International Journal of Phytopharmacology

www.onlineijp.com

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

Research Article

COMPARISION OF AQUEOUS AND OIL EXTRACTS OF *Foeniculum vulgare* ALONG WITH METFORMIN ON UTERINE ENDOMETRIAL HISTOMORPHOLOGY AND STERODIAL HORMONES IN EXPERIMENTAL PCOS FEMALE RATS

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ABSTRACT

Polycystic ovary syndrome (PCOS) is an inflammatory-based metabolic disease associated with insulin resistance. The effect of both *fennel* aqueous (FAE) and oil (FOE) extracts of *foeniculum vulgare* seed were investigated upon uterine endometrial histomorphometry and the amount of steroid hormones in experimental PCOS. We evaluated the effects of these extracts on both uterus and hormone levels in PCOS rat model. The levels of estrogen, progesterone, LH and FSH were tested in rats with PCOS and without PCOS. Frothy female rats were randomly divided into 8 different groups and were treated with different doses of both FAE and FOE along with metformin to investigate the effects of *fennel* extracts. Two different doses of *fennel* (100 mg / kg body weight and 150 mg / kg body weight) were used in treatments. After 63 days of the treatment, all blood samples were taken for biochemical and histological studies. The results of this study showed that *fennel* could have protective effect on uterine tissue in rats with PCOS. *Fennel* aqueous extract (FAE) reduced estrogen hormone, tissue thickness and increased progesterone and uterine endometrial thickness in PCOS rats more than *fennel* oil extract (FOE) in both doses so that FAE with the higher dose polycystic ovary could normalize the problems caused by PCOS in female mice. Metformin that is a commonly used drug for PCOS treatment decreased serum estrogen and elevated serum Progesterone in PCOS rats. It is concluded that *fennel* in both extracts with high dose had a significant effect upon PCOS treatment in rats even in some cases it could show more notable effect than metformin.

Key words: Female rats; *Fennel*; Polycystic ovarian syndrome; Uterus.

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INTRODUCTION

Polycystic ovary syndrome is considered as the most common endocrine disorder being observed in 6-10 % of women in reproductive ages. It is associated with insulin resistance, hyperandrogenism, subfertility and adverse cardiovascular risk factors. The pathogenesis of PCOS remains uncertain and it can be multifactorial containing interactions between genetic and environmental factors such as fetal exposure to androgens, obesity and sedentary lifestyle (Conway E *et al.*, 2010). Menstrual irregularities, polycystic ovaries, biochemical and clinical hyperandrogenism are used to characterize PCOS (Carmona E *et al.*, 2010). Some drugs have been reported to be useful in treating PCOS such as

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Home page: http://onlineijp.com/

DOI: http://dx.doi.org/10.21276/ijp.2017.8.4.6

Quick Response code

Received:29.09.17 **Revised**05.10.17 **Accepted**:11.10.17

metformin; one of commonly used therapies for this matter. It increases insulin sensitivity, decreases the level of androgens and improves the process of ovulation in patients with PCOS (Lord JM *et al.,* 2003; Legro RS *et al.,* 2013). Considering the side effects of chemical drugs and their application in treatment diseases which can cause complex problems, herbal medicines have been being broadly used as effective therapies for the prohibition and remedies of various health conditions for centuries by almost every known culture. The growing spread of research in the field of medicinal plants has been reported (Garodia P *et al.,* 2007). Up to now, the treatment of PCOS has been focused on using effective plants from which *fennel* belonging to the family of Apiaceae is traditionally used as medicinal herbal therapy for the treatment of various illnesses such as epileptic disease, seizures carminative, digestive, lactogogue and diuretic, respiratory and gastrointestinal disorders. It has been represented that *fennel* extract contains different polyphenolic compounds with tremendous antioxidant activity that cause *fennel* to be a useful plant in treating various diseases (Cahng SH *et al.,* 2013; Kooti W *et al.,* 2015). Ostad reported that *fennel* was effective on uterine contractions as a model for improving painful menstruation. In this study; *fennel* essential oil reduced the severity of uterine contractions. Essential oil of *fennel* was found to contain numerous medicinal and antioxidant activities against some diseases (Kooti W *et al*., 2015). Pai has reported that *fennel* oil extract contains some effect against Candida albicans and some other bacterial infections (Pai MB *et al.,* 2010). There have not been many studies according to the effects of *Foeniculumvulgare* on treatment of PCOS so far, for this reason the aim of this study was comparison and determination of metformin along with aqueous and oil extract of *fennel* on uterine endometrial, estrogen and progesterone, LH and FSH level changes in female rat serum with PCOS as well as determination of *fennel* effect (with two different doses) upon the epithelium and endometrial uterus thickness.

MATERIALS AND METHODS

Ethics statement

Ethics approval was taken from the local ethic committee.

Plant extracts

The *fennel* seed was prepared from West Azerbaijan province and then confirmed by the expert of Herbarium laboratory. For aqueous extract, an amount of 100 g of *fennel* seeds were crushed and extracted with distillated water (200 mL) under reflux. After filtration, the solvent was evaporated in incubator to obtain some powder residue (3 g). Thus, from each 100 g of dry weight aqueous extract of *fennel*; 3 g of dried powder was obtained. The essential oil was extracted according to the method described by Viuda-Martos with some modifications. Briefly, the dried *fennel* seeds (100 g) were ground and hydrodis-tilled for 4 h using a Clevenger-type apparatus. The oily layer obtained on top of the aqueous distillate was separated, dried with anhydrous sodium sulfate and filtration. The oil was kept in a dark glass bottle at 4◦C. The dose used for rat treatment was 100 and 150 mg FOE kg^{-1}BW.

Experimental protocol

For this study, 40 Wistar female rats weighing 180-200 g were purchased from the animal house of the faculty of science, Urmia University and they were treated in accordance with the law of care and use of experimental animals during the experiment. They were kept in cages with free access to food and water at 23-25 ˚C relative humidity of 30 ± 5% and light/dark cycle for 12 h. Animals were divided into 8 groups of 5 categorizing into 8 groups including group 1: control group (C) Recipient of normal diet; group 2: Polycystic ovary syndrome group (P) treated with polycystic ovary syndrome by intramuscular injection of estradiol valerate (4 mg / Kg BW); group 3: Polycystic ovary syndrome-*fennel* aqueous extract 1 (P + FAE1) treated with aqueous extract of *fennel* (100 mg / kg BW) after induction of an ovary syndrome; group 4: Polycystic ovary syndrome - *fennel* aqueous extract 2 (P + FAE2) treated with *fennel* aqueous extract (150 mg / kg BW) after induction of an ovary syndrome; group 5: Polycystic ovary syndrome - *fennel* oil extract 3 (P + FOE1) being treated with oil extract of *fennel* (100 mg / kg BW) after induction of an ovary syndrome; group 6: Polycystic ovary syndrome - *fennel* oil extract 4 (P + FOE2) treated with oil extract of *fennel* (150 mg / kg BW) after induction of an ovary syndrome; group 7: The healthy mice treated with aqueous extract of *fennel* (150 mg / kg BW) (F); group 8: Polycystic ovary syndrome- Metformin 2 (P + M) treated with metformin (150 mg / kg BW). All the treatments were done by gavage feeding procedure.

Biochemical analysis

The test animals were treated for 63 days. At the end of the day 63, all the animals were analyzed for phase assignment; the animals were anesthetized in their estrous phase using chloroform. The blood was taken from heart after autopsy for making heart clear enough for blood collection. The blood samples were transferred to tubes and immediately centrifuged (3000 rpm/10 min). Serum samples were stored at -20 ºC in frozen form for biochemical analyses. Both Auto-analyzer device (RA-1000) and commercially available kits (Pars Azmoon, Tehran, Iran) were used to test the level of estrogen and progesterone hormones.

Histological study

For histological study, the uterine tissues were removed and fixed in formol 10%. After tissue

processing, the samples were blocked in paraffin separately to be tested flowingly. Transverse serial sections (5-µm thick) were prepared and 1/10 parts selected, transferred to slides and stained by hematoxylin and eosin (H&E). The sections were investigated microscopically for the evaluation of histopathological changes (Hemati A *et al.,* 2012).

Statistical analysis

The obtained data were displayed as mean ± standard deviation (S.D.) and were examined for their statistical significance of difference with student t-test, one-way analysis of variance (one-way ANOVA) and the post hoc test dunnet and Tamhane's (depends on both normality and homogeneity of the absorbance values) utilizing SPSS 16.0. P-values less than 0.05, 0.01 and 0.001, were considered significant statistically (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

RESULTS

In this study, the average of progesterone serum concentration was measured in all test blood serum as group P (4.71 ± 0.13 ng/ml, $P < 0.001$) showed a significant decrease in the concentration of progesterone in comparison to the other tested groups shown in figure 1. In both groups, including P + FAE1 (6.35 ± 0.17 ng/ml, $P < 0.05$) and P + FOE1 (6.07 ± 0.32 ng/ml, $P < 0.05$) treated with lower dose of *fennel* extracts (100 mg / kg BW), a slight increase was observed in comparison with group P (4.71 ± 0.13 ng/ml) but this difference did not reach statistical significance. Apart from this, P + M (13.85 ± 0.38 ng/mL $P < 0.001$), showed higher effect in progesterone increasing level in comparison to the other test samples while it showed very close level to both P + FAE2 (13.83 ± 0.21 ng/mL $P < 0.001$) and P + FOE2 (13.67 ± 0.25 ng/mL $P < 0.001$). These three test groups represented very remarkable progesterone inducing level in comparison to the group P. In the case of extract type comparison, both extracts with higher doses including P + FAE2 (13.83 ± 0.21 ng/mL) and P + FOE2 (13.67 ± 0.25 ng/mL) could increase the induction of progesterone secretion significantly. Both FAE1 (6.35 ± 0.17 ng/mL) and FAE2 (13.83 ± 0.21 ng/mL) (aqueous extracts of *fennel*) effected the concentration of progesterone more than FOE1 (6.07 ± 0.32 ng/mL) and FOE2 (13.67 ± 0.25 ng/mL) respectively with slight differences. On the other hand, group P (3.72 ± 0.17 ng/ml, $P < 0.001$) represented a notable induction of estrogen serum concentration comparing to the other test groups as shown in figure 1. Both P + FAE1 and P + FOE1 groups (3.05 ± 0.02 ng/mL and 3.09 ± 0.31 ng/mL, $P > 0.05$), treated with lower dose of *fennel* extracts (100 mg / kg BW), did not show significant changes in comparison to group P (3.72 ± 0.17 ng/mL) respectively while P + FAE2 and P + FOE2 groups (1.92 ± 0.16 ng/mL, $P < 0.001$ and 2.12 ± 0.32 ng/mL, $P < 0.001$), treated with higher doses of *fennel* extracts (150 mg / kg BW), showed remarkable decrease in the concentration of estrogen comparing to group p respectively. P + FAE2 (1.92 ± 0.16 ng/mL, $P < 0.001$) could decrease the concentration of estrogen hormone much stronger than the other test groups. Apart from this, the thickness of epithelium was measured. The group P (336.66 ± 0.18 µm,$P < 0.001$) showed the thickest average of epithelium thickness in comparison to all test groups. Both groups including P + FAE1 (322.46 ± 0.12 µm,$P < 0.001$) and P + FOE1 (326.66 ± 0.18 µm, $P< 0.001$), treated with lower doses of *fennel* extracts (100 mg / kg BW), showed significantly thicker epithelium thickness rather than the groups treated with higher doses (150 mg / kg BW) including P + FAE2 (159.16 ± 0.04 µm) and P + FOE2 (162.86 ± 0.16 µm). The groups P + M (158.46 ± 0.04 µm, $P > 0.05$), P + FAE2 (159.16 ± 0.04 µm, $P > 0.05$) and P + FOE2 (162.86 ± 0.16 µm, $P > 0.05$) showed almost very close epithelium thickness to the control group (156.16 ± 0.42 µm). The average thickness of the endometrial uterus was also tested for all test groups and the results displayed that the group P (220.76 ± 0.17 µm, $P< 0.001$) showed a significantly thinner endometrial uterus thickness compared to the other test groups except the groups treated with lower doses of *fennel* including P + FAE1 (240.28 ± 0.25 µm, $P > 0.05$) and P + FOE1 (248.62 ± 0.09 µm, $P > 0.05$). On the other hand, the groups treated with lower dose of *fennel* represented a notable thicker endometrial thickness in comparison to the other test groups. The thickest endometrial uterus thickness belonged to P + FAE2 (428.23 ± 0.48 µm, $P < 0.001$) which represented a significant difference in comparison to the group P (220.76 ± 0.17 µm) are shown in figure 2. Serum LH and FSH changes were also analyzed and the results displayed that LH concentration was higher in P (1.29 ± 0.22mlU/ml, $P < 0.001$) than the control group (0.55 ± 0.08 mlU/ml) notably. Both groups treated with high dose of *fennel* (150 mg / kg BW) including P + FAE2 (0.59 ± 0.03 mlU/ml, $P < 0.001$) and P + FOE2 (0.61 ± 0.03 mlU/ml, $P < 0.001$) represented a significant decrease in concentration of LH average in comparison to the group P (1.29 ± 0.22mlU/ml), on the other hand, they had similar level of LH with control group (0.55 ± 0.08 mlU/ml), and F (0.61 ± 0.04 mlU/ml) in serum as there was no significant difference observed. While the groups treated with lower dose of *fennel* coded P + FAE1 (0.85 ± 0.06 mlU/ml,$P < 0.01$) and P+FOE1 (0.87 ± 0.03 mlU/ml, $P < 0.01$) had higher level of LH in serum than control group (0.55 ± 0.08 mlU/ml), there was also a significant difference between the mentioned groups with the ones treated with higher level of *fennel* including P + FAE2 (0.59 ± 0.03 mlU/ml, $P < 0.001$), P + FOE2 (0.61 ± 0.03 mlU/ml, $P < 0.001$) and F (0.61 ± 0.04 mlU/ml, $P < 0.001$). According to the results of FSH level in serum of

different groups, group P (0.23 ± 0.03 mlU/ml, $P < 0.001$) showed a significant decrease in level of FSH compared to control group (0.33 ± 0.03 mlU/ml). The groups treated with high level of *fennel* (150 mg / kg BW) including P + FAE2 (0.35 ± 0.08, mlU/ml, $P < 0.001$) and P + FOE2 (0.29 ± 0.01, mlU/ml, $P < 0.001$) showed a remarkable increase in level of FSH comparing to group P (0.23 ± 0.03 mlU/ml). Apart from this, P + FAE1 (0.32 ± 0.02 mlU/ml, $P < 0.01$) also showed a noticeable increase in the level of FSH comparing to the group P. The group F (0.42 ± 0.35mlU/ml, $P < 0.001$), displayed the highest level of FSH serum in comparison to the other test groups which are all shown in figure 3.

Fig 1. Comparison of mean changes in serum progesterone and estrogen in different experimental and control groups. All values represent the mean ± standard deviation (3 test).
***p < 0.05*; ***p < 0.01*; ****p < 0.001*.**

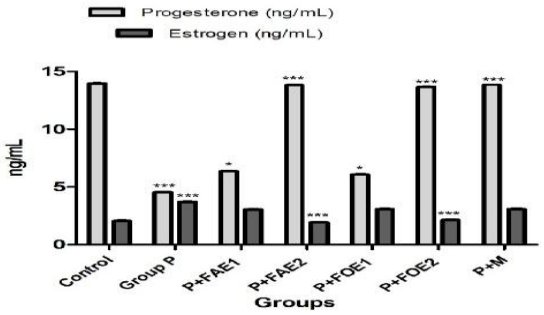


Fig 2. Comparison of mean changes in endometrial uterus and epithelium thickness v in different experimental and control groups. All values represent the mean ± standard deviation (3 test). ***p < 0.05*; ***p < 0.01*; ****p < 0.001*.**

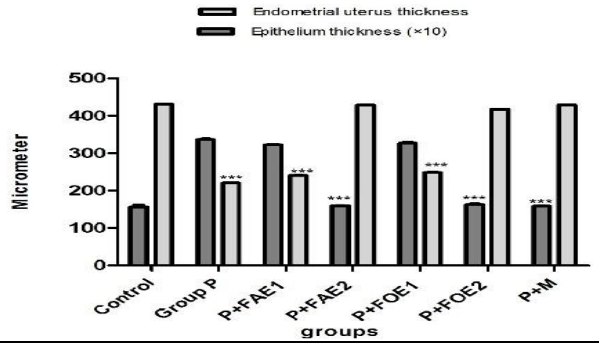


Fig 3. Comparison of mean changes in serum LH and FSH in different experimental and control groups. All values represent the mean ± standard deviation (3 test). ***p < 0.05*; ***p < 0.01*; ****p < 0.001*.**

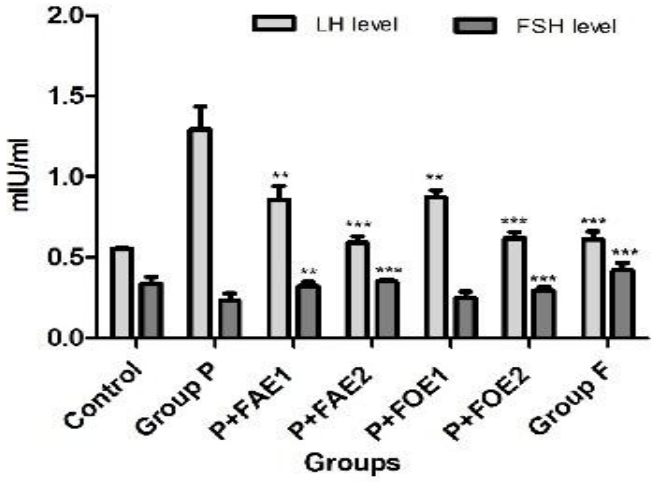


Fig 4. Overview of polycystic Ovary Hematoxylin and Eosin (H&E) painting and 400x magnificence

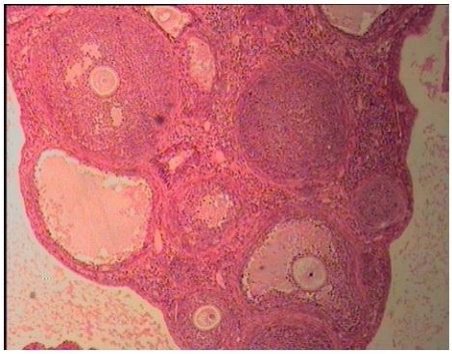
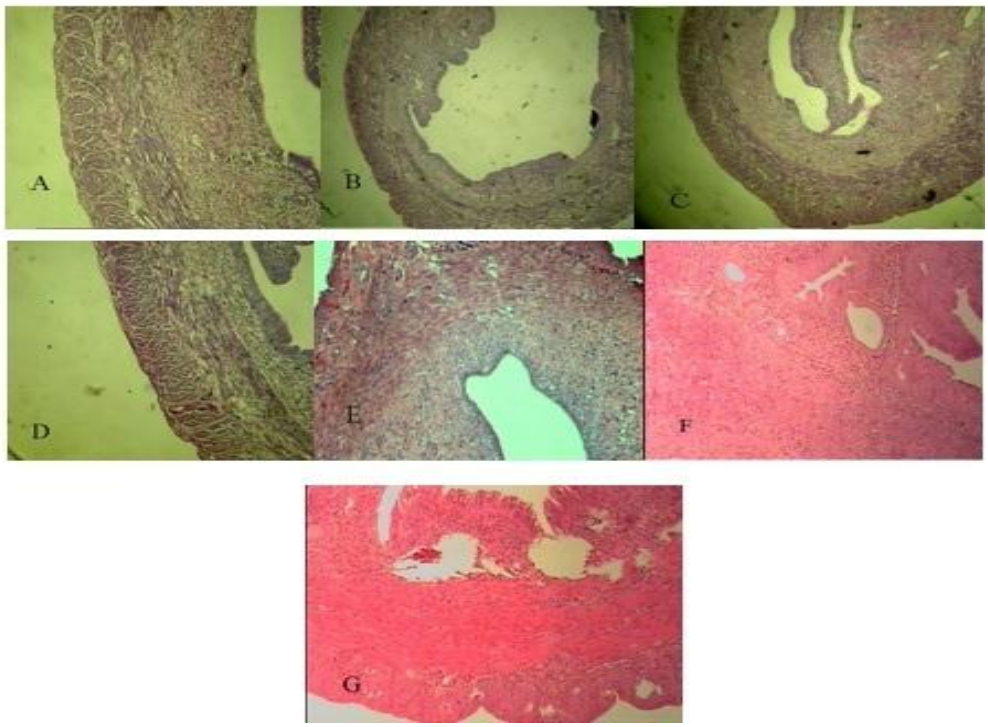


Fig 5. Cross section of the uterine tissue with Hematoxylin and Eosin (H&E) painting and 400x magnification in different test groups



A: The control group in which both epithelial height and endometrial of the uterus are in normal size.
B: The P group in which epithelial height and endometrial of the uterus have increased and decrease respectively.
C: P + FAE2 group treated with high dose of *fennel* (150 mg / kg BW) in which epithelial height and endometrial of the uterus have decreased and increased respectively (similar to control group)
D: P + FAE1 group treated with lower dose of *fennel* (100 mg / kg BW) in which epithelial height and endometrial of the uterus have decreased and increased respectively compared to the group P
E: P + M treated with metformin in which epithelial height and endometrial of the uterus have decreased and increased respectively (similar to control group)
F: P + FOE2 group treated with high dose of *fennel* (150 mg / kg BW) in which epithelial height and endometrial of the uterus have decreased and increased respectively (similar to control group)
G: P + FOE1 group treated with lower dose of *fennel* (100 mg / kg BW) in which epithelial height and endometrial of the uterus have decreased and increased respectively compared to the group P.

AUTHOR CONTRIBIUTIONS

B.A. and S.S. participated in study design and performed ELISA. B.A. and S.S. participated in study design, analyzed the data. B.A., M.A. and M.C. wrote, reviewed and edited the manuscript.

CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome**.**

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Cite this article:
Behnaz Aslanipour, Somayye Sadrefozalayi, Murat Alan, Mehmet Calan. Comparision of Aqueous and Oil Extracts of *Foeniculum vulgare* Along with Metformin on Uterine Endometrial Histomorphology and Sterodial Hormones in Experimental PCOS Female Rats. *International Journal of Phytopharmacology*, 2017; 8(4):173-178.
DOI: http://dx.doi.org/10.21276/ijp.2017.8.4.6