

THE EFFECTS OF *ANETHUM GRAVEOLENS L.* ON FEMALE REPRODUCTIVE SYSTEM OF RATS

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ABSTRACT

Effects of *Anethum graveolens L.* (dill) extracts on female reproductive system were studied in fifty four wistar female rats with regular estrous cycle in 6 groups. The experimental groups were fed 0.045 g/kg and 0.45 g/kg of aqueous extract and 0.5 g/kg and 5 g/kg of ethanol extract for 10 days. The sham group was fed with solvent and controls received no treatment. The estrus cycle changes were determined by daily vaginal smear changes. At the beginning and at the end of experiment blood samples were withdrawn to determine the blood estradiole and progesterone concentration. The ovaries were removed and granulosa lutein cells were studied under transmission electron microscopy. Treatment with high dose of the extract resulted in a significant increase in duration of the estrous cycle and diestrus phase and the progesterone concentration. Smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER) and mitochondria were increased in granulosa lutein cells in high dose groups. While more studies are required to clarify the properties of this herb, results of this investigation show that dill can be used as an either regulatory agent of menstrual cycle of women with irregular cycle or as an antifertility agent.

Keywords: *Anethum graveolens L.*, Estradiole, Progesterone, Corpus luteum.

INTRODUCTION

For *Anethum graveolens L.* or dill, a member of the Umbeliferae family, pharmacological effects such as antibacterial (1-3), antimycobacterial (4), antihyperlipidaemic, antihypercholesterolaemic (5), antiproliferative (6), antioxidant (7), cancer chemopreventive effects (8) and toxicological efficacy against the flesh fly (9) have been reported. Dill can improve gastric irritation (10), and as a folk remedy, has been used to improve flatulence, indigestion, stomachache, insomnia and colic (11, 12) and in females, as traditional medicine, it increases mother's milk production and promotes menstruation. While high doses of dill has been mentioned to weaken sexual and decreases spermatogenesis in males (12, 13), effects of this herb on reproductive system has not been reported.

Many steroidal and non-steroidal compounds have been and are being used as contraceptive and antioviulatory agent to control fertility. Though they act as potent antifertility agents, but they are not free from marked side effects. The major side effects are gastrointestinal and severe and painful uterine contractions, irregularity in the menstrual cycle for a long time, mammary and other tissue cancers. Hence, search for new potent antifertility materials with minimal side effects are in progress.

In this study effects of *Anethum graveolens L.* extracts on the estrous cycle, serum concentration of female steroid hormones and ultrastructural

changes of granulosa lutein cell of corpus luteum which are central cells of secretion of hormones in rat as an animal model was investigated.

MATERIALS

Anethum graveolens L. seeds were collected from the neighboring area of Eghlid in Fars Province, southern Iran, and identified by a botanist in Biology Department of School of Sciences of Shiraz University. The Voucher specimen was preserved for reference in the Herbarium of Biology Department with 1015 serial number. Kits for determination of progesterone and estradiole were from Spectria, Finland. 80% Ethanol, paraformaldehyde, 25% glutaraldehyde, Sodium cacodylate, osmium tetroxid, toluidine blue and uranyl acetate were obtained from Merck Company, Germany. TAAB resin was from TAAB co., England. For sectioning of corpus luteum the ultramicrotome Reichert-Jung-Ultracut made in England and for examination of sections transmission electron microscope Philips EM 300 made in Holland were used.

METHODS

Extract preparation

Dill seeds from other seeds were separated by stereomicroscope. Seeds were then powdered and for preparation of ethanolic extract, 100 grams of powder and 300 ml of 80% ethanol, and for the preparation of aqueous extracts, 100 grams of powder and 300 ml

of distilled water were percolated for 25 hours. Subsequently, the mixture were filtered and concentrated under reduced pressure by a rotary evaporator. The yield (w/w) of the aqueous and ethanol extract were 8.2% (g/g) and 4.5% (g/g) respectively.

Animals and extract administration

Fifty four Wistar female rats weighting between 150-200 g were obtained from the animal house of Razi Institute of Shiraz. The animals were adapted to the laboratory for two weeks prior to beginning of the experiments and vaginal smears were examined daily for selection of rats with normal estrous cycle. Animals were maintained at controlled temperature (22-24 °C) and a period of 12 hours light, and 12 hours darkness. Rats had free access to food and tap water. Animals were weighed before and after experiment. Principles of laboratory care established by the National Institute of Health (NIH Publication, No. 85-23, revised 1985) were followed. Female rats with regular estrous cycle were divided into 6 groups (9 animals in each group) of control, sham, low daily (LdA) dose (0.045 g/kg or 0.008 g/ml), and high daily (HdA) dose, (0.45 g/kg or 0.083 g/ml) as maximum non-fatal dose (10), for aqueous extract and low daily (LdE)dose,(0.5 g/kg or 0.09 g/ml) and high daily (HdE)dose, (5g/kg or 0.92 g/ml) as maximum non-fatal dose (10) for ethanol extract. Sham group received equal volumes of distilled water under similar condition and the control group was used for consideration of feeding stress. The mentioned dose of extracts were suspended in 1 ml distilled water and administrated orally by needle gavages for 10 days (2 regular estrous cycles) when animals were in estrous phase of estrous cycle. Vaginal smears were prepared in the morning (9-10 AM) during the experimental period.

Hormonal assay

In order to determine the variation of plasma concentration of estrogen and progesterone, at the beginning of the experiment, blood samples were collected from the tail vessel. Animals were anesthetized by diethyl ether and the lateral or ventral tail vein was punctured with a 23 mm needle gage and heparanized canola. After the last administration, while the animals were in estrous phase, each rat was anesthetized using diethyl ether and blood samples were taken by dorsal aorta puncture (approximately 4 ml). The blood samples were centrifuged for 15 minute (2000 rpm) and serum portions were separated. The progesterone concentration of samples were determined by radioimmunoassay solid phase method and estradiole concentration were measured by Elisa method using Spectria Kit in the Research Center of Nemazee Hospital.

Electron microscopy

Rats were sacrificed under deep anesthesia and after removal of their left ovaries corpus luteums were extracted and fixed in Carnovsky solution composed of 4%paraformaldehyde, 25% glutaraldehyde and 0.3 M cacodylate buffer with a PH of 7.3, postfixed in 1% osmium tetroxide, embedded in TAAB resin, sectioned at a thickness of 1 µm and stained with toluidine blue. Ultra-thin sections of 60–70 nm thickness of granulosa lutein cells were obtained and stained with uranyl acetate and counterstained with lead citrate and examined with a transmission electron microscope (14, 15).

Statistical analysis

The concentration of hormones and their cyclic variation in different groups were analyzed by One way ANOVA and Scheffe and Tukey test ($\alpha=0.05$). The animal weights and hormonal changes before and after experiments were analyzed by pair t-test ($\alpha=0.05$).

RESULTS AND DISCUSSION

Anethum graveolens extract increased duration of diestrus phases and total time of estrous cycle in high dose groups but had no significant effect in duration of estrus and proestrus phases. Normally, estrus cycle in rat lasts for 4-5 days (16) but in this study, the estrus cycle increased to 11.5 and 11.8 days in HdA and HdE groups respectively. Diestrus phase in control group was approximately 2 days but in rats of HdA and HdE groups elongated to 8.2 and 9.7 days respectively. The extract of *Calotropis procera*, *Asclepiadaceae* family, and *Rivea hypocrateriformis*, *Convolvulaceae* family, have also been reported to increase the estrus cycle in rats (17, 18).

There were no significant statistical differences in amount of serum estradiole between experimental, control and sham groups but the serum progesterone concentration increased significantly in high dose treatment group compared with control and sham groups. Also no significant statistical differences were observed between pre and post experimental serum concentration of estradiole but progesterone concentration increased significantly in HdA and HdE groups. A comparison between low dose and high dose of aqueous and ethanol extracts showed no significant differences. These results confirmed the prolongation of luteal phase of estrus cycle and the high activity of corpus luteum. This extract may stimulate the Hypothalamus-Hypophysis-Gonad and elevates the progesterone secretion and causes higher activity of the corpus luteum. For determination of any changes in the organelles after detection of any increase in serum concentration of progesterone, an ultrastructural study was performed. The photomicrograph of granulosa lutein cell of the control group in Fig. 1 showed euchromatic nucleus with inconspicuous

nucleolus, clear cytoplasm having diffuse free ribosome and polysomes, large and companionate lipid droplets and some mitochondria. In sham group no obvious changes were detected but the number of mitochondria and polysomes were increased. In the LdA and LdE groups compared to sham group, the cisterna of RER increased where some of them were branched and the nucleus was considered more heterochromatic. In HdA and HdE groups, the changes were clearer

than the sham group. Heterochromatic nucleus, increase in the lipid droplets, RER and mitochondria, condensation of the SER and crenation of the nuclear membrane and shrinkage of the nucleus were visible. Increase in the SER and mitochondria numbers showed that these cells were activated for steroidal material synthesis. It demonstrated the role of RER in protein synthesis and has been used as enzymes for production of hormone and also Relaxin (19, 20).

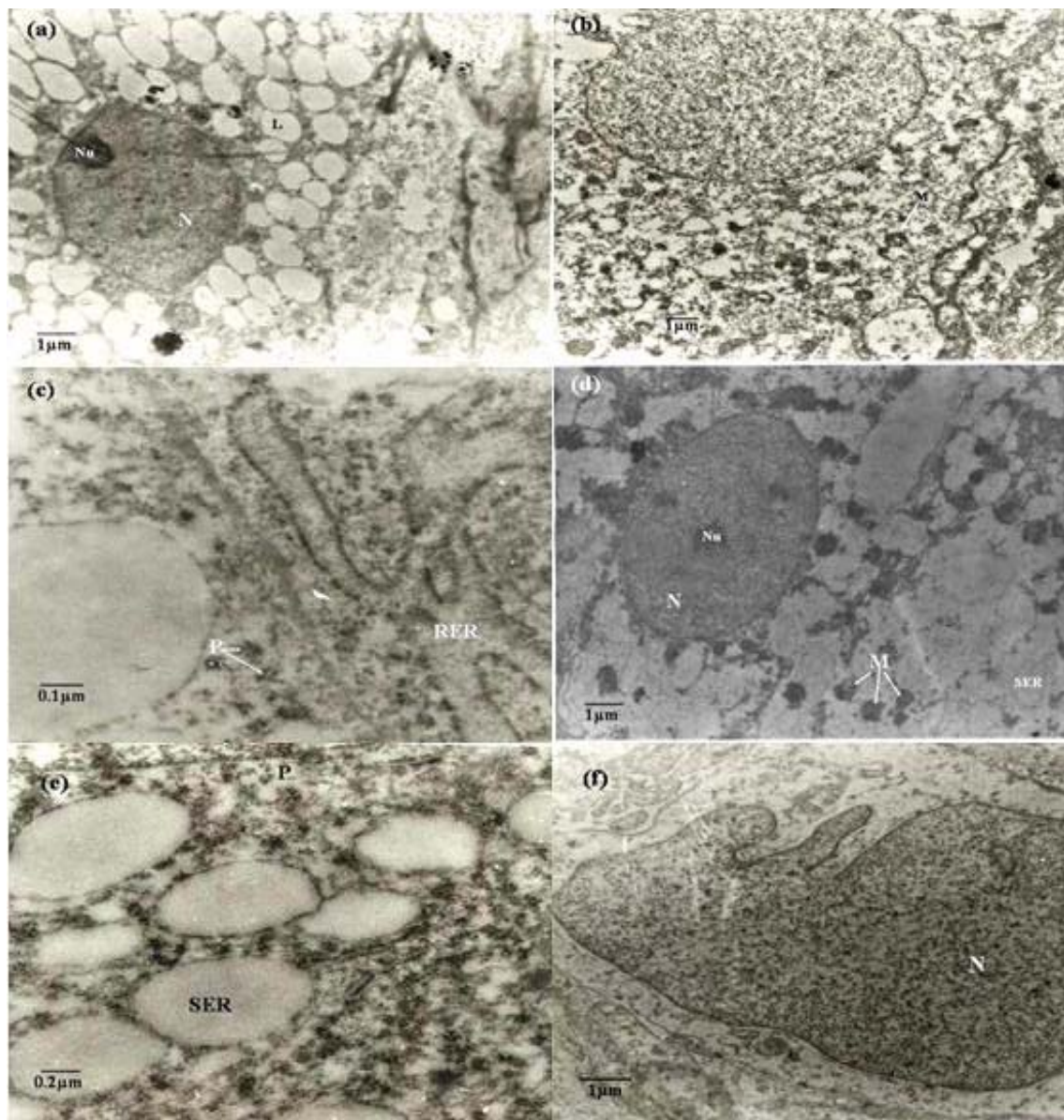


Figure 1. Electron micrographs of granulosa lutein cells of corpus luteum of female rats fed with *Anethum graveolens* L. (a) Granulosa lutein cell of control group. The cytoplasm contains numerous lipid droplets (L). The nucleus (N) showed prominent nucleolus (NU); (b) Granulosa lutein cell of S group, large number of mitochondria (M); (c) Granulosa lutein cell of LdA group, the branched rough endoplasmic reticulum (RER) and dilated smooth endoplasmic reticulum were clarified. The arrow indicates polysomes (P); (d) Granulosa lutein cell of HdE group. The cytoplasm contain numerous mitochondria and great dilated smooth endoplasmic reticulum (SER); (e) Granulosa lutein cell of HdE group with the large number of polysomes and dilated SER; (f) Granulosa lutein cell of HdE group with crenation of nuclear envelope

Table 1. The Mean and SD of duration of estrous cycle and diestrus phase (day) of control, sham, low dose (LdA) and high dose (HdA) of aqueous extract, low dose (LdE) and high dose (HdE) of ethanol extract of female rats fed with *Anethum graveolens L.*

| | Control | Sham | LdA | LdE | HdA | HdE |
|----------------|------------|------------|-----------|------------|---------------|---------------|
| Diestrus phase | 1.88± 0.11 | 2.11± 0.11 | 4.22±1.34 | 3.33± 0.94 | 8.22± 1.22 * | 9.77±0.36 * |
| Estrus cycle | 5.05± 0.15 | 6.61± 0.46 | 7.77±1.24 | 6.77± 1.03 | 11.55± 1.30 * | 11.77± 1.57 * |

P<0.05 when compared with control and sham groups

Table 2. The Mean and SD of serum concentration of estradiole and progesterone (Pg/ml) of control, sham, low dose (LdA) and high dose (HdA) of aqueous extract, low dose (LdE) and high dose (HdE) of ethanol extract of female rats fed with *Anethum graveolens L.*

| | Control | Sham | LdA | LdE | HdA | HdE |
|--------------|------------|------------|------------|------------|--------------|---------------|
| estradiole | 23.15±2.90 | 24.87±2.78 | 21.34±0.08 | 19.01±1.45 | 20.62± 2.01 | 41.71±0.66 |
| progesterone | 11.51±1.94 | 14.27±2.00 | 14.13±3.11 | 18.15±3.05 | 26.67±1.36 * | 30.46± 0.43 * |

* P<0.05 when compared with control and sham groups

Table 3. The Mean and SD of intergroups differences of estradiole and progesterone (pg/ml) of control, sham, low dose (LdA) and high dose (HdA) of aqueous extract, low dose (LdE) and high dose (HdE) of ethanol extract of female rats fed with *Anethum graveolens L.*

| | Control | Sham | LdA | LdE | HdA | HdE |
|--------------|------------|------------|------------|------------|-------------|--------------|
| estradiole | -1.45±3.10 | -0.43±2.86 | -5.42±8.36 | -8.92±4.63 | 4.07±2.49 | 16.46±9.55 |
| progesterone | -2.28±3.26 | -1.91±2.47 | -5.96±3.62 | -3.48±4.64 | 17.75±2.00* | 16.45±4.14 * |

P<0.05 when compared with control and sham groups

The crenation of nuclear envelope observed in granulosa lutein cells may be the sign of hasty aging in these cells (21, 22). No significant statistical differences of body weight were observed before and after experiments in all of groups.

From the results of this investigation it may be concluded that dill extract can change the duration of luteal phase of estrus cycle by elevation of progesterone concentration, therefore it is

useful and effective agent for treatment of irregularity in the menstrual cycle and as a natural contraceptive agent but extensive investigations are still requested.

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