EFFECT OF *FOENICULUM VULGARE*. MILL SEED EXTRACT ON THE GENITAL ORGANS OF MALE AND FEMALE RATS

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Summary : Following the oral administration of acetone extract of Foeniculum vulgare (fennel) seeds for 15 days is male rats, total protein concentration was found to be significantly decreased in testes and vas deferens and increased in seminal vesicles and prostate gland. There was a decrease in activities of acid and alkaline phosphatase in all these regions, except that alkaline phosphatase was unchanged in vasa. In female rats. oral administration of the extract for 10 days led to vaginal cornification and oestrus cycle. While moderate doses caused increase in weight of mammary glands, higher doses increased the weight of oviduct, endometrium, myometrium, cervix and vagina also. The results confirm the oestrogenic activity of the seed extract.

Key words : F. vulgare seed extract, male rats, female rats, genital organs, acid phosphatase, oestrogenic activity, alkaline phosphatase

INTRODUCTION

Fennel, *Foeniculum vulgare Mill.* (*F. vulgare*) is a well known Umbelliferous plant. The seeds of this plant have been known to promote menstruation, facilitate birth, alleviate the symptom of male climateric and increase libido (1). The essential oil of fennel possesses emmenagogue and galactagogue properties (5). The main constituent of fennel oil is anol or dimethylated anethole which is estrogenic (12) and causes the growth of lobule alveolar system in the mammary glands of immature female rabbits (4). It also induces estrus in mice (3).

In absence of specific information, this investigation was undertaken to assess the effect of fennel seed extract on the weight of testes and accessory sex glands and enzymes like acid and alkaline phosphatases in male rats. Observations were also made on oestrus inducing effect and on weight of mammary glands and parts of genital tract in female rats.

MATERIAL AND METHODS

F. vulgare seeds were procured locally, dried in shade and powdered. The powdered material was extracted with acetone by soxhlation. Acetone was allowed to evaporate and residue thus obtained was dissolved in known volume of 1% ethanol for oral administration.

Studies in male rats : Mature male rats of Wistar strain (150-200 *g*) were maintained in a well ventilated, humidity and temperature controlled animal house, with 14 hrs of light and 10 hrs of darkness schedule. They had free access to tap water and standard rat pallet diet (Hindustan Lever Ltd., India).

The animals were either treated with vehicle only (1% ethanol), or the extract (150 μg/100 *g* or 250 μg/100 *g*) every day for 15 days orally. Each group comprised of 5 animals.

After 24 hrs of last ingestion the animals were sacrificed by decapitation. The testes, vas deferens, seminal vesicles and prostate were dissected out, cleaned and weighed. The organ weights were expressed in terms of *mg*/100 *g*, body weight. Parts of tissues from each organ were homogenised in 0.1 M Tris - HCl buffer (pH 7.5) and centrifuged at 10,000 x g at 4°C for 15 min. The supernant obtained was used for the enzyme assays.

Total protein was estimated following the method of Lowry *et al.* (9). The activities of acid (ACP) and alkaline phosphatases (ALP) were estimated by the method of Andersch and Szezpinski (2) using p-nitrophenyl phosphate as substrate. In this method tartarate inhibition of acid phosphatase enzyme activity has been taken for measuring prostate specific acid phosphatase. Data were statistically analysed using Students' 't' test.

Studies in female rats : To evaluate the effect of extract on vaginal cornification, inbred adult Wistar rats (3-4 months old, 150$\pm$20 g) were maintained as described above. Females histologically showing regular 4-5 days cycle were selected for experiment.

The animals were bilaterally ovariectomised under light ether anaesthesia. After 15 days vaginal smears were taken. All animals showed leucocytic cells indicating diestrus phase. From day 16 the extract was administered (PO) at 3 dose levels (50, 150 and 250 μg/100 *g*) respectively daily for 10 days. Animals in control group received only 1% ethanolic solution. Vaginal smears of all the animals were taken every morning (9.00 to 11.00 AM) and stage of oestrous cycle was identified microscopically. 24 hrs after the last dose, all animals were sacrificed by decapitation. Oviducts, uteri, cervix,

vagina and mammary glands were dissected out, cleaned, blotted on a filter paper and quickly weighed. The wet weights of organs were expressed as *mg*/100 *g* body weight.

RESULTS

Male rats : There was no significant change in either the final body weight nor the organ weights after the administration of *F. vulgare*, seed extract.

High dose administration induced in the seminal vesicles and prostate a significant increase in the protein concentration but caused a marked decrease of protein in vas deferens and testes (Table I). However, the low dose induced a marked increase in protein concentration in the prostate only.

TABLE I : Effect of F. vulgare seed extract on protein concentration and phosphomonoesterases activity in genital tissue of male rats.

Parameters	*Treatment*	*Testis*	*Vas deferens*	*Seminal vesicle*	*Prostate*
Protein	Nil	11.8±1.01	13.8±1.39	10.9±0.87	11.3±1.11
(*mg*/*100 mg* tissue)	Low dose	10.2±1.27	11.0±1.64	13.2±1.71	15.1±1.20•
	High dose	9.7±0.84•	9.4±0.91•	19.2±1.22•••	20.5±2.02••
Alkaline	Nil	3.71±0.73	2.02±0.22	1.60±0.10	8.53±0.38
Phosphatase£	Low dose	1.68±0.08•	2.00±0.12	1.24±0.08•	4.38±0.06•••
	High dose	1.45±0.04•	1.98±0.10	0.62±0.07•••	1.73±0.08•••
Acid	Nil	2.32±0.24	1.41±0.11	1.41±0.09	2.54±0.53
Phosphatase£	Low dose	1.96±0.17	1.87±0.10	0.86±0.04•••	1.70±0.10
	High dose	1.34±0.17••	0.74±0.08••	0.44±0.04•••	0.50±0.04••
Prostatic acid	Nil	—	—	—	2.59±0.12
Phosphatase£	Low dose	—	—	—	1.14±0.09
	High dose	—	—	—	0.93±0.05•••

Each value is Mean (±S.E.M.) from 5 rats.

•$P<0.05$; ••$P<0.01$; •••$P<0.001$. Nil=1% ethanol solution; Low dose & High dose=150 µg/100 g and 250 µg/100*g* body weight/day/15 days of *F. vulgare seed extract.*

£Measured as *µmoles* of p-nitrophenol formed/hr/*mg* protein.

In testes, both doses had a marked inhibitory effect on ALP activity. However, ACP activity was found to be significantly reduced after high doses only.

In vas deferens, ACP was appreciably decreased by high dose treatment; ALP remained unaltered by the drug treatment.

In seminal vesicle, both doses decreased the ACP and ALP activity. In prostate, only high dose of the drug was effective in lowering the ACP and the prostatic specific ACP level. ALP was decreased significantly by both dosages.

Female rats :

Oestrous cycle : Effect of drug on the oestrous cycle was related to dose and duration of treatment. With 50 $\mu g/100\ g$ dose, 20% of rats showed vaginal cornification (estrus phase) on 9th day and 40% on 10th day. Higher dose (150 $\mu g/100\ g$) induced estrus in 20% rats on 5th day and 40% rats on 6th day; on 10th day 60% rats showed estrus phase. With 250 $\mu g/100\ g$ dose 20% of rats showed estrus phase on 4th day, 60% by 7th day, 80% by 8th day and all were in estrus phase on 9th day which persisted after 10th day also.

Body weight : During treatments no significant effect on the body weight was observed at all 3 dose levels.

Organ weights : The mammary gland weight was significantly increased by all doses studied (Table II). However, only the highest dose was effective in increasing the weights of the oviducts and myometrium. Weights of the endometrium, cervix and vagina were increased with middle and high doses.

TABLE II : Effect of *Foeniculum vulgare Mill. Seed extract on organ weights in* female castrated rats.

Treatment groups	*Organ weights (mg/100 g body wt.)*					
	Oviduct	*Endometrium*	*Myometrium*	*Cervix*	*Vagina*	*Mammary glands*
Group-I	23.60± 0.89	13.12± 0.44	29.04± 0.60	49.04± 2.90	97.23± 0.11	36.80± 0.52
Group-II	24.40± 1.03	13.43± 0.63	29.10± 1.16	55.28± 2.70	96.13± 0.72	38.60± 0.46•
Group-III	26.16± 1.54	15.25± 0.35••	31.21± 0.69	57.28± 0.97••	106.33± 1.50••	40.14± 1.28•
Group-IV	27.80± 0.99••	17.07± 0.62•••	35.12± 1.05•••	63.08± 1.12••	109.15± 1.89•••	40.20± 0.59••

Each value is Mean (±S.E.M.) from 10 rats.

•$P<0.05$; ••$P<0.01$; •••$P<0.001$.

Group I=1% ethanolic solution, alone; Groups II, III, IV were given the extract (50, 150 and 250/$\mu g/100\ g$ respectively) every day for 10 days.

DISCUSSION

Male rats : It is evident from the results that *F. vulgare* seed extract affects the functional integrity of testes and accessory reproductive organs in adult male rats by its probable oestrogenic property. Deficient androgen production suppress the protein synthesis, as it is an androgen dependent process (7). The observed decrease in protein concentration in the vas deferens in high dose group indicates the drug induced deficient androgen production. However, the increase in wet weights with associated protein concentrations in the prostate and seminal vesicles in high dose group may be due to the growth of fibromuscular tissues of these organs under the oestrogenic influence of the drug. This may represent a morphological sequelae of an imbalance between estrogen and androgen in these androgen target tissues (8).

ACP is a lysosomal enzyme and has been considered as a useful parameter for evaluating the androgen target gland function (10). ALP plays a comprehensive role in spermatogenesis, testicular hormone synthesis, in intermediate carbohydrate metabolism, and in metabolism of fructose (6). Both the enzymes are under androgenic control (11). The decreased activities of these enzymes in most regions of male genital tract indicates the deleterious effect of drug at high dose levels.

Female rats : Cornification of the vagina in the oestrous cycle is caused by estrogen or estrogen-like substances (7). The vaginal cornification under the influence of *F. vulgare* seed extract reflects the estrogenic nature of the drug.

The increase in oviductal, uterine, cervical, vaginal and mammary gland weights after the drug treatment also indicates accelerated growth under the inherent oestrogen–like activity of the drug.

The present study reveals the oestrogenic activity of *F. vulgare* seeds extract from its property to induce estrus and to increase target organ weights in female rats; it also probably acts as an antagonist to the androgens in male rats.

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