Aqueous Methanolic Bark Extract of *Oroxylum indicum* Inhibited Testosterone induced Prostate Hyperplasia in Rat

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**ABSTRACT**

*Oroxylum indicum* is a frequently reported traditional medicinal plant known to possess antiproliferative and antitumor activity. The present study investigated the effect of crude methanolic bark extract of *Oroxylum indicum* on testosterone induced benign prostate hyperplasia (BPH) in rat. Adult male rats were given either corn oil or testosterone dissolved in corn oil and testosterone with aqueous methanolic bark extracts of *Oroxylum indicum* (10, 50 and 100 mg/kg/day) for 14 days. The inhibitory effect of *Oroxylum indicum* on testosterone induced hyperplasia was evaluated by prostatic index and histopathological examination. Serum marker of liver injury (alanine aminotransferase, ALT and aspartate aminotransferase, AST) and liver histopathological examination were also conducted. Compared with testosterone induced BPH model group, *Oroxylum indicum* extract treated groups exhibited significant reduction in the prostatic index. *Oroxylum indicum* treated group also exhibited reduced hyperplasia of prostatic epithelium likewise finasteride treated group. Aqueous methanolic extract of *Oroxylum indicum* significantly inhibited testosterone induced prostate hyperplasia thus indicated the presence of efficient ingredients which can be used for the treatment of BPH.

**Keywords:** Benign Prostate Hyperplasia, *Oroxylum indicum*, Testosterone, Finasteride, Rat

**INTRODUCTION**

Benign growth of the prostate gland is a common disease affecting elderly population. If left untreated the risk of developing benign to malignant transformation is quite obvious under clinical situation. Benign prostate hyperplasia (BPH) disease is characterized by uncontrolled proliferation of the prostate epithelial cells and stromal cells, which results in increased prostate size.[3] As prostate enlarges, it constricts the urethra and reduces urine outflow, thus creating lower urinary symptoms (LUTS).

LUTS includes symptoms such as increased urinary frequency, urgency, nocturia leads to an increased risk of obstructions of the urethra, urinary retention and urinary infections.[5]

The pathogenesis of BPH is not completely elucidated. It is believed that dihydrotestosterone (DHT), a metabolite of testosterone plays the crucial role in the pathogenesis of BPH. The metabolic conversion of testosterone into DHT in prostate is catalyzed by the action of 5α reductase.[3] DHT production is increases with aging, thereby enhancing prostate growth and hyperplasia.[8] This fact was further confirm with the development of 5α reductase inhibitors, namely finasteride and dutasteride, which when given to BPH patients significantly reduced prostate size and DHT level in the prostate.[9,4] However, this conventional treatment for BPH is restricted because of associated side effects, such as erectile dysfunction, loss of libido, dizziness, gynaecomastia and upper respiratory tract infection.[7]
Plants are important sources of therapeutic drugs and play a significant role in the survival of the tribal and ethnic communities.\[^9\]\^\[^9\] Northeast region of India is a home to many diverse plant species with numerous medicinal properties, some of which are yet to be investigated. Previous reports have documented the ethno pharmacological use of many plant species used by indigenous population of this part of India.\[^8\]

*Oroxylum indicum* is one of the plants frequently reported to be used in traditional health practices. Apart from its uses as hepatoprotective plant, *Oroxylum indicum* extract also shown to possess effective antiproliferative and anti-tumor activity.\[^9\]–\[^11\] However, no study has tested the efficacy of *Oroxylum indicum* in a testosterone induced BPH in rat model. The present study was undertaken to test the efficacy of aqueous methanolic extract of *Oroxylum indicum* in the treatment of BPH in rat model. The study also compares the relative efficacy of *Oroxylum indicum* against currently available drug finasteride, a 5α reductase inhibitor used for treatment of BPH.

### MATERIALS AND METHODS

#### Chemicals

Testosterone, corn oil vehicle (Sigma-Aldrich), and Finasteride (Dr. Reddy) were used.

#### Preparation of *Oroxylum indicum* extract

The plant material *Oroxyllum indicum* was collected from the university campus and was authenticated by Prof. A. K. Das, dept of Botany, Rajiv Gandhi University (Voucher No: LBC/REGU/2013/01). The fresh stem bark of *Oroxylum indicum* was washed 4-5 times with tap water to remove salts, epiphytes, sand etc and allowed to shade dried and powdered. The powder was extracted with methanol, then evaporated under vacuum desiccator and dried and powdered. The powder was extracted with water. The solution was filtered with a whatmann no 49 filter paper and used as mentioned.

#### Animals, induction of BPH and treatment

Adult male Sprague-Dawley rats (n = 30) between 12–14 week of age were housed in polycarbonate cages with rice husk as a bedding material with a 12-h light/dark cycle and were fed standard laboratory diet and water ad libitum. Body weights were measured at the onset of the experiment and at the time of termination to measure the weight gain or loss during the study. All animals remained healthy throughout the experiment. Animal care was in accordance with institutional guidelines and complied with National Institutes of Health policy. After 7 days of acclimatization, rats were divided randomly into five experimental groups and one negative control group of 5 animals each. Briefly, BPH was induced in experimental groups by daily s.c. injection of testosterone (10mg/kg) dissolved in corn oil from day 0 to day 7 (induction phase). Negative control animals received s.c. injections of corn oil alone on the same schedule. The dosage and duration of testosterone treatment was based on the reports by Vikram et al., 2011.\[^12\] After 7 days of BPH induction, animals were randomly divided into five different experimental groups. One group continued testosterone (10mg/kg/day) for the rest of the experimental period, while another group of rats was administered daily i.p. injections of the 5α-reductase inhibitor, finasteride (5mg/kg/day) along with testosterone. The dosage of finasteride was based on previous study\[^13\]–\[^15\] The other three groups of animals were given daily i.p. injections of aqueous methanolic extract of *Oroxylum indicum* at the dose of 10, 50 and 100mg/kg/day along with testosterone for the rest of the experimental period. Rats were weighed and killed under ketamine hydrochloride (10mg/kg) anesthesia on the morning of day 21. Blood samples were collected by cardiac puncture on the last day of experiment i.e. on 21\(^{st}\) day. Serum was separated by centrifugation and stored at -20 °C for further analysis. Whole prostates were removed immediately, weighed, and fixed in neutral buffered formalin (10%), dehydrated, cleared in xylene and embedded in paraffin for histological analysis.

#### Prostatic index and histomorphometrical examination of prostate

The prostatic index (%) and the percentage inhibition of prostate gland growth by different treatments was calculated by following equation after Ali et al., 2013.\[^16\]

\[
\text{Prostatic Index (PI)} = \left( \frac{\text{Prostate Weight (g)}}{\text{Final Body Weight (g)}} \right) \times 100
\]

\[
\% \text{Percentage of Inhibition} = 100 \left( \frac{(T - NC)}{(PC - NC)} \right) \times 100
\]

The prostate acinar epithelial height was calculated in 50 high power fields (HPF) using ocular micrometer per animals and presented as mean ± SD.

#### Estimation of serum marker of toxicity and histopathological examination of liver

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level were estimated by utilizing...
commercial kits (Coral system, Goa, India). Liver was fixed in neutral buffered formalin, embedded in paraffin and 5 μm sections were prepared. Slides were stained with hematoxyline and eosin, observed under light microscope and photographed.

**Statistical analysis**

Data are presented as mean ± SD. The significance of mean differences in the numerical data of both control and test group of population were subjected to ANOVA.
(Analysis of Variance, one way) followed by post hoc tukey test. The mean differences between control and treated animals were considered significant at P-value not less than 0.05 (P<0.05).

RESULTS

The representative gross morphology and relative weight of the prostate glands from different experimental groups is presented in Fig 1. A and B. The relative prostate weight is an important indicator in BPH treatment. Administration of testosterone (10mg/kg/day, s.c.) for 21 days significantly elevated the relative prostate weight (0.48 ± 0.04%, P<0.001), when compared with normal control rats (0.11 ± 0.04%). Finasteride treatment caused significant reduction in the relative prostate weight (0.31 ± 0.04%, P<0.01), compared with the testosterone induced BPH model group. Intraperitoneal administration of aqueous methanolic extract of *Oroxylum indicum* significantly reduced relative prostate weight in a dose dependent manner when compared to testosterone alone treated group (0.43 ± 0.07%, 0.35 ± 0.07%, 0.32 ± 0.03%, P<0.05, 0.01; Fig 1. B). The percentage inhibition was found to be 46% for finasteride treated animals. The percentage inhibition was found to be 46% for finasteride treated animals.

The histological sections of the prostate gland from different experimental groups were presented in Fig 2. A-D. There were no much changes in the histoarchitecture of the prostate tissue in the normal control group. The prostate tissues were tightly packed with flattened cuboidal regular size epithelium with regular acinar folding (RAF) arrangement (Fig 2. A). In the BPH model group irregular acinar folding with intraluminal projections were observed. The amount of connective tissue was well marked with increase oval acini size. Stromal proliferation and glandular hyperplasia with epithelial proliferation and nuclear stratification have been observed (Fig 2. B). Treatment with 5α reductase inhibitor finasteride has found to inhibit epithelial hyperplasia in the prostate acini (Fig 2. C). Treatment with aqueous methanolic extract of *Oroxylum indicum* (50 and 100mg/kg/day) reduces testosterone induced glandular hyperplasia and epithelial proliferation as evidence by decreased in the number of intraluminal projections (Fig 2. D).

Testosterone treatment produced significant change in the epithelial height of the prostates of the BPH rats (P<0.001, Fig 3. B) when compared with the normal control rats (Fig 4. A). However, treatment with *Oroxylum indicum* significantly (P<0.01, Fig 4. D) decreased the epithelial height when compared with the BPH model group. Administration of finasteride (5mg/kg) for 14 days also significantly reduced the epithelial in the prostate acinar cells (P<0.01; Fig 3. C). The above findings indicate marked restoration of disrupted histoarchitecture by *Oroxylum indicum* when compared with BPH control.

No significant differences in body weight gain and relative liver weight was observed during the present study (data not shown). The serum ALT and AST level, potential marker of liver injury was estimated from different experimental groups to ascertain the level of liver injury and presented in Fig 4. A. No significant difference in the serum level of ALT and AST was recorded in the present study. Histological analysis of liver tissues also confirmed absence of any visible effect of *Oroxylum indicum* extract on liver of the experimental animals (Fig 4. B). Thus it confirms that continuous treatment with aqueous methanolic extract of *Oroxylum indicum* possess no observable liver toxicity in the present experimental condition.

DISCUSSION

In the present study, the relative efficacy of *Oroxylum indicum* as potential candidate for treatment of BPH was investigated in a testosterone induced BPH model in rat. Testosterone is often used for development of BPH in experimental animals and it is known to metabolized into DHT in the prostate tissues by 5α-reductase which known to play the crucial role in prostate hyperplasia. With increase in age, production of DHT increases thus leads to the development of BPH and LUTS in aged males. Currently 5α-reductase inhibitors like finasteride and dutasteride is being used for the treatment of BPH. These 5α-reductase inhibitors reduces the testosterone and DHT level in serum and prostate, which results in a reduction in prostate size and ultimately provides relief from the lower urinary tract symptoms related to BPH.

Prostate enlargement is used as one of important marker of BPH. In the present study, BPH model group animals experienced significant increases in relative prostate weight compared with the control untreated group of animals. In contrast, administration of aqueous methanolic extract of *Oroxylum indicum* resulted in a significant reduction in relative prostate weight compared with BPH animals. These results were consistent with histopathological examinations of prostate tissues. BPH animals experienced both stromal proliferation and glandular hyperplasia in the prostate, whereas animals treated with *Oroxylum indicum* showed mild glandular hyperplasia. These findings suggested that aqueous methanolic extract of *Oroxylum indicum* possess constituents which may be important for future drug discovery for effective treatment for BPH.
Figure 2. Representative histological microphotograph of prostate glands from different experimental groups: (a) control, untreated, (b) Testosterone (10 mg/kg/day) treated, (c) Testosterone (10 mg/kg/day) and finasteride (5 mg/Kg/day) treated and (d) Testosterone (10 mg/kg/day) and Oroxylum indicum methanolic extract (10, 50 and 100 mg/kg/day) treated group of animals. Arrow indicates the occurrence of dysplasia in the prostate acini of experimental animals.

Figure 3. Representative histological microphotograph of prostate glands from different experimental groups showing epithelial height: (a) control, untreated, (b) Testosterone (10mg/kg/day) treated, (c) Testosterone (10mg/kg/day) and finasteride (5mg/Kg/day) treated and (d) Testosterone (10 mg/kg/day) and Oroxylum indicum methanolic extract (10, 50 and 100 mg/kg/day) treated group of animals. (e) Prostate epithelial height in different experimental groups, data presented are mean ± SD of five animals per group. ***P<0.001, **P<0.01 and *P<0.05.

Figure 4. (A) Serum ALT and AST level and (B) representative histological microphotograph of liver from different experimental groups. No significant alteration in serum marker of liver injury (ALT and AST) was observed in any group during the experimental period, which was further confirmed by histopathological study of the liver tissues.
However 5α-reductase inhibitors also produce serious side effects. This urged the need to investigate alternative treatment for treating BPH with fewer side effects. Many previous studies reported the alternative and complimentary therapy such as *Serenoa repens*, lauric acid and myristic acid etc for BPH and LUTS. In line with these investigations we have tested the efficacy of *Oroxylum indicum* aqueous methanolic extract in the pathogenesis of testosterone induced prostate hyperplasia in rat.

The findings of the present study like the reduced relative prostate weights and the prostate histopathological examination together indicated that aqueous methanolic extract of *Oroxylum indicum* inhibits the progression of BPH in rats and these effects were closely associated with a reduction in glandular cellular proliferation in BPH rats.

**CONCLUSION**

In conclusion, i.p. administration of aqueous methanolic extract of *Oroxylum indicum* significantly decreased the prostate size and prostate hyperplasia in the testosterone induced BPH rat model. The present investigation also studied the effect of aqueous methanolic extract of *Oroxylum indicum* on liver function tests. Our study has shown that serum ALT and AST, potential biomarkers of liver injury remain unaltered by continuous administration of *Oroxylum indicum* aqueous methanolic extract for 14 days. Histopathological examination of liver also confirmed that *Oroxylum indicum* had no observable adverse effect on liver at selected doses. Together these findings indicate that aqueous methanolic extract of *Oroxylum indicum* may effectively inhibit the development of BPH.

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**CONFLICT OF INTEREST STATEMENT**

The author declares that there is no conflict of interest.

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