Ancient Science of Life, Vol No. V ,No. 2 October 1985, Pages 129 - 132

EFFECT OF FOENICULUM VULGARE SEED EXTRACT ON MAMMARY GLANDS AND OVIDUCTS OF OVARIECTOMISED RATS

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Received: March 06, 1985 **Accepted: June 30, 1985**

ABSTRACT: *The effect of acetone extracts of Foeniculum vulgare Mill., seeds at different dose levels (50/ug, 150/ug and 250/ug/100gm body wt.) on mammary glands and oviducts of castrated rats was investigated. The extract was found to increase nucleic acids and protein concentration as well as the organ weights in both the tissues. The medium and high doses were very effective. The results confirm the estrogenic nature of the seed extract.*

INTRODUCTION

Foeniculum vulgare Mill. (Fennel) is an aromatic herb belonging to the family Umbelliferae. The seeds are used in the indigenous system of medicine as an emmenagogue and galactogogue[1]. The compound anol or anethole, which is the major active compound of fennel oil, is considered to be an active estrogenic agent[2] due to its structural resemblance to diethylstilbesterol, a synthetic estrogen[3]. Our preliminary studies have shown the acetone extract of the fennel seeds to induce vaginal cornification in adult ovariectomised female rats and also to exhibit anti – androgenic effect in adult male rats[4]. Further, anol has also been reported to cause growth of lobule – alveolar system in the mammary glands of female immature rabbits[5]. In the absence of specific information on the effect of fennel seed extracts on the female genital tissues and on the mammary glands, the present investigation was undertaken in the oviduct and mammary glands of ovariectomised female rats administered with acetone extracts of fennel seeds at different doses, so as to understand the biochemical changes induced in these tissues.

MATERIALS AND METHODS

Foeniculum vulgare (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.

Adult female albino rats of Wistar strain (3 – 4 months old; 120 – 160 gms) were maintained in a well ventilated animal house with a constant 14 hrs light and 10 hrs darkness schedule. They had free access to tap water and standard rat pellet diet (Hindustan Lever Ltd; India). Females

histologically showing regular 4 – 5 days cycle were selected for experimentation.

A batch of animals were bilaterially ovariectomised under light either anesthia. 15 days after ovariectomy, the animals were divided into 4 groups of 5 animal each.

Group 1 : Control – intact animals showing estrus phase.

Group 2 : Ovariectomised controls – receiving 1% ethanolic solution.

Group 3 : Ovariectomised + 50 µg / 100 g. body wt. / day of *F. vulgare* seed extract / 10 days.

Group 4 : Ovariectomised + 150 µg / 100 g. body wt. / day of *F. vulgare* seed extract / 10 days.

Group 5 : Ovariectomised + 250 µg / 100 g. body wt. / day of *F. vulgare* seed extract / 10 days.

The intact controls were sacrificed on the day they exhibited estrus phase. The ovariectomised controls as well as the experimental were sacrificed by cervical dislocation 24 hrs after the last dose administration. The oviducts and mammary glands were dissected out, cleaned out, cleaned, blotted on a filter and paper quickly weighed. The wet weights of organs were expressed as mg/10g. body weight. The tissues were stored at -20^{o}C until further determination of protein and nucleic acids.

Total protein was estimated by the method of Lowry *et. al*[6]. The nucleic acids are extracted by the method of Schneider[7] and DNA was estimated according to Burton[8] and RNA was determined by the orcinol reaction of Ceriotti[9]. Protein and nucleic acid wet tissue. The data were analysed statistically using Students 't' test.

RESULTS

Table. 1 shows the effect of *F. vulgare* seed extract on organ weights and protein and nucleic acid concentrations in mammary glands of castrated rats. Results indicate the dose dependent increase in DNA, protein and mammary glands weight by the drug treatment. The RNA concentration were markedly elevated by medium ($p<0.05$) and high doses ($p < 0.01$) only. While the protein / DNA ratio showed a linear increase with increasing doses of the extract, the RNA / DNA ratio exhibited significant decrease with the low and high doses. The levels of all the biochemical parameters studied in the experimental were comparable to the values in the estrus animals (Group – 1).

Results presented in Table.2. also indicate the dose dependent increase of DNA, RNA and Protein concentrations as well as the oviducted weights, after the drug administration at medium and high doses. Low dose administration caused an increase in protein levels only. The trend in Protein / DNA and RNA / DNA ratios were similar to that observed in the mammary glands.

TABLE. I

Effect of *F. vulgare* seed extract on organ weight, protein and nucleic acid concentrations in mammary glands of ovariectomised rats.

GROUPS	WEIGHT (mg/100 g. body wt.)	DNA	RNA	PROTEIN	RNA/DNA Ratio	PROTEIN / DNA Ratio
		(mg/100 mg tissue)				
Group 1	40.54 ± 0.88	2.24 ± 0.16	1.854 ± 0.08	29.54 ± 2.32	0.830	13.188
Group 2	36.80 ± 0.52	1.54 ± 0.20	1.161 ± 0.03	3.32 ± 0.46	0.751	2.147
Group 3	38.60 ± 0.45*	2.03 ± 0.28	1.407 ± 0.12	5.55 ± 0.54*	0.702	2.771
Group 4	40.14 ± 1.28*	2.01 ± 0.04*	1.534 ± 0.08*	12.02 ± 1.01***	0.763	5.980
Group 5	40.20 ± 0.59*	2.49 ± 0.38**	1.637 ± 0.14**	40.71 ± 3.98***	0.655	16.291

Each value is a Mean ± S. E. M. of 5 experiments.

Gr. 1 = Intact estrus animal; Gr. 2 = Ovariectomised vehicle treated; Gr. 3 to 5 = Ovariectomised +50 µg; 150 µg; 250 µg; *F. Vulgare* seed extract / 100 g body wt. / day / 10 days, respectively.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs Group.2.

TABLE. II

Effect of *F. vulgare* seed extract on organ weight, protein and nucleic acid concentrations in oviducts of ovariectomised rats.

GROUPS	WEIGHT (mg/100 g. body wt.)	DNA	RNA	PROTEIN	RNA/DNA Ratio	PROTEIN / DNA Ratio
		(mg/100 mg tissue)				
Group 1	31.46 ± 1.45	1.18 ± 0.03	2.04 ± 0.13	15.84 ± 0.12	1.733	13.422
Group 2	23.60 ± 0.90	0.91 ± 0.05	1.02 ± 0.09	3.84 ± 0.40	1.123	3.875
Group 3	24.40 ± 1.03	1.01 ± 0.13	1.98 ± 0.13	5.34 ± 0.31 *	1.071	5.277
Group 4	26.16 ± 1.54	1.27 ± 0.07**	1.61 ± 0.19*	8.61 ± 0.85 ***	1.262	6.748
Group 5	27.80 ± 0.99	1.42 ± 0.91 ***	1.63 ± 0.12 **	41.19 ± 2.15 ***	1.145	28.946

Each value is a Mean ± S. E. M. of 5 experiments.

Gr. 1 = Intact estrus animal; Gr. 2 = Ovariectomised vehicle treated; Gr. 3 to 5 = Ovariectomised + *F. Vulgare* seed extract 50 µg; 150 µg; 250 µg. / 100 g body wt. / day / 10 days, respectively.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs Group.2 animals.

DISCUSSION

Our study indicates that *F. vulgare* seed extracts at three different doses increased the oviductal and mammary gland weights as well as the nucleic acid and protein concentrations in a dose dependent mammer. Since, the increment of wet weight, nucleic acids and protein concentration of genital tissues in females are correlated with heightened estrogen action[10-13], stimulation of these parameters in the oviducts and mammary glands by the administration of *F. vulgare* seed extract under the present experimental condition may point out the estrogenic nature of compound. It is also evident from this study that the potent dose is 250 µg / 100 g. dose is most effective in including persistent estrus in 100% of rats when administered for 9 days, confirming the above observation on dose potency.

Evidences has been accumulating that estrogen can induce mitosis[14], but progesterone suppresses estrogen induced mitosis in rat uterine[15] and vaginal epithelium[16]. Since, DNA component of the cell undergoes appreciable metabolic turnover during mitosis and since most of the genital tissue cells undergo mitosis and synthesis of DNA during the estrus cycle particularly after distrus[17], the increased level of DNA of rat oviduct and mammary glands may indicate stimulation of mitosis by the drug administration.

The increase in RNA concentration has been taken to be a measure for relative protein synthetic activity of mammary glands[18]. Recently, Larson and Smith[19] have shown the mammary RNA to be responsible for the synthesis of all structural proteins during mammary growth. Similarly, Means and O'Malley[20] have suggested that RNA synthesis medicates enzyme synthesis and cellular hypertrophy while the DNA synthesis reflects the increased cell replication in the Chick Oviduct. The present study reveals the estrogen like activity of *F. vulgare* seed extract in stimulating nucleic acids and protein levels in oviduct and mammary glands suggestive of hypertrophy and hyperplasia in the tissues.

Thus, the present study indicates not only the anabolic nature of the seed extract but also confirms the growth promoting estrogen – like activity of the *F. vulgare* seed extract.

ACKNOWLEDGEMENT

The financial assistance by the University Grants Commission for this work is gratefully acknowledged.

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