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Study of Foeniculum vulgare (Fennel) Seed Extract Effects on Serum Level of Estrogen, Progesterone and Prolactin in Mouse

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Abstract

Objective: The Foeniculum vulgare (FVE) or fennel has a long history of use as both a food and medicine. The seed of this plant has been used to promote menstruation, alleviate the symptoms of female climacteric, and increase the number of ovarian follicles. The aim of this study is to evaluate the fennel extract effects on serum level of estrogen, progesterone and prolactin in female mice.

Materials and Methods: A total of 28 virgin female albino mice were divided into four groups (n = 7). Groups 1 and 2 (experimental groups) were administered FVE at 100 and at a concentration of 100 and 200 mg/kg for 5 days, intraperitoneally. Group 3 (negative control) received ethanol and Group 4 (positive control) received normal saline. Animals were scarified at 6th day, sera were collected and the level of estrogen, progesterone, and prolactin hormones was analyzed using Elisa Kit.

Results: Data analysis revealed that there is a significant difference in the mean level of serum estrogen, progesterone and prolactin between four different groups. P value in experimental groups compared with the control groups was ($P < 0.0001$).

Conclusion: Fennel extract can increase the serum level of estrogen, progesterone, and prolactin in female mice; it can be introduced as a novel medicine for treatment of infertility.

Keywords: Estrogen, Foeniculum vulgare, Progesterone, Prolactin, Mouse

Introduction

Infertility is defined as the inability to achieve pregnancy in a year of unprotected, regular intercourses (1). Inappropriate diet, obesity, smoking, psychological stress, and genital tract infections are some important factors, which can result in infertility (2,3). About 15% of young couples in different societies suffer from infertility (4). Female factors are responsible for about 40% of infertility, among different factors, ovulatory dysfunction seems to be one of the most important one, which will be identified in about 15% of infertile couples (5). Current treatment for infertility includes ovulation-inducting medicines and assisted reproductive technology (ART), which have own side effects and higher cost, so most infertile couples cannot afford for these treatments (6). Therefore, some people look for folk medicine. Traditional medicine has a long history for the treatment of female and male infertility for more than thousands of years (5).

Foeniculum vulgare (FVE), known as Fennel, is a well-known medicinal plant native to the Mediterranean area (7). It cultivated in different regions of Europe and Asia, and much is imported from India, China and Egypt (8). The FVE fruit has a long history of use as both food and medicine. Traditionally, it is believed that the plant acts as a carminative (assists with flatulence control) and increase breast milk production (6). It has been

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reported that this plant can also enhance libido, facilitate birth, alleviate the symptoms of the male climacteric, promote menstrual flow, and soothe indigestion and cough (6).

FVE also possesses emmenagogue and galactagogue properties (6). It has been previously reported that FVE used in the pediatric colic and some respiratory disorders due to its anti-spasmodic effects. Seeds of fennel are used in folk medicine for treatment of dysmenorrhea due to its probable anti-spasmodic effects (9).

Fennel seed consists of trans-anethole, estragole, fenchone, and α-phellandrene and the relative concentration of these compounds varies considerably depending on the phenological state and origin of the plant (7).

Fennel has a long history as an estrogenic agent with low toxicity and lack of documented carcinogenicity (10), therefore it can be re-introduced into modern medical usage. Furthermore, anti-inflammatory, anti-diabetic, anti-tumor, and many other activities of this plant have been revealed in different studies (6). Our previous study reported that fennel seed extract at concentration of 100 and 200 mg/kg can increased the number of different ovarian follicles dramatically, but we did not measure the level of different sex hormones, which probably are responsible for this ovulation-induction effect of the plant in our last research. Hence, the aim the present study is to determine whether the administration of fennel seed extract can alter the serum level of estrogen, progesterone, and prolactin.

Materials and Methods

Fennel seeds were purchased from local markets authenticated by a botanist (School of Pharmacy, Tabriz University of Medical Sciences). The extract was prepared according to Word of Health Organization protocol for preparation of an alcoholic extract (11). Briefly, 100 g of fruit was shed-dried, powdered and added to 1000 ml of 70% ethanol (v/v) and left to macerate at room temperature for 20 h. The basin was slowly rotated during this time. After filtration, ethanol was evaporated at low pressure at 30 °C.

The Ethical Committee of Tabriz University of Medical Sciences approved all procedures used in this study. A total of 28 virgin female albino mice with the weight range of 25-30 g were used. The animals were fed with standard laboratory chow and water during the experiment. We used the Whitten effect for co-cycling animals and controlled vaginal changes for determining estrous cycle (12).

Pro-estrus mice were divided into four groups (n = 7) (13). Animals in experimental groups (1 and 2) were received fennel extract at 100 mg/kg (Group 1) and 200 mg/kg (Group 2) for 5 days, intraperitoneally (11). Group 3 (negative control) received ethanol as the extract solvent. Group 4 received normal saline and was considered the normal group. Ethanol was administered in the same volume as Groups 1 and 2. Animals in all groups were sacrificed on the 6th day of the study.

A simple method for determining estrus cycle stage is known as a vaginal lavage or vaginal smear. The 4 stages can be distinguished by observation of cell type characteristic and differences in cell density. A small amount of a physiological saline solution is inserted into the mice vagina with a disposable pipette, removed, placed on a slide and examined under a microscope.

After determining the estrogenic effect of FVE extract, we examined for estrogen, progesterone and prolactin hormones with Elisa Kit, purchased from Glory Company, London England.

Statistical analysis

Statistical analyses of data were performed using a one-way analysis of variance and Tukey's post-hoc test. $P < 0.050$ was considered as statistically significant.

Results

The effects of fennel seed extract on serum estrogen level

Data analysis revealed that there is a significant difference in serum estrogen level between different groups ($P < 0.0001$). The mean ± standard error (SE) in the experimental groups (1 and 2), Group 3 (negative control) and Group 4 (normal control) were 11.71 ± 4.43, 25.99 ± 9.82, 23.36 ± 8.83 and 14.70 ± 5.55 pg/ml (Figure 1).

The effects of fennel seed extract on serum progesterone level

As revealed in figure 2, there is a significant difference in the mean level of serum progesterone between four different groups ($P < 0.0001$). The mean ± SE in the experimental groups (1 and 2), Group 3 (negative control) and Group 4 (normal control) were 6.21 ± 1.40, 8.45 ± 1.15, 3.70 ± 1.11 and 3.28 ± 0.58 ng/ml, respectively (Figure 2).

The effects of F. vulgare seed extract on serum prolactin level

There is a significant increase in serum prolactin levels between different groups, as it can be found from figure 3. The mean ± SE in the experimental groups (1 and 2), Group 3 (negative control) and Group 4 (normal control) were 1.48 ± 0.58, 2.27 ± 0.85, 1.31 ± 0.49 and 1.36 ± 0.51 ng/ml respectively and P value in experimental groups compared with the controls showed a significant increase ($P < 0.0001$) (Figure 3).

Discussion

This study revealed that: (1) Fennel extract at a concentration of 100 mg/kg and 200 mg/kg can increase the estrogen serum level. (2) This extract at the above-mentioned concentration can increase the amount of progesterone level in comparison to control groups and finally, (3) Fennel extract at these applied concentrations can increase the prolactin serum level compared to controls.

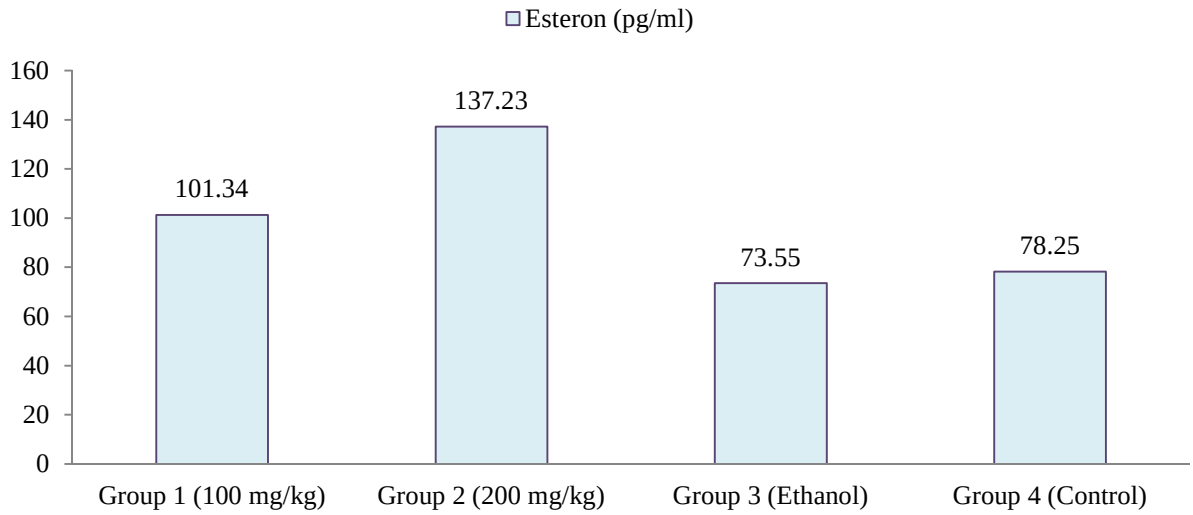

Figure 1. Comparison of the mean level of serum estrogen between groups

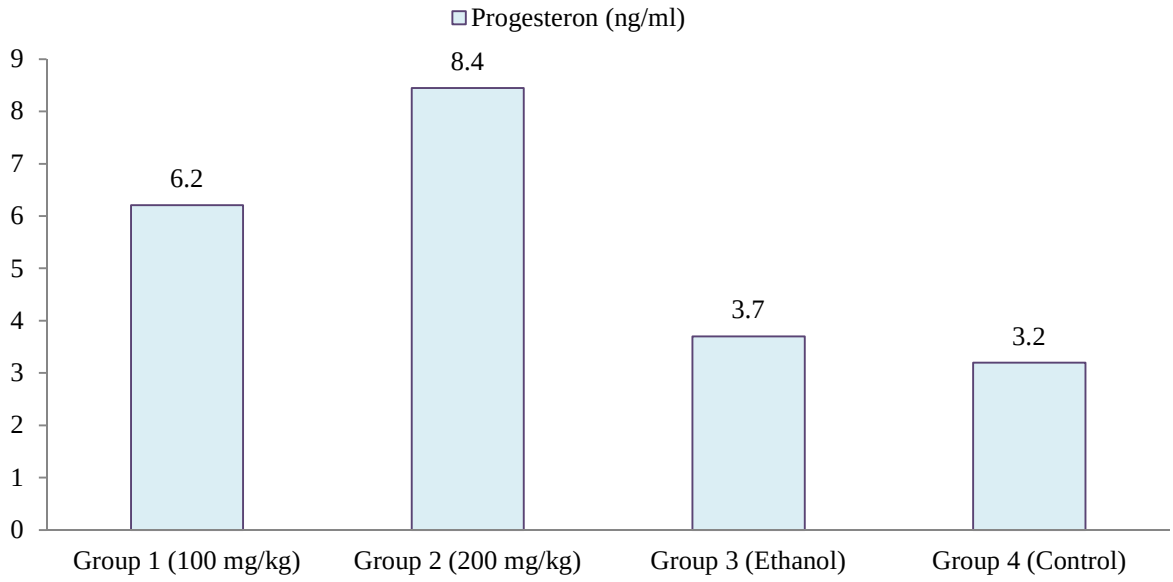

Figure 2. Comparison of the mean level of serum progesterone between groups

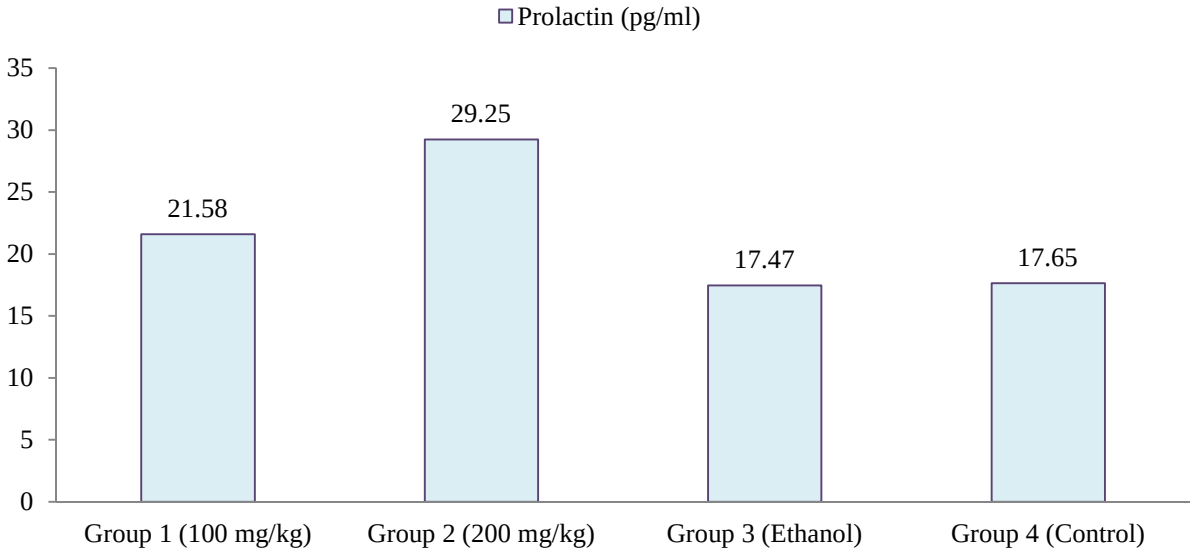

Figure 3. Comparison of the mean level of serum prolactin between groups

Infertility is a major and multifaceted worldwide problem, which its prevalence is increasing in both developed and developing countries (14,15). This problem which involved about 15% of young couples is defined as the inability to achieve a viable pregnancy within a year of regular and unprotected intercourse (16). Nowadays, different therapeutic methods such as medicines, surgery, and diverse ART techniques are used, but they do not always result in a viable pregnancy (17). Due to the cost and social burdens, some couples seek for alternative holistic therapies such as traditional herbal medicine. It has been reported that about 40% of infertility cases occur due to female factors (5). These factors include endometriosis, polycystic ovary syndrome and ovulatory dysfunction. Among them, the latte reconsidered as one of the most crucial factors, which identified in about 15% of infertile couples and accounts for more than 40% of infertility in women (18).

In recent years, application of alternatively medicine, especially herbal medicine, has been considered widely in the treatment of infertility due to its fewer side-effects and lower costs (17). Herbs such as Fructus lycii, Radix Morindae officinalis, Epimedii and many others have been used, for this reason (5).

F. vulgare known as fennel is a famous umbelliferous plant, which is being used for its anti-inflammatory, anti-spasmodic, analgesic, and laxative effects in folk medicine (19). Fennel oil contains different ingredients such as anol or dimethylatedanethole, which may have some estrogenic activity (20). Another component of this seed oil is flavonoids, a type of phytoestrogen. Some studies revealed the estrogenic actions of phytoestrogens both in in vivo and in vitro conditions (21). In another study, it has been reported that fennel can reduce the severity of dysmenorrheal and in another study reported that fennel has the same effect as Mefenamic acid in dysmenorrheal pain relief (22).

Previous study of our team showed that Fennel considerably increased the number of graafian, antral and multilaminar follicles at concentration of 100 and 200 mg/kg. Fennel extract analysis identified the presence of diosgenin, which is an estrogenic compound (6). Due to above mentioned results, this study has been designed to find more details about the possible mechanism of this plant seed extract on folliculogenesis and infertility. An imbalance between Luteinizing and follicular stimulating hormones can be identified as a reason for anovulatory, which identified as one of the ovulatory dysfunction (5). The initial treatment for ovarian stimulation is application of fertility drugs such as clomiphene citrate to induce ovulation, which has own adverse effects such as ovarian hyperstimulation or spontaneous abortion in some cases (23). Due to the above problems, more couples are interested in folk medicine to achieve pregnancy.

In this study, we reported that fennel extract at a concentration of 100 and 200 mg/kg can significantly increase the serum level of steroid hormones such as estrogen and progesterone in mice. Our data are in accordance to the results described by other researchers. Malini et al. reported that fennel seed extract had estrogenic activity on female, and male genital organs and the weight of these organs have been increased using this plant extract (23). There are also some evidence regarding to the role of this plant in estrus induction in mice (10).

Regarding the possible role of fennel extract on female reproductive system, Namavar et al. reported that this plant in a 2% concentration can be used safely to relieve primary dysmenorrheal (8). Another study by Ostad et al. revealed that fennel essential oil can result in uterine smooth muscle relaxation and may be of use for alleviation of dysmenorrhea sequelae (9). Accordingly, Delaram et al. reported that fennel extracts could reduce the severity of pre-menstrual syndrome (PMS), and they suggested administration of this extract for relieving the signs and symptoms of PMS (18).

In this study, we also reported that fennel extract at administered dosages can also increase the serum level of prolactin. In Iranian traditional medicine, some herbs such as fennel and caraway have been used to stimulate galactopoiesis and galactogenesis during pregnancy and lactation in this study; we also reported that fennel extract can increase the serum prolactin level in mice. To our knowledge, there is no scientific report on the effect of herbs on galactogensis and prolactin level. In this study, we revealed that fennel extract at the above mentioned concentrations can considerably increase the serum prolactin level in mice.

Conclusion

This study elucidated the fact that fennel extract can increase the serum level of estrogen, progesterone, and prolactin in female mice consistent with its use in folk medicine as a fertility enhancing agent. Further studies are suggested for understanding the exact mechanism(s) underlying these actions and probable changes in hormone receptors in genital organs.

Ethical issues

This research was approved by the Ethics Committee of Tabriz University of Medical Sciences, Iran.

Conflict of interests

We declare that we have no conflict of interests.

Acknowledgments

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