

EFFECTS OF *AZADIRACHTA INDICA* LEAVES ON THE SEMINAL VESICLES AND VENTRAL PROSTATE IN ALBINO RATS

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Abstract : Oral administration of 20, 40, 60, mg of dry *Azadirachta indica* leaf powder for 24 days resulted in decrease in the weights of seminal vesicles and ventral prostate, reduction in epithelial height, nuclear diameter and the secretory material in the lumen. Biochemically, there was a decrease in total protein, acid phosphatase activities. Seminal vesicles and ventral prostate being androgen dependent, the regressive changes histologically as well as biochemically, suggests the antiandrogenic property of the neem leaves.

Key words : seminal vesicles ventral prostate albino rats

INTRODUCTION

In recent years studies on antifertility property of plant extracts are receiving great attention (1) suggests the usefulness of their systematic exploration to prepare the contraceptives which interfere with the spermatogenesis in male ovulation, fertilization or implantation of ovum in uterus in females (2, 3).

Many plants which are common in India are reported to possess antifertility activity as a spermicidal, abortifacient or antiandrogenic. The leaves, fruits, roots, flowers and seeds of several plants are known to possess estrogen or antiandrogen like substances, which act on the reproductive system of male or female or

both and thus inhibiting their fertility (4, 5, 6, 7, 8).

Azadirachta indica (Syn: *Melia Azadirachta*) is an important medicinal plant cultivated throughout India and Burma (9, 10). It is extensively used as astringent, antiperiodic, antiprotozoal, antiparasitic, for cure of Leprosy, bronchitis, for healing ulcers in urinary passages and chronic fever etc. (9, 11, 12, 13). It has also been shown that *A. indica* possess antifertility property (8, 14).

Neem oil, a constituent from the seeds of *Melia Azadirachta* possess spermicidal and anti-implantation effects (15). However, the effect of this plant on the male reproductive system is not known.

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Our preliminary studies on the testis treated with *A. indica* leaves revealed regressive and degenerative changes in the germ cells and Leydig cells and reduced sperm count and sperm motility and increased abnormal sperms (16, 17). The present investigation reports the effect of *A. indica* leaves on the seminal vesicles and ventral prostate. It deals with the study of histoarchitecture and some basic biochemical parameters like total protein, total free sugar, acid phosphate (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities of seminal vesicle and ventral prostate.

METHODS

The leaves of *A. indica* were collected locally, dried in shade, coarsely powdered and quantitatively suspended in distilled water for oral administration (Gavage) to albino rats. 4-5 months old adult male albino rats of Wistar strain weighing 230-250 gms from rat colony of the Zoology Department were housed under well ventilated light-dark schedule with free access to food and water, were used.

The animals were divided into four groups, each consisting of 5 animals. Group I served as controls; Group II to IV treated with 20, 40, 60 mg of leaf powder, respectively, suspended in 1 ml of distilled water per day for 24 days. 24 hrs after the last dose, the animals were dissected and the surrounding connective tissue and blood vessels were removed, blotted and weighed. For routine histology the tissues were fixed in Bouin's fluid, dehydrated in alcohol and embedded in paraffin wax. Sections of 6 μ m thickness were cut and stained with Harris-

haemotoxylin and eosin. The epithelial height and nuclear diameter of the tissues were recorded using calibrated ocular micrometer.

For biochemical assays, 50-100 mg tissues were quantitatively homogenised in Tris-Hcl buffer (Phosphoric buffer 0.1 M pH; 7.2) and centrifuged at 800 g for 15 min. The supernatant was collected and used for the assay of total protein (18); free sugar, Glucose (Folin-Wu-method); acid and alkaline phosphatase (19); and Lactate dehydrogenase (20).

Student's 't' test was employed for statistical analysis.

RESULTS

Histological changes

Seminal vesicles (Table I; Fig. 1-4) In the seminal vesicle of control rats, the epithelium was disposed in a typical cryptic pattern. The epithelium was tall and columnar with a narrow muscle layer surrounding it. The lumen was fully distended with secretions. The height of the epithelium was $26.80 \pm 0.18 \mu$ m and nuclear diameter was $7.94 \pm 0.27 \mu$ m.

In all the treated rats the lumen and its secretions were reduced. The epithelial cells were short and cuboidal. The muscle layer had widened. The epithelial height was reduced to $17.33 \pm 0.48 \mu$ m (85%), $17.60 \pm 0.45 \mu$ m (66%) and $13.68 \pm 0.56 \mu$ m (51%) in 20, 40 and 60 mg treated groups, respectively. The nuclear diameters were also reduced to $5.78 \pm 0.18 \mu$ m (72%), $5.48 \pm 0.27 \mu$ m (69%) and $4.92 \pm 0.27 \mu$ m (62%),



Fig. 1 : T.S. of seminal vesicle of the control rat x 100

Fig. 2 : T.S. of seminal vesicle of the rat treated with *Azadirachta indica* leaf powder 20 mg daily for 24 days x 100.

Fig. 3 : T.S. of seminal vesicle of the rat treated with *Azadirachta indica* leaf powder 40 mg daily for 24 days x 100.

Fig. 4 : T.S. of seminal vesicle of the rat treated with *Azadirachta indica* leaf powder 60 mg daily for 24 days x 100.

Note the lumenward manifestation of the Epithelium and reduction of secretory material in all the treated groups.

MU : Muscle layer

EP : Epithelium

SE : Secretory material

N : Nucleus

TABLE I : Effect of treatment *A. indica* on the seminal vesicle of accessory reproductive organs of male albino rats (Values are expressed as SEM of 5 animals).

Group	Epithelial height (Mm)	% change	Nuclear diameter (Mm)	% change	Protein content (mg/g)	% change	Free Sugar content (glucose)	% change	ACP (mM/hr/g)	% change	ALP (mM/h/g)	% change	LDH (μ m/h/g)	% change
I	± 26.80 0.18	100%	7.94 ± 0.27	100%	50.59 ± 2.26	100%	0.66 ± 0.07	100%	4.43 ± 0.09	100%	1.18 ± 0.02	100%	356.27 ± 1.98	100%
II	17.33*** ± 0.48	65%	5.78*** ± 0.18	72%	44.67* ± 1.23	88%	3.41*** ± 0.56	216%	3.69*** ± 0.09	83%	2.83*** ± 0.02	239.6%	877.12* ± 1.34	246%
III	17.60*** ± 0.45	66%	5.48*** ± 0.27	69%	39.44** ± 0.92	78%	4.05*** ± 0.43	613%	3.14*** ± 0.04	71%	3.18*** 0.02	269%	984.96*** 1.34	276%
IV	13.68*** ± 0.56	51%	4.92*** ± 0.27	62%	23.46 ± 2.34	46%	4.25 ± 0.55	644%	2.69 ± 0.10	61%	3.82 ± 0.02	324%	988.99 ± 2.86	277%

Treated groups are compared with Controls

*P < 0.05; **P < 0.01; ***P < 0.001

TABLE II : Effect of treatment *Azadirachta indica* on the ventral prostate of accessory reproductive organs of male albino rat. (Values are expressed as SEM of 5 animals).

Group	Epithelial height (μ m)	% Change	Nuclear diameter (μ m)	% Change	Protein content (mg/g)	% Change	Free Sugar content (mg/g) (Glucose)	% Change	ACP (mM/hr/g)	% Change	ALP (mM/hr/g)	% Change	LDH (μ M/hr/g)	% Change
I	34.40 ± 0.65	100%	9.85 ± 0.23	100%	70.74 ± 3.27	100%	0.76 ± 0.07	100%	5.54 ± 0.05	100%	1.05 ± 0.03	100%	372.33 ± 2.30	100%
II	26.93*** ± 0.74	78%	8.12*** ± 0.29	82%	48.71*** ± 2.47	69%	4.99*** ± 0.50	656%	3.25*** ± 0.06	59%	1.84*** ± 0.02	176%	717.99*** ± 1.25	192%
III	26.27*** ± 1.50	76%	8.06*** ± 0.21	82%	44.33*** ± 3.59	63%	6.26*** ± 0.45	823%	3.14*** ± 0.07	57%	2.18*** ± 0.02	207%	851.40*** ± 1.36	229%
IV	16.93*** ± 1.04	49%	6.62*** ± 0.27	67%	43.74*** ± 3.27	61%	6.64*** ± 0.20	878%	2.38*** ± 0.08	41%	3.17*** ± 0.01	302%	915.43*** ± 2.23	246%

Treated groups are compared with controls

***P < 0.001

respectively. The changes were statistically significant.

Ventral prostate (Table I; Figs. 5-8).

In control rats the ventral prostate consisted of a large number of distended

alveoli filled with secretion. The lumen was lined by low columnar cells with the layer thrown into crypts randomly. The spherical nuclei appeared to lie at the position close to the basement membrane. The epithelial height was $34.40 \pm 0.65 \mu\text{m}$ and the nuclear diameter was $9.85 \pm 0.23 \mu\text{m}$.

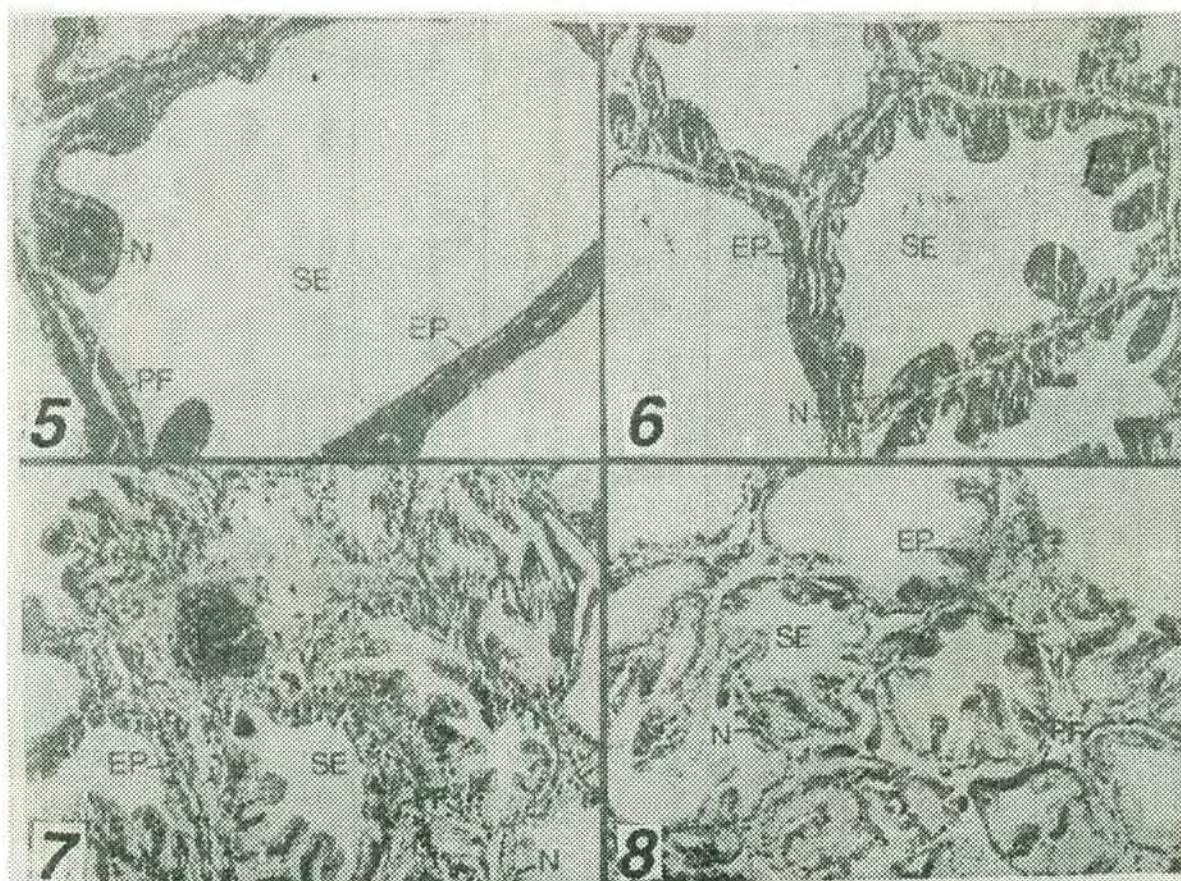


Fig. 5 : T.S. of ventral prostate of the control rat x 100.

Fig. 6 : T.S. of ventral prostate of the rat treated with *Azadirachta indica* leaf powder 20 mg daily for 24 days x 100.

Fig. 7 : T.S. of ventral prostate of the rat treated with *Azadirachta indica* leaf powder 40 mg daily for 24 days x 100.

Fig. 8 : T.S. of ventral prostate of the rat treated with *Azadirachta indica* leaf powder 60 mg daily for 24 days x 100.

Note the epithelial infolding and increases in the number of prostatic follicles in all the treated groups.

EP : Epithelium

SE : Secretory material

PF : Prostatic follicle

In all the treated rats the lumen was reduced in most of the prostatic alveoli. The number of prostatic alveoli were increased. The secretory material was scanty in the lumen and the epithelium had proliferated with crypts having invaded the lumen. (Figs. 7 and 8). The epithelial height was reduced considerably to $26.93 \pm 0.7 \mu\text{m}$ (78%), $26.27 \pm 0.50 \mu\text{m}$ (76%) and $16.93 \pm 1.04 \mu\text{m}$ (49%), the nuclear diameter were reduced to $8.12 \pm 0.29 \mu\text{m}$ (82%), $8.06 \pm 0.21 \mu\text{m}$ (82%) & $6.62 \pm 0.27 \mu\text{m}$ (67%) in 20, 40, and 60 mg treated groups, respectively.

Biochemical changes

The changes in biochemical composition viz., total protein content, free sugar, Glucose, ACP, ALP, and LDH are mentioned in Table II.

DISCUSSION

The plant products affecting the aspects of male reproduction, bring about the effect through either of two mechanisms namely, estrogenic and antiandrogenic effect (21). The target organs for estrogen of any origin in the male are the testis, epididymis, vas deferens, seminal vesicle and prostate. (22, 23).

The male accessory organs share a sensitivity to sex steroids and depend on androgens for their structural and functional properties. Androgens

consisting primarily of testosterone and dihydrotestosterone stimulate organ growth and secretory activity of the various accessory reproductive organs (24, 25, 26).

In the present study treatment of *A. indica* on the histology of seminal vesicle and ventral prostate reveals :-

1. a reduction in the weight,
2. a reduction in the epithelial height,
3. a reduction in the nuclear diameter and
4. a reduction in the secretory material in the lumen.

The seminal vesicle and ventral prostate are the target organs of androgen action. Castration diminishes the size and secretory activity of both seminal vesicle, ventral prostate and androgen administration reverses the inhibiting action (25). The accessory system of male ducts and glands are morphologically and physiologically dependent upon the production of androgens (24) and the androgenic activity may be more quickly and conveniently determined by the changes in histology and secretory activity of necessary reproductive glands (26). Seminal vesicle and ventral prostate being androgen dependent the regressive changes due to the treatment of *A. indica* leaves in the present study provides an indirect evidence for the antiandrogenic action of the neem.

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