Effect of *Benincasa hispida* Fruits on Testosterone-Induced Prostatic Hypertrophy in Albino Rats

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**ABSTRACT**

**BACKGROUND:** *Benincasa hispida* Cogn. has been used traditionally in India for the management of urinary disorders. The fruit of *B. hispida* is used as a diuretic and the seeds have been reported to possess antiangiogenic effects in prostate cells.

**OBJECTIVE:** The aim of the present study was to examine the effect of petroleum ether extract, ethanolic extract, and *B. hispida* seed oil on hyperplasia of the prostate induced by the subcutaneous administration of testosterone in rats.

**METHODS:** In vitro studies were performed to determine the 5α-reductase inhibitory potential of the extracts. The results of those studies paved the way for the pharmacologic screening of the extracts to assess their potential against testosterone-induced hyperplasia in rats. Nine groups containing 10 rats per group were created for this study. Hyperplasia was induced by administration of testosterone (3 mg/kg SC) for 14 days in all the groups except the vehicle-treated group. Simultaneous administration of petroleum ether extract (100 or 200 mg/kg PO), ethanolic extract (100 or 200 mg/kg PO), and *B. hispida* seed oil (20 or 40 mg/kg PO) was conducted. A standard 5α-reductase inhibitor (ie, finasteride) was used as a positive control. The weight of the rats was recorded on day 0 (ie, day 1 of the study) and on day 14, and the influence of testosterone and test extracts on the weight of the rats was determined. On day 14, rats were euthanized; prostates were dissected out, and weighed. The rats' prostate/body weight (P/BW) ratio was then determined. Histologic examinations were performed on prostates from each group.

**RESULTS:** The petroleum ether extract as well as *B. hispida* seed oil exhibited inhibition of 5α-reductase activity in in vitro studies. Ethanol extract did not exhibit significant inhibitory potential in vitro. Further in vivo study found that testosterone treatment significantly increased the rats' P/BW ratio in all the groups except the vehicle-treated rats, and this increase in weight was significantly inhibited in rats administered petroleum ether extract (100 and 200 mg/kg PO) and *B. hispida* seed oil (20 and 40 mg/kg PO). Ethanol extract did not exhibit any significant activity.

**Conclusions:** Petroleum ether extract and *B. hispida* seed oil inhibited testosterone-induced hyperplasia of the prostate in these rats. Further studies are

**Key words:** Benincasa hispida, Cucurbitaceae, benign prostatic hyperplasia, prostate/body weight ratio, testosterone.

**INTRODUCTION**

Benign prostatic hyperplasia (BPH) is the result of gradual overgrowth of the prostate gland, a gland that lies at the base of the bladder and encircles the urethra. The enlarged prostate impinges on the urethra and therefore BPH is generally associated with impairment in urinary function. It is reported that 80% of men aged >80 years suffer from BPH.

Benincasa (Cucurbitaceae) is a monotypic genus with a single species, Benincasa hispida Cogn. It is under cultivation in large areas in Indian states (ie, Uttar Pradesh, Punjab, Rajasthan, Bihar). Traditionally, the fruit of *B hispida* is used as a diuretic and the seeds have been reported to exhibit antiangiogenic effects on prostate cells.

*B hispida* seeds consist of steroids such as β-sitosterol and stigmas-5-ene-3-β-ol, alkaloids such as 5-methylcytosine, saponins, urea, citrulline, linoleic and oleic acids, minute amounts of triterpenoids known as isomultiflœrenol and cucurbitacin B, and proteins such as trigonelline, cofferain, and osmorin.

*Cucurbita pepo* (pumpkin) has been widely studied for its preventive actions against BPH. Carbin et al found that the actions of petroleum ether extract of the pulp of *C pepo* fruits may be attributed to the content of phytosterols that are known to interfere with the actions of dihydrotestosterone (DHT). Pumpkin seed oil has been found to improve prostate health. In Budapest, Hungary, 60 patients with BPH were treated with a prostate health supplement containing 300 mg of pumpkin seed oil extract. Twenty-six patients administered the health supplement for 10 months, 22 patients for ≥7 months, and 12 patients for ≥4 months at a dosage of 2 capsules 3 times daily in the first month and then 1 capsule 3 times daily. Based on the results of urodynamic tests and the evaluation of changes in subjective complaints, improvement was observed in >80% of the patients. There was an increase in urinary flow while dysuric complaints, frequent and painful urination, and frequency of nocturnal urination decreased. Being a member of the same family and having a similar phytochemical profile, *B hispida* might possess actions similar to *C pepo*.

In the prostate, DHT is produced from testosterone by the enzyme 5α-reductase. DHT is a potent androgen that promotes growth of the prostate. Although the pathogenesis of BPH is not completely defined, these androgens have been identified as playing an integral part in the disease process. Inhibition of the production or actions of DHT can result in the inhibition of growth of the prostate gland.

There is substantial clinical evidence that androgens and DHT play a key role in the development of BPH. Androgens are also involved in the development and progression of prostate cancer. Various approaches involving attenuation of androgenic stimulation of prostatic growth and use of 5α-reductase inhibitors such as finasteride and...
epristeride have been employed, but their use has been limited by multiple adverse
effects (AEs). However, the magnitude of therapeutic effect produced by finasteride
is relatively small, and a clinically significant benefit is observed in less than half of
untreated patients. Finasteride can cause AEs such as gynecomastia, impairment of
muscle growth, and severe myopathy, due to its structural similarity to steroidal hor-
omes. Therefore, it may be rewarding to look into traditional herbal medicines for
the management of BPH.

The present study was conducted to determine the antiandrogenic potential of
B hispida fruit on testosterone-induced hypertrophy in the prostate of rats. The in
vitro 5α-reductase inhibitory activity of the test extracts was first determined. In vivo
studies were then performed to validate the findings of the in vitro studies.

MATERIALS AND METHODS

PLANT MATERIAL
Fruits of B hispida were procured from local fields in Sagar (Madhya Pradesh, India).
The plant was authenticated by the Department of Botany, Dr. Harisingh Gour
University, Sagar, India. A herbarium has been deposited in the Department of Botany
and the accession number of the specimen is Bot/H/1994. Fruits were cut open with
a knife, seeds were separated from dried fruits manually, and the pulp was dried in the
sun to remove moisture. Seeds and dried pulp were coarsely powdered in a grinder.

PREPARATION OF EXTRACTS
Coarsely powdered pulp and seeds were fed in separate Soxhlet extractors and extracted
with petroleum ether (60°C–80°C). The solvent from the petroleum ether extract was
eliminated under reduced pressure (yield: petroleum ether extract, 1.67% w/w; seed oil,
15.6% w/w). Then, the defatted marc of the pulp was extracted with alcohol (95% v/v) to
obtain the ethanolic extract (EE) (yield, 4.55% w/w).

DRUGS AND CHEMICALS
Testosterone was donated by Sun Pharma Advanced Research Center, Vadodara,
Gujarat, India. Finasteride was purchased from Sigma Aldrich, St. Louis, Missouri.
EDTA, sodium phosphate, and sucrose were purchased from Himedia Pvt. Ltd.,
Mumbai, India. Ethanol (95%) was purchased from Bengal Chemicals Pvt. Ltd., Kolkata,
India. Methanol, ethyl acetate, and petroleum ether (60°C–80°C) were purchased
from Qualigens Fine Chemicals Pvt. Ltd., Mumbai, India. All other chemicals used
in the study were of analytic grade.

IN VITRO STUDIES
With a view to explore the possibility that the extracts may have some action on
prostatic hyperplasia, the extracts were screened for 5α-reductase activity, the key
enzyme involved in hyperplasia of the prostate. The in vitro studies measured the
5α-reductase inhibitory potential of the extracts, seed oil, and finasteride by deter-
mining the concentration of testosterone in the reaction mixture using HPLC. The
detailed methodology is described in the following sections.
Preparation of Enzyme Solution

Human prostate (about 200 mg) supplied from the local hospital of Sagar was minced in small pieces and homogenized in 10 mL of medium A (20 mM sodium phosphate, pH 6.5, containing 0.32 mM sucrose and 1 mM EDTA). The homogenate was centrifuged at 4000 rpm (716g) for 15 minutes. The supernatant was used as a source of enzyme. The concentration of enzyme in the supernatant was determined by the Bradford method of protein estimation.\(^{13}\)

Preparation of Test Materials

Testosterone (1 mM solution in ethanol) and extract/seed oil (1 mg/mL) were prepared in ethanol (95%) with gentle heating wherever necessary. The EDTA solution (10 mg/mL) was made in distilled water. Finasteride (10 \(\mu\)g/mL) was prepared in ethanol.

Determination of Optimum Concentration of Enzyme

Optimum enzyme concentration was determined by keeping the concentration of substrate constant and varying the concentration of enzyme. Testosterone solution (1 mM) was prepared in ethanol. The reaction mixture (1 mL) was prepared by combining testosterone solution (0.1 mL), enzyme solution (0.1–0.9 mL), and sodium phosphate buffer (20 mM). The reaction mixture was incubated at 37°C for 1 hour. The reaction was terminated by the addition of 2 mL of ethyl acetate. The reaction mixture was then shaken vigorously for 1 minute and the ethyl acetate layer was separated. It was evaporated to dryness, and the residue dissolved in 2 mL of methanol. Testosterone content in methanolic solution was estimated by HPLC (AT10, Shimadzu Corp., Kyoto, Japan). The column was eluted isocratically with a mobile phase of methanol:water (80:20) at a flow rate of 1.0 mL/min.\(^{16}\) The optimum amount of enzyme solution that converted testosterone to DHT was found to be 0.8 mL.

Determination of Inhibitory Concentrations of Extract

The reaction mixture (1.5 mL) was made by adding 0.1 mL of testosterone solution, 0.1 mL of EDTA solution, 0.1 to 0.5 mL of extract/seed oil solutions depending on the group, optimum amount of enzyme solution (ie, 0.8 mL), and sodium phosphate buffer (20 mM), to a final volume of 1.5 mL. Reaction mixture was incubated at 37°C for 60 minutes, and reaction was terminated by addition of 2 mL of ethyl acetate. The mixture was vortexed for 1 minute; the ethyl acetate layer was separated and evaporated to dryness. The residue was dissolved in methanol and made up to a volume of 2 mL with methanol. The residual testosterone content in methanol was determined by HPLC. The column was eluted isocratically with a mobile phase of methanol:water (80:20) at a flow rate of 1.0 mL/min.\(^{16}\)

In Vivo Studies

The results of the in vitro studies were encouraging because appreciable 5\(\alpha\)-reductase inhibitory activity was found in the petroleum ether extract and seed oil of \(B\) *hisipida*. Along with petroleum ether extract and seed oil, EE was also included in
the in vivo studies to assess its in vivo effects and to validate the findings of the in vitro studies.

**Animals**

Male Sprague-Dawley rats weighing 115 to 125 g (aged 2–3 months) were housed in polypropylene cages at room temperature (25 ± 2°C) and were fed a standard pellet diet (Brooke Bond, Lipton, India) with water supplied ad libitum. The protocol for animal experimentation was approved by the Institutional Animal Ethics Committee of Dr. Harisingh Gour University.

**Acute Toxicity Studies and Determination of Doses**

The petroleum ether extract, EE, and seed oil were subjected to acute toxicity studies to determine the dose for the in vivo studies according to the Organization for Economic Cooperation and Development guidelines. In all cases, a 2000-mg/kg oral dose of the test extract was found to be tolerable, as no mortality was observed during the study. On the basis of these studies, the doses of 100 and 200 mg/kg for petroleum ether and EE were selected. These studies along with the previous studies on C. pepo seed oil determined the dose of seed oil for the present study. Using 1000 mg of seed oil as the daily dose, rats were administered an oral dose of 2 or 4 mg/100 g (20–40 mg/kg) once daily for 14 days.

**Preparation of Extracts**

Petroleum ether extract and EE were suspended in Tween-80 solution (2% v/v) for oral administration. Rats were administered an oral dose of 100 and 200 mg/kg once daily for 14 days. Seed oil was diluted with arachis oil to prepare the required dose. Using 1000 mg of seed oil as the daily dose, rats were administered an oral dose of 2 or 4 mg/100 g (20–40 mg/kg) once daily for 14 days.

**Grouping and Treatment of Animals**

Testosterone (3 mg/kg daily) dissolved in arachis oil was administered as a subcutaneous injection. The rats were placed in 9 groups of 10 rats each to receive test extracts, testosterone, and Tween 80 (2% v/v) for the study period (ie, 14 days) as follows: group 1, arachis oil (SC) + Tween 80 (2% v/v PO) (control); group 2, testosterone (SC) + Tween 80 (2% v/v PO); group 3, testosterone (SC) + petroleum ether extract (100 mg/kg PO); group 4, testosterone (SC) + petroleum ether extract (200 mg/kg PO); group 5, testosterone (SC) + EE (100 mg/kg PO); group 6, testosterone (SC) + EE (200 mg/kg PO); group 7, testosterone (SC) + seed oil (20 mg/kg PO); group 8, testosterone (SC) + seed oil (40 mg/kg PO); and group 9, testosterone (SC) + finasteride (1 mg/kg PO).

**Body and Prostatic Weights**

Weight was measured a day before treatment was started (baseline) and at 14 days of treatment. On day 14, all animals were euthanized under light ether anesthesia. The prostates were immediately removed and weighed. Mean body weight and prostatic/body weight ratios were calculated for each group.
**Histologic Studies**

After prostatic weight measurements, the tissues were fixed in 10% formalin (in normal saline). After 24 hours, the tissues were subjected to histologic studies using microtome followed by hematoxylin and eosin staining. The slides were observed under a microscope and the images recorded. The investigator who read the histology specimens (A.N.) was blinded to the treatment groups.

**Statistical Analysis**

All results are expressed as mean (SD). Comparisons between groups were performed with the Dunnett test using GraphPad Prism statistical software (GraphPad Software Inc., La Jolla, California). $P < 0.05$ was considered to be statistically significant.

**RESULTS**

**In Vitro Studies**

The optimum concentration of the enzyme was found to be 0.8 mL (270.0 µg protein calculated by Bradford method of protein estimation).\(^{15}\) Varying concentrations of test substances were incubated with a constant amount of testosterone and enzyme in reaction mixture, and the residual testosterone content was determined after termination of the reaction with ethyl acetate. The residual testosterone content in the reaction mixture increased with increasing concentrations of petroleum ether extract, EE, and *B hispida* seed oil. The inhibitory concentration of 50% (IC\(_{50}\)) value was calculated by regression analysis. The IC\(_{50}\) calculated for petroleum ether extract, EE, seed oil, and finasteride was 0.15 mg, 4.26 mg, 0.18 mg, and 1.06 µg, respectively. Relative inhibitory potency of the test material was, therefore, finasteride > petroleum ether extract > seed oil > EE (Table I).

**In Vivo Studies**

The results of the in vitro studies paved the way for the pharmacologic screening of the extracts to assess their potential against testosterone-induced hyperplasia in rats.

**Determination of Prostatic Weight and Prostate/Body Weight (P/BW) Ratio**

In the group treated with testosterone alone, mean prostatic weight and P/BW ratio were significantly increased after 14 days of injections compared with the control group. The mean (SD) prostatic weights were 419.0 (11.5) mg and 253.5 (22.5) mg and P/BW ratios were 2.70 (0.12) and 1.62 (0.11), respectively, for the testosterone-alone group ($P < 0.01$ vs the testosterone + finasteride-treated group) and the control group ($P < 0.01$ vs testosterone + finasteride–treated group).

Table II summarizes the effects of petroleum ether extract (100 and 200 mg/kg PO), EE (100 and 200 mg/kg PO), seed oil (20 and 40 mg/kg PO), and finasteride (1 mg/kg PO) on prostatic hyperplasia induced with testosterone. In groups treated with petroleum ether extract (100 mg/kg PO), the mean (SD) prostatic weight and P/BW ratio were 338.0 (17.0) mg and 2.14 (0.06), respectively, and an increase in dose (200 mg/kg PO) resulted in a mean prostatic weight of 334.3 (16.3) mg.
Table I. 5α-Reductase inhibitory concentrations (IC$_{50}$) of the treatment groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>0.15 mg</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>4.26 mg</td>
</tr>
<tr>
<td>Benincasa hispida seed oil</td>
<td>0.18 mg</td>
</tr>
<tr>
<td>Finasteride</td>
<td>1.06 μg</td>
</tr>
</tbody>
</table>

and P/BW ratio of 2.12 (0.06). These observations were significant ($P < 0.01$) compared with the group treated with testosterone alone, suggesting the efficacy of petroleum ether extract and finasteride on testosterone-induced prostatic hyperplasia. In the group treated with EE 100 mg/kg PO, the mean prostatic weight and P/BW ratio were 411.0 (11.0) mg and 2.69 (0.01), respectively, and an increase in dose (200 mg/kg PO) resulted in a mean prostatic weight of 421.0 (12.4) mg and P/BW ratio of 2.69 (0.01). These observations were not significant statistically compared with the group treated with testosterone alone, whereas they were significant compared with the group treated with finasteride. In groups treated with B hispida seed oil 20 mg/kg PO, the mean prostatic weight and P/BW ratio were 343.2 (4.8) mg and 2.20 (0.04), respectively, and an increase in dose (40 mg/kg PO) showed a mean prostatic weight of 353.6 (14.9) mg and P/BW ratio of 2.18 (0.04). These observations were also significant ($P < 0.01$) compared with the group treated with testosterone + finasteride, suggesting the efficacy on testosterone-induced prostatic hyperplasia. In the case of the finasteride-treated group, the mean (SD) prostatic weight was 325.8 (5.7) mg and P/BW ratio was 2.04 (0.06). On the basis of mean prostatic weight and P/BW ratios, we also calculated the percent recovery in P/BW ratio by test groups compared with the group treated with testosterone alone. The increase induced by testosterone alone was considered to be 100% and all other test groups were compared with this reading, which was considered to be the control. The reduction in weight induced by test materials was compared with the group treated with testosterone alone. The formula used for calculation of percent recovery was as follows:

$$ \text{percent recovery by the test sample} = A - B, $$

where $A$ was the percent increase in prostatic weight induced by testosterone and $B$ was the percent increase in prostatic weight induced by the test sample.

The percent recoveries thus calculated for the group treated with petroleum ether extract at doses of 100 and 200 mg/kg were 51.85% and 53.71%, respectively. In the groups treated with EE, these recoveries were 0.93% and 0.92% for 100 and 200 mg/kg. Recovery with B hispida seed oil at doses of 20 and 40 mg/kg was 46.30% and 48.15%, respectively. Recovery in the group treated with finasteride (1 mg/kg) was 61.61%.
Table II. Effects of test extracts of *Benincasa hispida* on prostatic weight in rats with testosterone-induced prostatic hypertrophy. Data are mean (SD), unless otherwise indicated.

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight, g Baseline</th>
<th>Day 14</th>
<th>Prostatic Weight, mg</th>
<th>P/BW Ratio, 10⁻³</th>
<th>P/BW Ratio Increase After Treatment (P1–P2)</th>
<th>Increase in Prostate Weight, %</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle only)</td>
<td>115.5 (2.6)</td>
<td>155.3 (2.9)</td>
<td>253.5 (22.5) † ‡</td>
<td>1.62 (0.11) † ‡</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Testosterone alone</td>
<td>118.5 (2.7)</td>
<td>156.1 (3.0)</td>
<td>419.0 (11.5) †</td>
<td>2.70 (0.12) †</td>
<td>1.08</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Testosterone + petroleum ether extract 100 mg/kg</td>
<td>123.0 (2.8)</td>
<td>158.0 (2.7)</td>
<td>338.0 (17.0) †</td>
<td>2.14 (0.06) †</td>
<td>0.52</td>
<td>48.15</td>
<td>51.85</td>
</tr>
<tr>
<td>Testosterone + petroleum ether extract 200 mg/kg</td>
<td>122.5 (2.7)</td>
<td>157.9 (2.9)</td>
<td>334.3 (16.3) †</td>
<td>2.12 (0.06) †</td>
<td>0.50</td>
<td>46.29</td>
<td>53.71</td>
</tr>
<tr>
<td>Testosterone + EE 100 mg/kg</td>
<td>119.0 (3.3)</td>
<td>152.0 (3.4)</td>
<td>411.0 (11.0) †</td>
<td>2.69 (0.01) †</td>
<td>1.07</td>
<td>99.07</td>
<td>0.93</td>
</tr>
<tr>
<td>Testosterone + EE 200 mg/kg</td>
<td>121.5 (3.5)</td>
<td>156.0 (3.7)</td>
<td>421.0 (12.4) †</td>
<td>2.69 (0.01) †</td>
<td>1.05</td>
<td>99.05</td>
<td>0.92</td>
</tr>
<tr>
<td>Testosterone + seed oil 20 mg/kg</td>
<td>122.5 (2.7)</td>
<td>156.0 (3.8)</td>
<td>343.2 (4.8) †</td>
<td>2.20 (0.04) †</td>
<td>0.58</td>
<td>53.70</td>
<td>46.30</td>
</tr>
<tr>
<td>Testosterone + seed oil 40 mg/kg</td>
<td>126.0 (2.8)</td>
<td>162.2 (3.8)</td>
<td>353.6 (14.9) †</td>
<td>2.18 (0.04) †</td>
<td>0.56</td>
<td>51.85</td>
<td>48.15</td>
</tr>
<tr>
<td>Testosterone + finasteride 1 mg/kg</td>
<td>125.0 (2.7)</td>
<td>161.5 (2.9)</td>
<td>325.8 (15.7) †</td>
<td>2.04 (0.06) †</td>
<td>0.42</td>
<td>38.39</td>
<td>61.61</td>
</tr>
</tbody>
</table>

P/BW = prostatic/body weight; P1 = increase in P/BW ratio of vehicle-treated control group; P2 = increase in P/BW ratio of test group; EE = ethanolic extract.

*Percent recovery in P/BW ratio by test groups compared with the group treated with testosterone alone.

† *P < 0.01 versus the group treated with testosterone alone (Dunnett test). F = 135.49, df = (8, 81) 89 in the case of prostatic weight and F = 294.44, df = (8, 81) 89 in the case of P/BW ratio comparisons.

‡ *P < 0.01 versus the group treated with finasteride (Dunnett test). F = 135.49, df = (8, 81) 89 in the case of prostatic weight and F = 294.44, df = (8, 81) 89 in the case of P/BW.
**Histologic Examinations**

*Testosterone (3 mg/kg SC) + Tween 80 Group*

In the group treated with testosterone alone, tubules appear to have become wider compared with the control group (Figure, B and A, respectively). The walls of tubules are thickened and almost every tubule developed large involutions projecting into the lumen, reducing the volume of the lumen compared with the control group. The amount of the secretion in some tubules was increased. The connective tissue was compressed and blood vessels were dilated compared with the control. The distinct nucleus and normal sarcoplasmic texture were not visible. There were some connective tissue around the glands and papilla epithelial cells, and hematoxylin and eosin–stained secretions were visible in some cavities. The shape of the tubules was obliterated. Lumen was narrow, but at most places, the transitional nature of the epithelium persisted.

![Figure. Histologic examination of the effects of *Benincasa hispida* on testosterone-induced prostatic hyperplasia in rats (100x magnification). (A) Control group (Tween 80 [2% v/v PO] + arachis oil [3 mg/kg SC]); (B) testosterone alone (3 mg/kg SC); (C) petroleum ether extract 100 mg/kg + testosterone (3 mg/kg SC); (D) petroleum ether extract 200 mg/kg + testosterone (3 mg/kg SC); (E) ethanolic extract 200 mg/kg + testosterone (3 mg/kg SC); (F) *B hispida* seed oil 20 mg/kg + testosterone (3 mg/kg SC); (G) *B hispida* seed oil 40 mg/kg + testosterone (3 mg/kg SC); and (H) finasteride 1 mg/kg + testosterone (3 mg/kg SC).
CURRENT THERAPEUTIC RESEARCH

Testosterone + Petroleum Ether Extract (100 and 200 mg/kg PO)

In the groups treated with petroleum ether extract (100 or 200 mg/kg PO), vacuolization in the cells appeared to be clear (Figure, C and D). The nucleus was pyknotic (vacuole formation in outer layer of nucleus). Chromatin material in the nucleus was normally distributed. The lumens of the tubules were normal, and in some places the epithelium was slightly thicker than that of the control group. The lumens were filled with more eosinophilic secretions. Squamous cells were sparse in number, but more cuboidal and pear-shaped cells were present in the transitional epithelium. Involutions were few in number and even less than what were observed in the control group. Connective tissue between the tubules was reduced. Tubules appear to have large lumen. The stroma didn’t exhibit any analogy to the cells in the control group.

Testosterone + EE (100 or 200 mg/kg PO)

Both groups treated with EE appeared to be similar to the group treated with testosterone alone (Figure, E). No improvement in volume of the lumen, sarcoplasmic texture, or stromal distribution was visible in this group, except in the shape and size of the tubules compared with the testosterone-treated group. The epithelium was more maintained compared with the group treated with testosterone alone.

Testosterone + B Hispida Seed Oil (20 or 40 mg/kg PO)

In the groups treated with B hispida seed oil (20 or 40 mg/kg PO), better intraluminal secretions appeared and tubules had morphologic improvement in the texture (Figure, F and G). The epithelium was still wider and thicker. Compared with the group treated with testosterone alone, the stroma (composed of connective and smooth muscle cells) appeared normal. End secretory parts of the gland were more maintained than those in the testosterone-alone and petroleum ether extract groups. The appearance of the transitional epithelium resembled that of the control group. The involutions were thicker than those observed in the control group. However, some tubules had wider lumens with smooth transitional epithelium. Minor curvature in the epithelium was also observed. Involutions in the epithelium were fewer and thicker than that of the control group.

Testosterone + Finasteride (1 mg/kg PO)

With normal distribution of stroma, this group had a texture similar to that observed in the B hispida seed oil group. The projections were not as prominent as observed in the group treated with testosterone alone. Although finasteride appeared to antagonize the effects of testosterone to a certain degree, several cells with increased volume were observed throughout the transitional epithelium. Cells with swollen nuclei were prominent in many places. Reduced involutions in lesser number were observed (Figure, H).

Control Group (Arachis Oil)

Normal histologic features of prostate gland are visible, showing the tubules of variable diameter and irregular lumen (Figure, A). The lumens were filled with prostatic secretions. In connective tissue, blood vessels, and lymph vessels, the matrix was nor-
mal. In some places, aggregation of columnar cells was observed. The prostate gland was surrounded by a capsule; a thick layer of involuntary muscles with a distinct nucleus and normal sarcoplasmic texture was visible. Some pyramidal cells were also observed in the intermediate layers.

**DISCUSSION**

Testosterone is converted to more potent DHT by the enzyme 5α-reductase type 2 present in prostate homogenates. With the addition of petroleum ether extract, EE, and *B. hispida* seed oil in reaction mixtures, increased levels of unchanged testosterone were found in the reaction mixture, suggesting inhibition of enzyme action by these test materials. Furthermore, the inhibition of conversion by these materials clearly reflects that activity is blocked, and therefore more testosterone remains unchanged in the reaction mixture.

The results of the present investigation suggest that petroleum ether extract and *B. hispida* seed oil at different dose levels inhibited prostatic hyperplasia induced with an exogenous supply of testosterone in a rat model. In the group treated with testosterone alone, subcutaneous injection of testosterone increased prostatic weight and P/BW ratio compared with the control group (vehicle only).

The effects of testosterone and DHT on prostatic growth in rodents have previously been documented and used to assess the effects of drugs used for the treatment of prostatic hyperplasia, including saw palmetto fruit lipid extract. The results presented here suggest that petroleum ether extract and *B. hispida* seed oil were associated with the significant attenuation of prostatic hyperplasia in rats. The in vitro studies identified the mechanism of prevention of prostatic hyperplasia induced by testosterone. It is evident that petroleum ether extract and *B. hispida* seed oil have 5α-reductase-inhibitory activity. The histologic findings of recovery in the tubular latency and shape further support *B. hispida* as a strong investigational candidate for the management of prostatic hyperplasia. Further studies are necessary to confirm the effect of the drug on BPH in humans.

**LIMITATIONS**

The etiology of BPH in humans is heterogeneous, and no other species shows the same complexity of this disorder. Animal models of BPH studied to date do not appear to fully mimic the stromal and epithelial changes with BPH in humans. Therefore, in vitro and experimental models are of limited value for the study of BPH events in humans. Spontaneous animal models are limited to nonhuman primates and canines (hormone-induced BPH in canines appears to be an especially replicate model of human BPH), but ethical and economic problems have reduced the applicability of these models. In particular, BPH induced with testosterone or DHT does not reproduce all findings of BPH in humans because the pathogenesis of BPH is dependent on a functional androgenic signal involving several components (eg, testosterone synthesis in the testes, conversion of testosterone to DHT, transportation of DHT to target prostate tissues, binding of DHT to androgenic receptor and the subsequent gene modulation). BPH in humans also involves prostatic estrogens and α-adrenergic
receptors not fully reproducible in other models. Thus, the model used in the present study has limitations in predicting the effects of any treatment in the management of BPH in humans. A further limitation of this study was the lack of an objective scoring system for the histologic examinations; however, there is presently no gold standard scoring system for prostate histology.

CONCLUSION
The results of the present investigation suggest that petroleum ether extract and *B. hispida* seed oil at different dose levels inhibited prostatic hyperplasia induced by an exogenous supply of testosterone in a rat model.

ACKNOWLEDGMENTS
The authors have indicated that they have no conflicts of interest regarding the content of this article.

Dr. Nandecha carried out the in vitro and in vivo studies. Dr. Nahata assisted in the in vivo studies and worked on the histology of the specimens. Dr. Dixit provided guidance and intellectual input throughout the study.

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