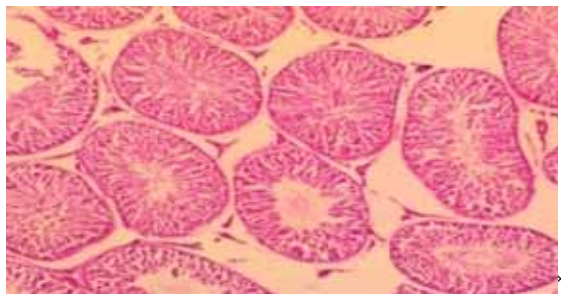


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Group C: Moderate depletion of germinal epithelium in few seminiferous tubules (black arrow) (H&E) **Group D:** Severe depletion of seminiferous tubule (black arrow) and interstitial congestion (white arrow) (H&E)



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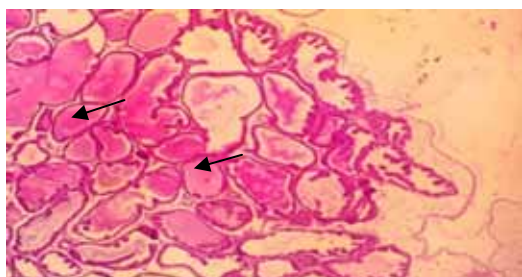
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Group E: Normal maturation of germinal epithelium in seminiferous tubules (H&E) tubules

Group F: Moderate depletion of germinal epithelium in seminiferous tubules (black arrow) (H&E)

Figure 1: Photomicrograph sections of testes

Histology of Prostate gland



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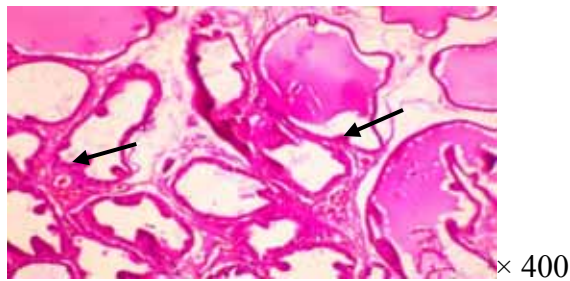


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Control: Mild prostate hyperplasia with cystic hyperplasia (black arrows)

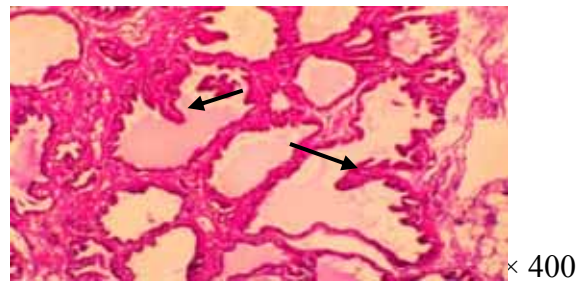
Group B: Severe hyperplasia with protusion of epithelium into the lumen(white arrows)

and moderate cystic hyperplasia

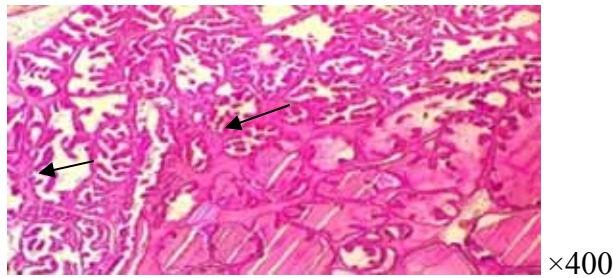


Group C: Moderate hyperplasia of the prostate gland (black arrows) (H&E)

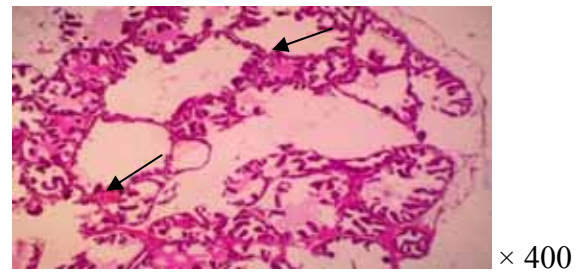
(black arrow) (H&E)



Group D: Severe prostate hyperplasia with epithelium extending into the lumen (black arrows) (H&E)



Group E: Marked prostate hyperplasia with epithelial protusion into the lumen (black arrows) (H&E)



Group F: Mild hyperplasia of the prostate (black arrows) (H&E)

Figure 2: Photomicrograph sections of prostate gland

4.0 Discussion

BPH is a common proliferative disorder and age-associated disease affecting 70% of men aged seventy years or over [43]. 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 [14, 44]. 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 [45, 46]

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 [47]. Although prostatic hyperplasia is present in all the groups, but 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 comparison with group B (testosterone group) is plausible, as histopathology results showed the presence of prostatic cysts only in the control and testosterone groups. Prostatic cysts are encapsulated cysts [48], and contribute to the serious health consequences of BPH by causing irritative or obstructive lower urinary tracts symptoms [49, 50]. The presence of cysts in the control and testosterone groups of this study can be associated with high serum estrogen level observed in the groups. Previous studies revealed the role of hyperestrogenization in the etiopathogenesis of the prostatic fluid accumulation within the excretory ducts [51, 52] and abundance of estrogen receptor beta which has anti-proliferative effect in the prostate [7].

The significant reduction observed in the weight of epididymis in Ap40mg/kg group can be linked with reduction in sperm count observed in the group. Study has indicated that presence of sperm in the epididymis contributes to epididymal weight [29]. Previous reports on the seed revealed that the seed extract causes reduction in sperm counts, degeneration of spermatozoa and vacuolation of epididymis [32].

Our study showed no significant difference in the weight of testes in the control group when compared with the Ap40mg/kg and Ap60mg/kg groups, but histopathology section showed germinal epithelium depletion of the seminiferous tubule which appeared more severe in Ap60mg/kg group than Ap40mg/kg group. The depletion of germinal epithelium observed in

these groups is associated with reduction in serum testosterone level recorded for the groups and this finding seems to be in line with the study that found reduction in germinal epithelial height in the testicle of animals treated with the seed extract [29]. Testosterone is an important regulator of spermatogenesis and maturation of germ cells [53]. The severe depletion of germinal epithelium of seminiferous tubule observed in testosterone group is due to conversion of exogenous testosterone administered into estrogen. Estrogen acts through feedback mechanism on hypothalamic pituitary gonadal axis and thus inhibits intrinsic production of testosterone. Previous study demonstrated that exogenous administration of testosterone to male animals produced atrophy of germinal epithelium with attendant suppression of spermatogenesis [54]. The significant decrease in epididymis sperm count observed in Ap60mg/kg group resulted from low serum testosterone level recorded in the group. Studies involving depriving testicle of testosterone suggest that androgens are essential for physiological maturation and survival of the spermatozoa in the epididymis [55]

Significantly lower serum testosterone and estrogen level in groups Ap40mg/kg, Ap60mg/kg, T+Ap40mg/kg and T+Ap60mg/kg suggests anti-gonadotropin effect of the seed extract. The seed extract has been reported in some studies to cause decrease in serum FSH, LH and testosterone levels [32] and also possesses anti-estrogenic activity [56].

Contrary to high prostate DHT levels observed in groups Ap40mg/kg, Ap60mg/kg, T + Ap40mg/kg and T + Ap60mg/kg of this study is the report which showed low DHT level in the prostate of rats treated with the seed extract [33]. 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 Ap40mg/kg and Ap60mg/kg groups similarly demonstrated significantly high prostate DHT level. This high prostate DHT level is believed to complement

the beneficial and anti-prostate growth associated with low serum estrogen level observed in the groups, hence inhibited cystic prostate hyperplasia in the groups. DHT and its metabolites are agonists that promote estrogen receptor beta activity [57, 58] and this receptor controls prostate development as it mediates anti-proliferative signalling in the prostate [12]. However, further study needs to be carried out on phytoconstituents of the seed and its effects on activity of other BPH markers and modulators such as 5-alpha reductase enzymes, prostate specific antigens, aromatase enzymes and androgen receptor.

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). *Abrusprecatorious* seed therefore has potential to reduce severity of testosterone-induced BPH.

Ethical Approval:

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

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