Effect Of *Oroxylum Indicum* Crude Bark Extract On Experimentally Induced Prostatic Hyperplasia In Sprague Dawley Rats

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Received: September 26, 2014; revised: December 10, 2014; accepted: December 14, 2014

Abstract: The present study was designed to investigate the effect of crude bark extract of *Oroxylum indicum* in experimental prostatic hyperplasia in rat. The male animals were treated either corn oil or testosterone (10 mgkg-1) dissolved in corn oil and testosterone with crude bark extracts of *Oroxylum indicum* (50 and 100 mgkg-1) consecutively for three weeks. The protective efficacy of *Oroxylum indicum* on prostate hyperplasia was illustrated by prostate weight, prostatic index, percentage of inhibition and histological examinations. Crude extract of *Oroxylum indicum* caused significant reduction in the prostate weight and prostatic index when compared to testosterone induced BPH model group. Crude extract treated group also ameliorated the hyperplasia of prostate epithelium in a similar manner as was observes in finasteride (5 mgkg-1) treated group. The study indicates that *Oroxylum indicum* significantly reduced the progression of BPH and it may be an another phytotherapeutial source of drugs in BPH treatment.

Key words: *Oroxylum indicum*, Prostate hyperplasia, Crude bark extract, Testosterone, Rat

Introduction

Benign prostatic hyperplasia (BPH) is a non-malignant, uncontrolled proliferation of the epithelial and stromal cells that occurs in the peri urethral transition zone of the prostate gland leading to lower urinary tract symptoms (LUTS) (Arruzabala *et al*., 2007; Pais, 2010). Symptoms of LUTS are associated with increased urinary frequency, urgency, sense of incomplete emptying and nocturia that leads to an increased risk of obstructions of the urethra, urinary retention and urinary infections (Miller and Tarter, 2009). BPH is one of the common prostate diseases experienced by around 85% of men above the age group of 45 years (Sarma and Wei, 2012).

The mechanism underlying the pathogenesis of BPH is not clearly understood, but androgen is known to play a significant role in the disease progression. In general, testosterone is converted to dihydrotestosterone (DHT) in prostate by 5α reductase enzyme (Carson and Rittmaster, 2003). The production of DHT is increases with ageing due to factors unknown that finally lead to growth of prostate size (Carson and Rittmaster, 2003). 5α-reductase inhibitors namely finasteride and dutasteride are used as a conventional drug for BPH treatment (McConnell *et al*., 1998; Clark *et al*., 2004). However, these drugs are restricted because of adverse side effects like erectile dysfunction, loss of libido, dizziness and gynecomastia (Bullock and Andriole, 2006). In this novel ground the phytotherapeutic and pharmaceutical agents of plant origin have proven to be an effective treatment options in BPH patients without or minimal side effects.

The plant *Oroxylum indicum* (L.) Vent. (Bignoniaceae) is a medicinally important forest tree species (Bignoniaceae) and is frequently reported to be used in traditional health practices (Rijal, 2008; Tangjang *et al*., 2011; Shankar *et al*., 2012). The available earlier reports showed that *Oroxylum indicum* possess strong hepatoprotective (Bharali *et al*., 2014), antioxidant (Siriwataname-tanon *et al*., 2010), antiproliferative (Lambertini *et al*., 2004) and antitumor (Mao, 2002) activities. The present investigation was designed to evaluate the effect of crude bark extract of *Oroxylum indicum* in rat BPH model.

Materials and methods

Chemicals

Testosterone, corn oil (Sigma-Aldrich) and Finasteride (FINAST, Dr. Reddy's Laboratories) were used. All other chemicals used during the experiment were of analytical grade.

Collection and preparation of plant material

The plant was collected on 25th March, 2013 from the Rajiv Gandhi University campus, and identified with the help of taxonomist from the Department of Botany, Rajiv Gandhi University. The voucher specimen (LBC/RGU/2013/01) was deposited at the Centre with Potential for Excellence in Biodiversity (CPEB), Rajiv Gandhi University for future reference. The fresh stem bark of *Oroxylum indicum* was washed, shade dried, powdered. 5 g of powder was dissolved in 100 ml double distilled water for overnight, filtered and kept at -20°C for further use.

Test animals

Adult male Sprague-Dawley rats weighing 80-140 g were used in the present study. Animals were procured from the stock animal facility of the department of Zoology, Rajiv Gandhi University. The animals were kept in polycarbonate cages and rice husk were used as a bedding material with twelve hour light/dark cycle. Animals were fed standard laboratory diet and water ad libitum. Animals were reared in accordance with institutional guidelines and complied with National Institutes of Health (NIH, US) policy. All animals remained healthy throughout the experiment.

Experimental design

After one weeks of acclimatization in laboratory condition, animals (n=5) were randomly divided into five groups. Group I remained as a negative control and received subcutaneous (s.c.) injection of corn oil only. BPH model was induced in experimental groups by daily subcutaneous injection of testosterone (10 $mgkg^{-1}$) dissolved in corn oil from day 0 to day 7 (induction phase). The dosage and duration of testosterone treatment was based on the reports by Vikram *et al.*, 2011. After one week of BPH induction, animals were divided into four different experimental groups. Group II served as BPH model and continued testosterone (10 $mgkg^{-1}$) for the rest of the experimental period, Group III received daily intraperitoneal (i.p.) injections of 5α-reductase inhibitor, finasteride (5 $mgkg^{-1}$) along with testosterone. The dosage of finasteride was based on previous study (Fitts *et al.*, 2004; Rudolfsson and Bergh, 2008). Group IV and V of animals were given daily intraperitoneal injections of crude bark extract of *Oroxylum indicum* (50 and 100 mgkg-1) along with testosterone. All animals were treated once daily for three consecutive weeks. Body weights were measured at the onset of experiment and at the time of termination to measure the weight gain or loss during the study. At the end of the experimental period the animals were anesthetized with ketamine hydrochloride and sacrificed. Blood samples were drawn from the cardiac puncture, serum was separated by centrifugation and stored at -20°C for further analysis. Whole prostate were removed immediately, weighed and fixed in neutral buffered formalin, dehydrated, cleared in xylene and embedded in paraffin for histological study.

Calculation of prostatic index and percentage of inhibition

The prostatic index and the percentage inhibition of prostate size was calculated by following equation (Ali *et al.*, 2013).

$$\text{Prostatic Index (PI)} = \frac{\text{Prostate Weight (g)}}{\text{Final Body Weight (g)}} \times 100$$

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

Where T, NC and T represents the prostatic index values of the treatment group, negative control and positive control respectively.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical significance was determined by Student *t*-test (paired)

using Microsoft Excel 2007 and the level of significance was set at $P < 0.05$.

Results

In the present investigation, treatment with the crude bark extract of *Oroxylum indicum* for three consecutive weeks significantly decreased the size of prostate gland in experimentally induced prostatic hyperplasia. This was evidenced by reduction in prostate weight and prostatic index. The prostate weight was significantly increased (0.712 ± 0.002, $P<0.01$) in BPH model group when compared to the negative control group (0.174 ± 0.051) group. Administration of *Oroxylum indicum* extracts (50 and 100 $mgkg^{-1}$ i.p.) significantly reduced the prostate weights (0.505 ± 0.029, 0.452 ± 0.014, $P<0.01$, 0.001) in a dose dependent manner when compared to the BPH model group. Similar effect was observed in finasteride (10 $mgkg^{-1}$ i.p.) treated group (0.375 ± 0.025, $P<0.001$), when compared with BPH model animals (Table 1).

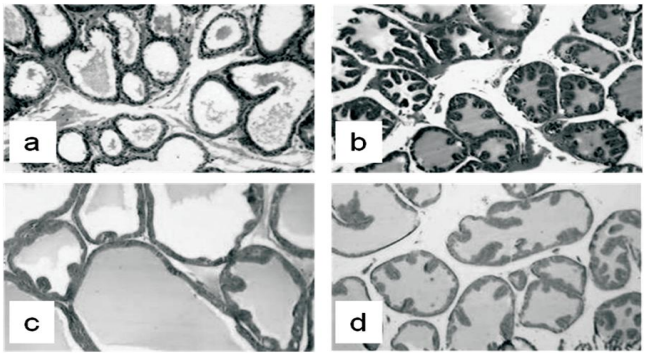

Fig. 1. Histological microphotographs of prostate gland from different experimental groups: (a) Negative control, (b) Testosterone (10 $mgkg^{-1}$) treated, (c) Testosterone (10 $mgkg^{-1}$) and finasteride (5 $mgkg^{-1}$) treated and (d) Testosterone (10 $mgkg^{-1}$) and *Oroxylum indicum* crude extract (50 and 100 $mgkg^{-1}$) treated groups. Arrow indicates the occurrence of epithelial layer thickness in the prostate of experimental animals.

Prostatic index of testosterone treated BPH group (0.469 ± 0.036 %, $P<0.01$) was significantly increased in the present study when compared to the negative control (0.170 ± 0.053 %) group. But treatments with *Oroxylum indicum* extract (50 and 100 $mgkg^{-1}$ i.p.) significantly decreased the prostatic indexes (0.341 ± 0.005 %, 0.291 ± 0.017 %, $P<0.05$, 0.01) compared to the BPH model group. As expected, administration of finasteride also significantly reduced the prostatic index (0.268 ± 0.017 %, $P<0.01$) when compared to BPH model animals. The percentage of inhibition was found to be 66.98 % for finasteride treated group. The percentage of inhibition for *Oroxylum indicum* treated groups were 42.45 % and 59.24 % respectively (Table 1). There was no significant differences in body weight of different experimental groups found in the present study.

The histological observation of BPH group exhibited typical features of prostate epithelial hyperplasia (Fig 1.b) when compared to negative control group. No abnormal changes in the histology of the prostate tissue was found in the negative

Table 1. Effect of crude bark extract of *Oroxylum indicum* on body weights and prostate enlargements in testosterone treated rats

Group	Initial body weight (g)	Final body weight (g)	Body weight difference (g)	Prostate weight (g)	Prostatic Index	% of inhibition
I	80 ± 10	102.5 ± 2.5	22.5 ± 12.5	0.174 ± 0.051	0.170 ± 0.053	-
II	131 ± 11	152.87 ± 12.37	21.87 ± 1.37	0.712 ± 0.002^{a}	0.469 ± 0.036^{d}	-
III	118.15 ± 1.85	139.675 ± 0.32	21.525 ± 2.17	0.375 ± 0.025^{b}	0.268 ± 0.017^{c}	66.98
IV	129.5 ± 4.5	147.835 ± 6.36	18.335 ± 1.86	0.505 ± 0.029^{c}	0.341 ± 0.005^{e}	42.45
V	141.267 ± 9.75	155.667 ± 14.36	14.4 ± 11.11	0.452 ± 0.014^{b}	0.291 ± 0.017^{c}	59.24

Group I: Negative control, Group II: BPH model (testosterone 10 $mgkg^{-1}$), Group III: testosterone 10 $mgkg^{-1}$ + finasteride 5 $mgkg^{-1}$), Group IV and V: testosterone 10 $mgkg^{-1}$ + *Oroxylum indicum* crude extracts 50 and 100 $mgkg^{-1}$. Values were expressed as mean ± SD (n=5). Statistical analysis is done by Student paired *t*-test. [a] $P < 0.01$ compared to negative control (Group I), [b] $P < 0.001$ compared to BPH model (Group II), [c] $P < 0.01$ compared to BPH model (Group II), [d] $P < 0.001$ compared to negative control (Group I), [e] $P < 0.05$ compared to BPH (Group II).

control group (Fig 1.a). The finasteride treated group also inhibited epithelial hyperplasia in the prostate acini when compared with the BPH group (Fig 1.c). Treatments with crude bark extract of *Oroxylum indicum* (50 and 100 $mgkg^{-1}$ i.p.) remarkably reduced testosterone induced epithelial hyperplasia and epithelial layer thickness as compared to the BPH induced group (Fig 1.d).

Discussion

The enlargement of prostate gland has been viewed as one of vital marker for the experimental evaluation of BPH (Veeresh Babu *et al*., 2010; Pais, 2010). In the present study, BPH model group exhibited significant elevated prostate weight and prostatic index compared with the negative control group. The finasteride treatment group also showed the lower prostate weight and prostatic index compared to the BPH model animals. In contrast, treatment of crude bark extract of *Oroxylum indicum* evidenced a significant reduction in prostate weight compared to the BPH model group. These results were consistent with histological examinations of prostate tissues. Many earlier studies also reported the effectiveness of alternative and complimentary therapy of plant origin such as *Serenoa repens* (Paubert-Braquet *et al*., 1996), *Semen vaccariae* (Zhang *et al*., 2013), *Cucurbita pepo* (Nawfal *et al*., 2011), coconut oil (Arruzabala *et al*., 2007), *Urtica dioica* (Nahata and Dixit, 2011) and *Boerhaavia diffusa* (Vyas *et al*., 2013). The findings of the present study supported the benefit of phytotherapy as demonstrated by earlier workers in the management of prostate hyperplasia in experimental animals.

In conclusion, intraperitoneal administration of *Oroxylum indicum* (50 and 100 $mgkg^{-1}$) in a BPH model animals significantly decreased the prostate size, prostatic index and prostatic hyperplasia. These findings indicate that crude bark extract of *Oroxylum indicum* may effectively reduce the progression of testosterone induced BPH. Altogether, the present study suggest firmly that the *Oroxylum indicum* bark extract possess constituents which may be important for futures potential of drug discovery for effectiveness treatment for BPH.

Acknowledgments

The authors are thankful to Coordinator, Center with Potential for Excellence in Biodiversity (CPEB-II) and HoD, Department of Zoology, Rajiv Gandhi University for providing necessary facilities.

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