Protective Effect of Prostane in Experimental Prostatic Hyperplasia in Rats

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ABSTRACT
Aim: Prostane, a polyherbal formulation, was evaluated for its efficacy on 5α-reductase inhibition, α-adrenergic antagonistic activity and testosterone-induced prostatic hyperplasia. Methods: 5α-reductase inhibition was evaluated using rat prostate homogenate as an enzyme source. Adrenergic antagonistic activity was evaluated using isolated rat vas deferens. Experimental prostatic hyperplasia was induced in rats by giving testosterone 3 mg/kg sc for 21 days. Results: Prostane dose-dependently inhibited 5α-reductase activity and exhibited α-adrenergic antagonistic activity. The treatment with Prostane at 250, 500 and 750 mg/kg body wt., po for 21 days significantly reduced the prostatic weight, the epithelial height and the stromal proliferation in experimental prostatic hypertrophy. Conclusion: It is concluded that Prostane is effective in the treatment of experimental prostatic hypertrophy in rats and may be evaluated in clinical trials on benign prostatic hypertrophy after necessary toxicological evaluation.

Keywords: Prostane; BPH; oxidoreductases; adrenergic alpha-antagonists; enzyme inhibitors; prostate; hyperplasia

INTRODUCTION
A non-malignant enlargement of the prostate, or benign prostatic hyperplasia (BPH), involves the proliferation of epithelium and fibromuscular tissue, commonly seen in aged men1,2. The consecutive constriction of the urethra may lead to reduction in urine flow rate, bladder outlet obstruction and symptoms of irritation. Apart from surgical treatments like transmural vaporization and prostatectomy, conventional treatments include 5α-reductase inhibitors, anti-androgens, LHRH agonists, etc., to reduce the symptoms of BPH, underscoring the importance of androgens in the pathogenesis of prostatic hyperplasia3,4. Complications with surgical prostatectomy and side effects with conventional therapy exclude their use as routine treatments for BPH5.

Ayurveda, an ancient system of Indian medicine, cites several plants that are useful in the treatment of urogenital disorders. Prostane, a herbal formulation consists of Tribulus terrestris Linn., Areca catechu Willd., Pedalium murex Linn., Caesalpinia bonducella Fleming and Asparagus racemosus Willd.
Tribulus terrestris and Pedalium murex have been reported to be good diuretics\textsuperscript{6-9}. In earlier studies, Pedalium murex and Areca catechu have shown 5α-reductase inhibitory activity (paper presented at the 13th ISAS National Symposium on Analytical Techniques-2001, 1998 Nov. 24-25, Bangalore, India, p. 171-173). Caesalpinia bonducella has been used in the treatment of hydrocele and glandular swelling\textsuperscript{10}. In the present study, Prostane was evaluated for its 5α-reductase inhibitory activity and α-antagonistic activity \textit{in vitro}, and its activity against experimental prostatic hyperplasia in rats.

**MATERIALS AND METHODS**

**Composition of Prostane:** Prostane, a polyherbal formulation, is a mixture of pulverized Tribulus terrestris (fruit 25%), Areca catechu (nut 25%), Pedalium murex (fruit 25%), Caesalpinia bonducella (root 15%) and Asparagus racemosus (root 10%). The constituent plants of the formulation were procured and were identified by Dr. R. Kannan, Botanist of The Himalaya Drug Co. A specimen of each plant was deposited in the herbarium of the R&D Center.

**In vitro study on Prostane for 5α-reductase inhibition:**

**Enzyme preparation:** Enzyme preparation and 5α-reductase inhibitory activity were done by the method described by Ashina et al.,\textsuperscript{11} with minor modification. Male Wistar rats weighing 250-275 g were anesthetized with ether, and the prostates were dissected and homogenated at 5% concentration with 100 mmol/L phosphoric acid buffer solution at pH 6.5 containing 250 mmol/L of sucrose, 1 mmol/L of dithiothreitol (DTT) and 1 mmol/L of EDTA. The homogenate was centrifuged at 10000 x g for 30 minutes and the supernatant was taken as a source of enzyme. Testosterone was dissolved in ethanol at a concentration of 10 mmol/L. A 50 mmol/L NADPH was prepared in potassium phosphate buffer at pH 6.5.

**Preparation of extractum:** Prostane powder (10 g) was extracted with 100 mL of water in a water bath at 100\degree C. The fluid extract was then filtered and dried; the residue (Prostane extractum) was used for the experiment. The extractive value of Prostane extractum was 10% (10 g of Prostane powder yielded 1 g of Prostane extractum).

**Estimation of 5α-reductase inhibitory activity:** Various concentrations of Prostane extractum were prepared in triplicates in 2.0 mL aliquots of potassium phosphate buffer. Testosterone 100 μL, NADPH 100 μL and 500 μL of enzyme solution were added. The mixture was incubated at 37\degree C for 3 hours and reaction terminated by the addition of 4.0 mL ethylacetate. The mixture was vortexed for 1 minute and the ethyl acetate layer was separated and taken for the estimation of dihydro-testosterone (DHT).

**Estimation of DHT by gas chromatography:** Dihydro-testosterone was estimated by the gas chromatography method\textsuperscript{12} with minor modification. A Netel Micro 9100 gas
chromatograph attached with a flame ionization detector was used. The instrument was equipped with a stainless steel column packed with 3% OV-17 (2 m length and 1/8” I.D.). The temperature of the oven, injector and detector were set at 220, 250 and 280°C, respectively. Nitrogen (IOLAR-1) was used as a carrier gas at a flow rate of 30 mL/minute. Twenty µL of the ethyl acetate layer was injected into the gas chromatographic column and the peak area of the DHT was calculated. The formation of DHT was taken as a measure of 5α-reductase activity since the enzyme reduced testosterone to DHT. The inhibitory activities of various concentrations of Prostane extractum were compared with finasteride, a 5α-reductase inhibitor. The amount of DHT formed in the presence of the enzyme (control) was considered 100%, and formation of DHT in the presence of Prostane extractum was then compared with the control.

*In vitro study on the α-adrenergic antagonistic activity:* Male Wistar rats weighing 275-300 g were used. The animals were anesthetized with ether. The vas deferens were then dissected free from extraneous tissue and suspended in an organ bath containing Tyrode solution gassed with 95% O₂ and 5% CO₂ mixture at 37°C. Contraction of the tissue was recorded isotonically using a lever transducer attached to the Polyrite (Medicare model 207, Ambala, India).

Following an equilibration period of 30 minutes, contractions were induced by administration of norepinephrine (NE) at various concentrations, viz. 0.5, 1.0, 2.0 and 4.0 µg/mL with or without test drug (Prostane extract) and the contractions were recorded.

*In vivo study on the activity against experimental prostatic hyperplasia:* Forty male Wistar rats weighing 275-300 g were selected and randomized into five groups of 8 animals each. The animals were housed in standard laboratory conditions at a temperature of (22 ± 3)°C, relative humidity 50-55%, and 12-hour light-dark cycle. Drinking water and synthetic pelleted diet (Lipton India Ltd., Mumbai) were supplied *ad libitum* throughout the study period. The animals received the following treatments:

Group I: Olive oil 1 mL•kg⁻¹•d⁻¹, sc for 21 days as vehicle control.

Group II: Testosterone undecanoate (TU) 3 mg•kg⁻¹•d⁻¹, sc in olive oil for 21 days¹³.

Group III: Prostane powder 250 mg•kg⁻¹•d⁻¹, po and TU 3 mg•kg⁻¹•d⁻¹, sc in olive oil, both for 21 days.

Group IV: Prostane powder 500 mg•kg⁻¹•d⁻¹, po and TU 3 mg•kg⁻¹•d⁻¹, sc in olive oil, both for 21 days.

Group V: Prostane powder 750 mg•kg⁻¹•d⁻¹, po and TU 3 mg•kg⁻¹•d⁻¹, sc in olive oil, both for 21 days.

On the 22nd day, the rats were euthanised under diethyl ether anesthesia. The prostate glands were collected, and the weight of ventral, dorsal and total prostates were recorded. After being weighed, the prostates were fixed in 10% neutral buffered formalin (NBF). The formalin fixed tissues were then processed by paraffin technique, and sections of 5 µm thickness cut and stained by the routine H & E method.
**Statistical analysis:** The data were expressed as Mean ± SEM. The results were analyzed statistically using the ANOVA and Student’s ‘t’ test. The minimum level of significance was set at *p*<0.05.

**RESULTS**
Prostane extractum showed 5α-reductase inhibitory activity in a dose-dependent manner (Table 1). It also revealed α-adrenergic antagonistic activity and shifted the dose-response curve of NE towards the right (Figure 1). Testosterone administration at a dose of 3 mg/kg to rats for 21 days resulted in a significant increase in the weights of the total prostate as well as dorsal and ventral prostates as compared to the normal control. Treatment with Prostane powder inhibited prostate enlargement dose-dependently. A significant prevention in prostatic enlargement was observed at 500 and 750 mg/kg dose levels (Table 2). Sections of prostate in Group I showed secretory luminal cells lined with a single layer of low columnar epithelium and the acini were filled with pale eosinophilic material (Figure 2). In Group II the epithelial cells showed an increase in

<table>
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<tr>
<th>Treatment</th>
<th>DHT formation (µmol/L)</th>
<th>Inhibition (%)</th>
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<tbody>
<tr>
<td>Control (with enzyme)</td>
<td>242.62 ± 10.72</td>
<td>0</td>
</tr>
<tr>
<td>Prostane</td>
<td>10 mg</td>
<td>179.17 ± 6.62&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>20 mg</td>
<td>81.76 ± 4.82&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30 mg</td>
<td>32.51 ± 4.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Finasteride</td>
<td>10 µg</td>
<td>88.45 ± 6.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>20 µg</td>
<td>32.01 ± 5.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30 µg</td>
<td>21.64 ± 4.62&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>*p*<0.001 as compared to control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Prostate weight (mg/100 g b. wt.)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Ventral</td>
</tr>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>64.04 ± 5.58&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>Testosterone (3 mg/kg)</td>
<td>99.62 ± 9.21</td>
</tr>
<tr>
<td>III</td>
<td>Testosterone (3 mg/kg) + Prostane (250 mg/kg)</td>
<td>85.36 ± 7.45</td>
</tr>
<tr>
<td>IV</td>
<td>Testosterone (3 mg/kg) + Prostane (500 mg/kg)</td>
<td>67.87 ± 6.55&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>Testosterone (3 mg/kg) + Prostane (750 mg/kg)</td>
<td>63.88 ± 5.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*p*<0.01 as compared to Group II.
height and number (hyperplasia) and fibrovascular stromal proliferation (Figure 3). These epithelial cells showed regular arrangement, but were sometimes thrown up into papillary folds. In Groups IV and V (Prostane 500 and 750 mg/kg), a reduction in epithelial cell height and restriction of stromal proliferation was noticed (Figure 4).

**DISCUSSION AND CONCLUSION**

It is well established that 5α-reductase is an enzyme abundantly found in the nuclear membrane microsomes of prostatic epithelial cells that is involved in the conversion of testosterone to DHT. An increased production of DHT results in the development of prostatic hyperplasia\textsuperscript{14,15}. 5α-reductase inhibitors reduce tissue DHT concentration without interfering in the sexual function since they block only the formation of DHT\textsuperscript{16}.

It is generally accepted that α-adreno-receptors mediate the contractile response of the prostate and are responsible for about 50% of the prostatic urethral pressure in BPH patients. Thus, α\textsubscript{1}-adrenoreceptor antagonists are widely used in the treatment of BPH\textsuperscript{4}. The rationale for using α\textsubscript{1}-blockers to treat BPH is based on the physiology and pharmacology of the prostate smooth muscle. α\textsubscript{1}-blockers presumably decrease the resistance along the prostatic urethra by relaxing the smooth muscle component of the prostate. In the present study, the 5α-reductase and α-adrenergic inhibitory effects of Prostane suggest that the preparation may be useful in the treatment of benign prostatic hyperplasia.
Testosterone administration resulted in an increase in prostatic weight and the histological study revealed a proliferation of epithelium and stromal connective tissues, which is in concurrence with the earlier studies\textsuperscript{2,13,17}. Prostane dose-dependently reduced the testosterone-induced prostatic hyperplasia as indicated by the reduction in prostatic weight and epithelial cell height; similar changes were observed with androgen deprivation\textsuperscript{18}.

In conclusion, this study demonstrates the $\alpha$-adrenoreceptor antagonistic and 5$\alpha$-reductase inhibitory activities of Prostane, as well as its effect in reducing the prostatic weight, the epithelial height and the stromal proliferation in experimental prostatic hypertrophy in rats. The authors believe that Prostane may be passed on to clinical trials in the treatment of benign prostatic hypertrophy after necessary toxicological evaluations.

**REFERENCES**


