The Effect of Hydro-Alcoholic Extract of Fenugreek Seeds on Female Reproductive Hormones in Mice

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Abstract

Fenugreek is a plant from Fabeaceae family which is used for many medical plans. This study was conducted to study the effect of fenugreek seed's extract on reproductive system of female *Balb/C* mice .To this, mice's were divided in five groups: control, placebo, and three experimental groups. Each group had ten mice which their estrous cycles were synchronized for starting the study. Control group did not receive any drug. Placebo group received normal saline, and experimental groups were injected in peritoneum by 50,100, and 200 mg/kg extract every day until 20 days. After finishing injection, blood samples were taken. Hormone measuring (including FSH, LH, estradiol, and progesterone) was done using one way variance analysis of SPSS program at 95% probability level. Ovary slides were prepared and were studied using optical microscope. Obtained results, showed significant reduction in FSH, and LH levels and also significant increase in stradiol level in all experimental groups, but progesterone level was increased only in second experimental group. Histology results of ovary showed significant reduction in folliculogenesis of all three experimental groups. Also, increase in number of corpus luteum was highly significant. Furthermore, destruction of ovary tissues was observed in second experimental group. According to results, the extract of fenugreek seed stopped folliculogenesis trend and destroyed ovary tissue which shows its anti fertility effect in female mice.

Keywords: Fenugreek, reproductive hormones, mice

1. Introduction

Traditional medicine is a nature based science which is inherited from ancestors and includes plants, minerals and animal matters which are used as drugs [1] . In traditional medicine, pharmaceutical plants have a special place. Medicinal plant is a plant which has specific effective matters, is used in prevention or cure of illness, and has been mentioned in one of national valid Pharmacopoeia [2]. The importance of pharmaceutical plants is more obvious today and scientists of various countries are trying to identify medicinal plants, their properties and effective matters. Fenugreek is one of the old plants which have a wide range of medicinal properties: reducer of blood sugar and fat, anti diabetes, pain reliever, and anti cancer, increase in sex abil-

ity, and increase in milk production and worm killer [3] . Scientific name of fenugreek is *Trigonella foenum -graceum* L. which *trigonou* is derived from Latin and means triangle (because of triangle leaves), and *foenum-graceum* which means Greek hay is because of its different uses in ancient Greek. Also, because two seed pods are produced from main stem's nodes, these plants has been called "bull's horn" or "Goat horn " [4] . This plant is an annual, bush plant with 10 to 50 cm length which is sown in various regions of world like small Asia, Iran, Egypt, Algeria, India, Morocco, Italy, and Spain. The region of this plant has been known west of Asia [5] . Economical products of fenugreek are seeds and leaves, and reproduction of this plant is done by seeds which are sown in clay, calcareous lands in September. Seeds are sown together with clover seeds. Harvesting time is from June to July after harvesting, stems are being cut from the bottom and being dried [6] . This plant is one of the oldest pharmaceutical plants.

It was used in old Egypt as incense, and for mummifying corpses and also for easy confinement and increase in milk production. Even nowadays Egyptian woman use this plant for curing menstrual pain, as a tea for stomach problems of tourists, and also as a complement matter for wheat and corn flours for baking breads and confectionaries [7] . In ancient Chinese drugs, seeds of fenugreek were used as strengthen drug [8] . The nature of plant and its seeds is dry and warm. Seeds are used as sterilizer, mild laxative, diuretic, in bronchial inflammation treatment, leprosy treatment, the treatment of hemorrhoids and mouth deodorant [9] . Also, it is laxative, anti inflammatory, joint pain reliever, pulmonary and bone tuberculosis. Meanwhile, it is used for increase in weight [6] . Seeds of fenugreek have constant oils, essence, alkaloids, flavonoids, saponin, sapogenin, mucilage, free amino acids, carbohydrate, fiber, phosphorus, sulfur, lecithin, iron, calcium, magnesium, potassium, sodium, coumarin, tannin, resin, pectin, niacin, and carotenodic compounds.

Fenugreek has many pharmacological effects: results of researches show that diabetic mice (induces by stereptozotocine) which were cured by seed extract of fenugreek seeds, had an increase in weight and a reduction in ratio of kidney weight to body weight [10] .Also, reduction in blood fats was ascribed low carbohydrate absorption and fat absorption was ascribed to active presence of fibers [11]. Results of studies on wild mice showed that fenugreek increased excreting of acids and neutral stroles, and then saved cholesterol of body was decreased [12]. The effect of fenugreek on fat index of diabetic patients with high cholesterol showed that this plant reduced fats significantly [13]. Significant changes were observed in total cholesterol, LDL and triglyceride but no in HDL level. Flavonoides of fenugreek seeds have anti oxidant activities which play their roles via hydrogen reduction and deletion single oxygen. A lot of fenugreek seed poly phenoles prevent oxidative hemolysis and peroxidation of induced fats (by hydrogen peroxide in laboratory) of human's red cells. This study was conducted to evaluate the effect of hydroalcoholic extract of fenugreek on reproduction physiology of female mice.

2. Materials and Methods

2.1. Extraction

To prepare the extract of seeds, they were grinded completely and 30 g of obtained powder was poured in a sterilized erlen, 40 cc of physiological serum was added to it, and was located in a

cool place. After 24 hours, erlen contents were mixed completely using a shaker for five minutes. Then, after filtering the solution by filtrative paper and calculating extract residual in solution, concentration of extract in base solution was determined and doses were prepared.

2.2. Animals

Female mice (*Balb/C*) in weight range of 25-40 g were taken from animal division of Isfahan Medical University. Samples were kept in similar conditions of water, food, light, temperature and moisture to environment adaption. These similar conditions were continued in injection time too.

2.3. Study treatments

Samples were divided to five groups with 10 mice in each group: one control group, one placebo group, and three treatment groups. Mean weight of all groups were 30 ±5 g and each group was kept in a separate cage. Prepared extract was injected in three concentrations according to body weight:

Control group: non injected

Placebo group: 9% normal saline

group1: 50 mg/kg extract of body weight

group2: 100 mg/kg extract of body weight

group3: 200 mg/kg extract of body weight

Ten Injections were done between 8-10^{am} in a twenty days period (every other day) . One day after progesterone injection, extract injection was started and one day after the last injection, blood sampling and autopsy were done. For studying drug effect on samples, all mice must be in similar estrous cycle. To this, 0.5 micro gram of cloprostenol was injected in peritoneum and three microgram of progesterone was injected under skin of all samples. One day after that, extract injection was started and one day after last injection, blood samples of heart were prepared to study variation of sexual hormones, and also autopsy was done to ovary histology of samples.

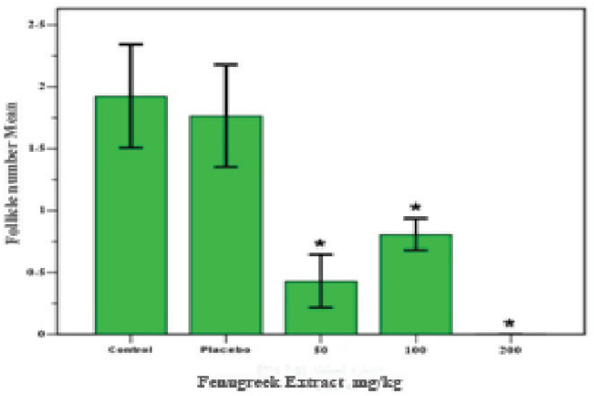
2.4. Statistical analysis

Obtained data were analyzed using one way variance analysis of *SPSS 11.5* program, and mean comparison was done using Duncan test.

3. Results

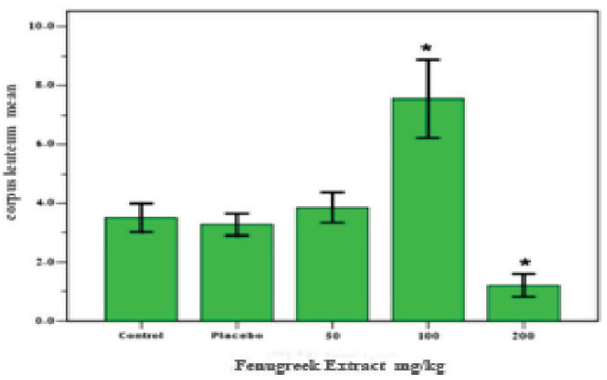
3.1. Number of graafian follicles

Counting graafian follicles of prepared tissue slides and mean comparison of groups using Duncan test ($p \leq 0.05$) showed that all experimental groups had significant reduction in proportion to control group and third group had the least.



Graph 1. The number of graafian follicles in various groups

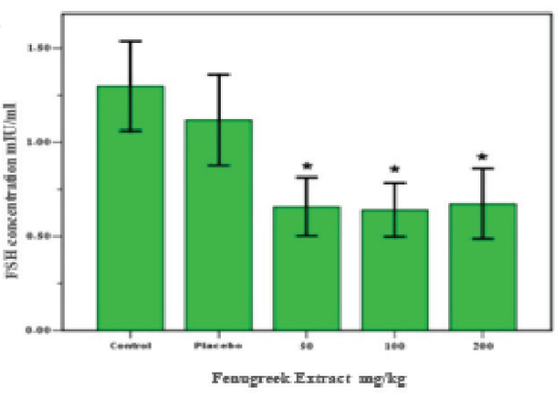
Results of counting corpus leuteum of tissue slides showed significant increase in second experimental group (100 mg/kg) and significant reduction in third group (200 mg/kg) in proportion to control but there was no significant difference between first group (50 mg/kg) and control.



Graph 2. The number of corpus leuteum in various groups

3.2. FSH amount

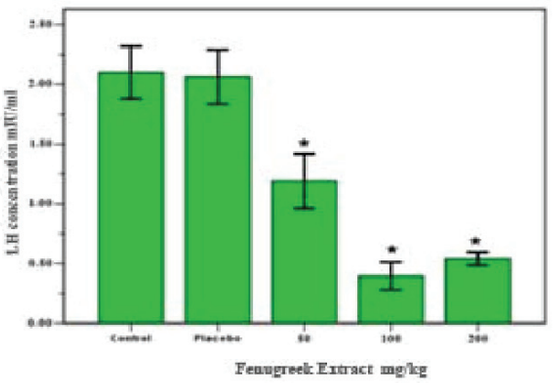
Comparing FSH level (mIu/ml) in blood serum of experimental and control groups using Duncan test ($p \leq 0.05$) showed significant reduction all three experimental groups in proportion to control.



Graph 3. FSH amount of various groups

3.3. LH amount

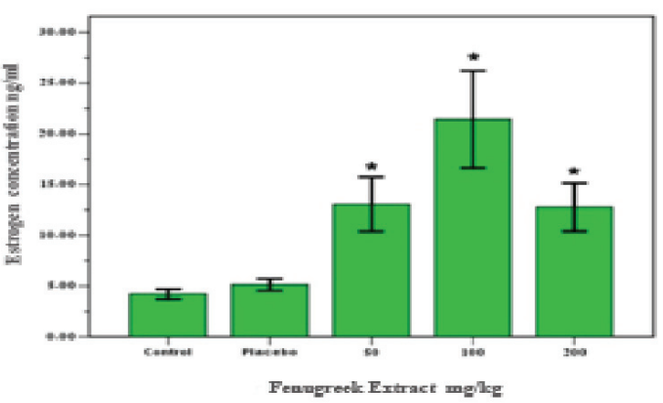
There was significant reduction in LH level (mIu/ml) of all experimental groups according to mean comparison results.



Graph 4. LH amount of various groups

3.4. Estrogen amount

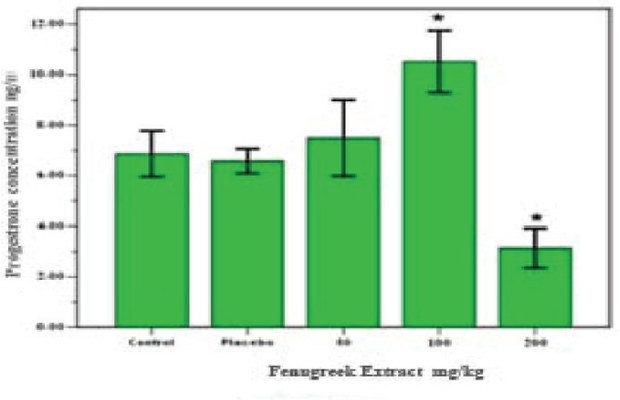
Mean comparison of estrogen level in blood serum of experimental and control groups using Duncan test ($p \leq 0.05$) showed significant increase in hormone level of all three experimental groups and second group (100mg/kg) had the highest hormone level.



Graph 5. Amount of Estrogen hormone in various groups

3.5. Progesterone amount

Mean comparison results of progesterone level in blood serum of experimental and control groups showed increases in first group (50 mg/kg) and second group (100 mg/kg) which this increase was significant only for second group. Also, third group (200 mg/kg) showed significant reduction in proportion to control group.



Graph 6. Amount of progesterone hormone in various groups

4. Summary and conclusion

Obtained results show significant reduction in number of graafian follicles in all three experimental groups (50,100, and 200 mg/kg) . This reduction is more obvious in third experimental group which is in agreement with previous results [3] . In their study, because of some compounds like sapogenin and diosgenin extant in fenugreek seeds, which are precursor of progesterone and testosterone reducer, sperm production was decreased [3].

By decreasing FSH level in follicle liquid (one of the follicle growth affecting factors) $IGFBP_{4.5}$ will be increased and action of proteases will be prevented, then, FSH antagonists will be increased and follicle will be evolved with atresia. GnRH stimulates also $IGFBP_{4.5}$ production in granulosa cells of follicle and reduces $IGFBP_{4.5}$ proteases, then, causes follicle atresia. Probably, secreting Aromatase preventing protein from dominant follicle is effective on the other follicles and cause growth stop and atresia. Furthermore, low concentration of leptin in follicle liquid may have negative effect on growth and ripening of ovules. Results of studies show that Nitric oxide (NO) prevents growth of follicles and repining of ovocytes via inducing apoptosis [14] . According to results, amount of FSH hormone decreased significantly in all experimental groups. High concentration of progesterone and low concentration of estrogen prevent follicle stimulating hormone. Meanwhile, it seems that opioid peptides of brain are mediums of this negative feedback [15] . On the other hand, inhibin hormone of dominant follicle prevents FSH secreting from pituitary, in very specific way. considering the results, LH hormone was reduced significantly in all experimental groups which can be because of :

Linoleic acid (CLA) has reducing effect on LH amount via decreasing leptine. Due to quite definite and significant relationship between leptine and nitric oxide in LH releasing from pituitary, Leptine reduction will be led to reduction in nitric oxide and then GNRH releasing [14] .

Fenugreek increases prolactin via affecting serotonergic system which will be led to prevention in GnRH releasing and then reduction in LH[14] .according to results, estrogen hormone was increased in all experimental groups. Seed extract of this plant has Gitogenin, Trigonelline, and Quercitin which have estrogen making activity. It seems that these three compounds play important roles in increasing estrogen by their biological activities. Considering the results, amount of progesterone hormone was increased significantly in second experimental group and was decreased significantly in third group. These increase and decreases are similar to increase and

decrease in corpus leuteum of second and third groups. On the other hand, progesterone increase can be ascribed to diosgenin compounds of fenugreek which are progesterone precursor.

5. References

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