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Effects of *Foeniculum vulgare L.* extract on anxiety-like behaviors in ovariectomized mice

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Original Article

Abstract:

Background and Aims: Anxiety is one of the symptoms of menopause, which is due to stopping of ovarian activity. The aim of this study was to investigate the effect of *Foeniculum vulgare L.* extract on anxiety-like behaviors in ovariectomized mice.

Methods: Eighty female mice weighing 25-30 g and aged 6-8 weeks were divided into 10 groups of eight each, controls: the group without ovariectomy given distilled water, positive controls: the ovariectomized group receiving 40 µg/kg of estradiol valerate, the groups without ovariectomy given F. vulgare extract by gavage at 200, 500, and 750 mg/kg, the ovariectomized group given distilled water, the ovariectomized groups given F. vulgare extract at 200, 500, and 750 mg/kg, and the ovariectomized group given diazepam at 1 mg/kg. Elevated plus maze (EPM) and open field were used to investigate anxiety in mice. The level of malondialdehyde (MDA) and total antioxidant capacity (TAC) of the brain and serum were also measured.

Results: In ovariectomized mice, the number of entries to the closed arms of EPM was significantly increased compared with control group ($P<0.05$). Treatment of ovariectomized mice with 500 and 750 mg/kg of the extract significantly increased the

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number of entries to the open arms and significantly decreased the number of entries to the closed arms of EPM ($P<0.05$). The time spent in the center and the crossing squares in the open field significantly decreased in ovariectomized mice compared with control group and treatment with estradiol significantly increased them ($P<0.05$). In ovariectomized mice, serum and brain MDA levels significantly increased and their TAC significantly decreased compared with control group. Different doses of extract, estradiol and diazepam significantly reduced MDA levels and increased TAC in both brain and serum ($P<0.05$).

Conclusion: vulgare extract may ameliorate the symptoms of anxiety in ovariectomized mice by reducing oxidative stress.

Keywords: Foeniculum vulgare L., Anxiety, Antioxidant capacity, Malondialdehyde

INTRODUCTION

One of the natural periods of women's life is menopause in which menstruation stops and there will be no more possibility of fertility. Menopause starts at the age of about 45-55 years and occurs due to secretion of less amounts of hormones such as estrogen by ovaries, leading to menopause signs. First, irregular menstruation occurs and then it will stop. The women without one year of menstruation are considered as postmenopausal (1).

The ovarian activity toward gonadotropin is omitted, and their operation is decreased with aging. Meanwhile, menstruation does not occur; menopause occurs and leads to the reduction of sex hormones and the increase of follicle stimulating hormone (FSH). Estrogen reduction due to menopause results in problems such as flushing, night sweating, boredom, irritability, trembling, memory disorders, reduction of memory and brain speed processing, dizziness, anxiety, and depression, some of which are developmental among postmenopausal women (2). Anxiety is considered as one of the common disorders that are viewed as motivational and natural feelings that compatible signs of acute stress response. Anxiety appears when there is a fear of an individual (coherence). However, when anxiety is unbalanced and become acute, it is regarded as an incompatible response and a psychological disease (3).

Various factors such as lifestyle, genetic, and sex hormones affect anxiety due to the menopause. Research has demonstrated that high levels of estrogen decrease anxiety (3). It has been reported that post-menopausal women become anxious and an alternative treatment with estrogen can improve their anxiety (4). However, Oxidative stress is defined as an imbalance between increased reactive oxygen species (ROS) production and inadequate antioxidant defense, a condition characterized by an overload in oxidants, which may culminate in cellular dysfunction. Previous studies have demonstrated that oxidative stress contributes to aging and age-related processes that often accompany menopause (5).

Foeniculum vulgare L (fennel) is a flowering plant of Apiale, Apiacea and Foeniculum family. F. vulgare is a fragrant grassy plant with two or more years old and 2 m high, the fruit of F. vulgare is 6-10 mm long and 2-3 mm wide, has a fusiform track and green to light brown shape (6). This plant has estrogen-like compounds (phytoestrogens) and has warm nature. F. vulgare can increase milk due to the phytoestrogens, and it is the best treatment for menstruation stoppage. In longer treatment with estrogen during menopause improves endometrioses, breast cancer, and heart diseases. Therefore, researchers seek out alternative treatments to estrogen to prevent its complications (3).

addition, F. vulgare is anti-obesity and can remove vagina inflammation and flushing of menopause. Besides that, if it used with Echium amoenum, it can stop menstruation interruption. This plant is antispasmodic and anti–meteorism and improves irritable bowel and reduces food appetite (7). Generally, all the F. vulgare parts including stem, leaves, and fruit are used. The oily fruit of the plant includes 63% water, 95% protein, 10% fat, 13.4% mineral materials, 18.5% fiber, and 42.3% carbohydrates. The oil extracted from F. vulgare fruit includes 4% palmitic acid, 22% oleic acid, 14% linoleic acid, and 6% petro cyclic acid. This fruit has 4-6% essential oil with differing compounds and fragrance based on the plant growth location (8). There are over 30 types of terpenes in F. vulgare essential oil; anethole is the most important of these terpenes that comprises 50-80% of the essential oil

(9). F. vulgare fruit includes flavonoids, phenolic acids, hydroxyl acids, coumarin, and tannin. F. vulgare contains phenol, flavonoids, and phenolic acids. Phenolic acids include chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid, and rosmarinic acid. Flavonoids have eriositrin, quercetin, iso quercetin, rutin, hyperacid, nicotyphlorin and kaempferol (10). The aim of this study was to investigate the effect of *Foeniculum vulgare L.* extract on anxiety-like behaviors in ovariectomized mice.

Methods

Extraction:

F. vulgare samples were collected and then botanically authenticated in the Herbarium Unit at Medical Plants Research Center of Shahrekord University of Medical Sciences (No. 721). Then, they were (the whole samples: 1000 g) were crashed gently and macerated. A powdered plant was distilled in water poured in an Erlenmeyer to make a pasty form due to the existence of carbohydrate and water absorption; in addition, sufficient amount of boiling water was added in some phases. The gained extraction was filtered after 72h, and it was concentrated in a rotary evaporator. Then, the extract was dried at 37 ºC (11).

Laboratory animals and grouping:

A total of 80 female mice weighing 25-30 g and aged 6-8 weeks were kept at 25 ºC, under 12/12 h light/dark cycle with free access to the same food and water that were used in this study(from Tehran Institute Pasteur).

The mice were anesthetized for ovariectomy by 90 mg/kg of ketamine and 4.5 mg/kg of xylazin. After complete anesthetization, the abdomen was trashed, and then the surgery point was sterilized. Ovaries (follicle red tissue linked to oviduct tube) were removed gently. After that, the internal and external were stitched separately. Finally, the penicillin was injected to prevent infection. After three days, for six days, the vagina smear of the mice was prepared and evaluated by light microscope. If the fern design was not observed, the ovariectomy would be considered to be performed correctly (12).

The groups were as follows:

Group 1 (control group): The group without ovariectomy given distilled water as gavage;

Group 2 (positive control): The ovariectomized group given 40 µg/kg of estradiol valerate as gavage;

Groups 3-5: The groups without ovariectomy given F. vulgare extract at 200, 500, and 750 mg/kg as gavage;

Group 6: The ovariectomized group given distilled water as gavage;

Groups 7-9: The ovariectomized groups given F. vulgare extract at 200, 500, and 750 mg/kg; and

Group 10: The ovariectomized group given diazepam at 1 mg/kg (1).

In Khazaei et al study, five different doses of the extract were administered to five groups of mice (five mice/group). After 24 hours, there were no deaths in the animals that received the plant extract at 1, 10, 100 and 500 mg/kg, and one death was reported at 1000 mg/kg [13]. Doses of drugs were selected based on our pilot study. All drugs and extracts were dissolved in distilled water. All steps of experimentation were carried out in accordance with the regulations of the University and the Guide for the Care and Use of Laboratory Animals of National Institutes of Health (ethics code: IR.SKUMS.REC.1397.31).

Elevated plus maze (EPM):

EPM is an accredited test for evaluation of anxiety in rodents. The apparatus is made of black opaque plexiglass and includes two open (30×5×0.25 cm) and enclosed (30×5×15 cm) arms, which are connected by a platform area (5×5 cm) located at 50 cm above the floor. Testing room was appropriately illuminated and kept quiet. Mice were separately placed in the center of the EPM facing to open arm and standard indexes of anxiety evaluation were videotaped for 5 min. The indexes included the time spent on the open and closed arms, the number of entries to the open and closed arms, and the total number of entries to the arms. The apparatus was sterilized with 70% ethanol after testing each mice (1).

Open field test:

The open chamber is a glass house sized 45*45 cm and 45 cm height, that is divided into 16 equal squares and is located in the middle of a silent room. To perform the test, at least 1 hour before the test, animals were put in the experiment room. Each animal was left in the open field for 10 min for familiarization one day before the test. The next day, the animal was put in the middle square, and for 6 min the travelled distance and the time spent in the center and margins were recorded (1).

MDA levels of serum and brain:

Two hundred µl of serum or homogenate brain tissue was mixed with 1.5 ml of 20% acetic acid, 1.5 ml of TBS (0.8%) and 200 µl of 8.1% sodium dodecyl sulfate. The samples

were then boiled in boiling water for 60 minutes. The samples were then cooled and 1 ml of distilled water and 5 ml of a n-butanol-pyridine mixture were added and cooled. The mixture was then centrifuged at 4000 rpm for 10 minutes and the optical absorbance of the supernatant was recorded at 523-nm wavelength (14).

Evaluation of total antioxidant potential:

To measure serum and brain tissue antioxidant capacity, three solutions were used by FRAP method consisting of buffer (1.55 ml sodium acetate, 8 ml concentrated acetic acid that was diluted with distilled water to 500 ml), ferritin chloride solution (270 mg FeCl 3 (6 $_2$) whose volume increased to 50 ml by adding distilled water) and Triazine solution (47 mg Triazine solved in 40 ml of HCL 40 mM). The solution was prepared by adding 10 ml of solution number 1, 1 ml of solution number 2, and 1 ml of solution number 3. Twenty five µL of serum of homogenated liver and kidney tissue was added to the solution and left at 37 ºC for 10 min; then, the optical absorbance was recorded at 593-nm wavelength (14).

Data analysis: Data analysis was performed by using SPSS 16. The one-way ANOVA and Tukey's test were used for the comparison of mean values. The results were expressed as the mean±standard deviation (SD). $P<0.05$ was considered significance level.

Results

EPM

There was no significant difference in time of entries to the open arm between ovariectomized and control mice. However, there was a significant difference in number of entries to the closed arms between ovariectomized mice and control group. The treatment of overiectomized mice with 500 and 750 mg/kg of the extract caused a significant increase in entry time to the open arm of EPM and decrease in entry time to the closed arm (Figure 1).

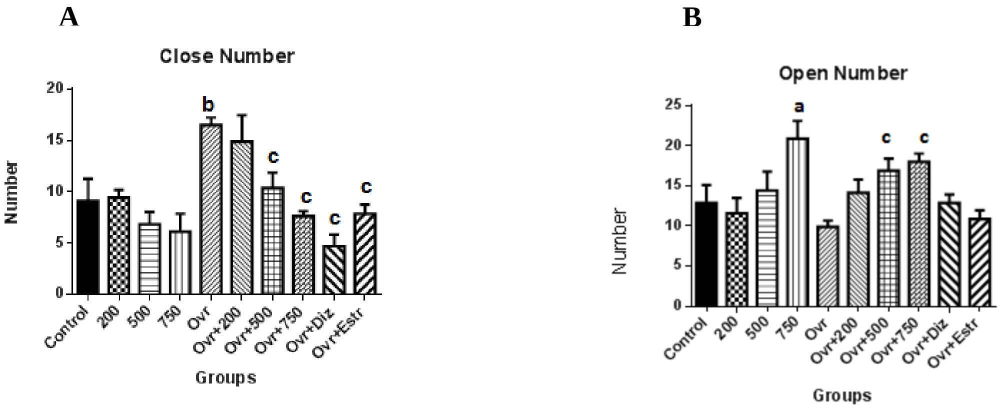

Figure 1: Comparison between the entry time number to open arm (A) and close arm (B) in elevated plus maze test; a: significant difference between control group and extract group (P<0.01); b: significant difference between control group and ovariectomized mice (P<0.01); c: significant difference between ovariectomized group and ovariectomized groups given extract, diazepam, and estradiol (P<0.01). All values are expressed as mean±SE.

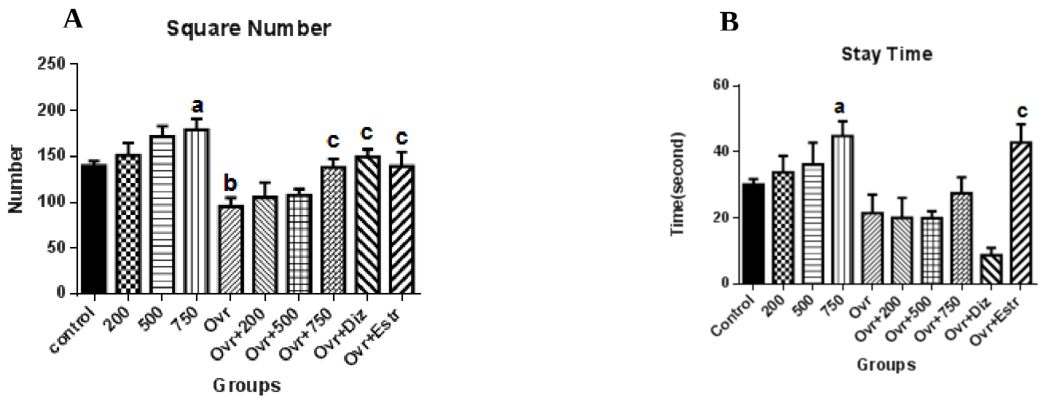

Figure 2: The comparison of time spent at central squares (A) and crossing squares (B) in open field test; (a) significant difference between control group and extract groups (P<0.01); (b) significant difference between control group and ovariectomized group (P<0.05); (c) significant difference between ovariectomized group and ovariectomized groups that received extract, diazepam, and estradiol (P<0.01).

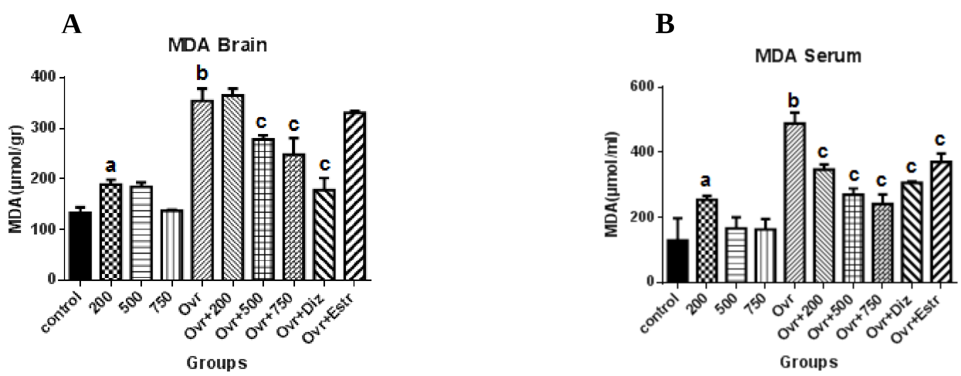

Figure 3: the comparison of MDA serum (A) and brain (B) in experimental groups. (a) significant difference between control group and extract groups (P<0.05); (b) significant difference between control group and ovariectomized group (P<0.001); (c) significant difference between ovariectomized group and ovariectomized groups given extract, diazepam and estradiol (P<0.01).

Open field test

Figure 2 demonstrates that the time spent at the center of open field test in ovariectomized mice had no significant difference to the control group. The treatment of ovariectomized mice with estradiol caused a significant increase in time spent at the center of the open field test. The number of crossing squares in open field test in ovariectomized mice significantly decreased compared with control group. The treatment of ovariectomized mice with 750 mg/kg of the extract and also estradiol and diazepam caused a significant increase in crossing squares in the opened field test.

MDA level

Serum and brain MDA levels were significantly higher *in* ovariectomized mice than in the control group. The treatment of ovariectomized mice with different doses of the extract, estradiol, and diazepam significantly decreased serum MDA levels. The treatment of ovariectomized mice with 500 and 750 mg/kg of the extract and also diazepam significantly decreased brain MDA levels (Figure 3).

Antioxidant capacity

The antioxidant capacity of serum and brain tissue in ovariectomized mice significantly decreased compared with control group. The treatment of ovarictomized mice with different doses of the extract, estradiol and diazepam caused a significant increase in the antioxidant capacity of serum and brain (Figure 4).

Discussion

Our findings demonstrated that ovariectomy of mice caused an increase in anxiety behaviors such as the time for entering the EPM closed arm. Besides that, anxiety behaviors in the open field test were seen in mice, including the decrease of time spent in the center and the crossing squares. In the present study, mice were studied two weeks after ovariectomy, and they exhibited anxiety-like behaviors in the open field test and EPM. In the present study, the treatment of ovariectimized mice with 500 and 750 mg/ kg doses of F. vulgare extract have improved anxiety behaviors as the significant increase number of entries to the EPM open arm and reduction number of entries to the EPM closed arm. In control given 750 mg/kg of the extract, the entries number and time spent on the EPM open arm was more than that in the control mice. F. vulgare may have antioxidant properties to reduce anxiety-like behaviors. According to the findings of this research, the treatment of ovariectomized mice with estradiol caused a significant reduction in entries time to the closed EPM arm, a significant increase in entries time to the open EPM arm, and a significant increase in time spent at center and crossing squares in the open field test.

In the study of Alimohammadi et al. (2015), a significant decrease of time spent and number of entries to the open arms was observed in anxious mice in comparison with the control group (3). Moreover, Honari et al. (2012) has reported anxiety-like behaviors such as the decreased number of entries and time spent in the open arms in ovariectomized mice [1]. Hi et al. (2014), announced the anxiety-like behaviors in the ovariectomized mice as rearing, exploration and passing time reduction from squares of the open field (15).

Generally, with regard to the development of anxiety-like behaviors and depression in ovariectomized rats, several reports are available based on the species of rats, type of behavioral tests and time of the test. According to Estrada–Camarena et al. (2011), depression-like behavior was seen after one week from ovariectomy; however, no depression–like behavior was seen 3 and 12 weeks after ovariectomy (16). In female Long-Evans rats, anxiety and depression behaviors were seen six months after ovariectomy (17). In The study of Li et al., anxiety and depression behaviors due to ovariectomy were observed at the fifth week after surgery in Sprague Dawley rats (15). Alimohammadi et al. (2015) have reported anxiety behaviors in mice after one month (3).

In the studies of Kishore et al. (2012) and Vafaei et al. (2010), the treatment of animals with F. vulgare extract significantly increased the number of entries and time spent at the open arms of EPM. In the open field test, the F. vulgare extract caused a significant increase in the number of standing on feet and the number of crossing squares (18). Kishore et al. and Vafaei et al. reported the anti-anxiety effects of F. vulgare extract at lower dose in control mice (19). However, the present research demonstrated that these differences might be due to the difference in treatment period and also extracts' phytochemical compounds. In a double–blind randomized clinical (control) trial by Ghazanfarpour et al. (2018), 60 postmenopausal women were under investigation by F. vulgare or placebo. According to their findings, anxiety and stress levels showed significant changes after the treatment; however, a significant decrease in anxiety and depression was seen among depressed patients following F. vulgare treatment. The researchers attributed the observed effects of F. vulgare to phytoestrogenic compounds (2). In the present study, the anti–anxiety effects of F. vulgare extract were seen in ovariectomized mice. One strength of our study is the investigation of anti–anxiety activity of medicinal compounds in animal models in order to investigate their mechanisms of actions, i.e., in the present research the role of oxidative stress in anti–anxiety activity of F. vulgare extract was investigated.

In ovariectomized mice, serum and brain MDA levels, as lipid peroxidation index, were significantly higher compared to the control group. Besides that, there was a significant reduction of brain and serum antioxidant capacity in ovariectemized mice that shows the weakness of the antioxidant defense system.

It seems that the oxidative stress due to menopause and reduction of estrogen levels play a vital role in psychological and mode disorders. Recent studies have shown that estrogen exhibits antioxidant activity that is due to the phenol form of its structure. Phenol structure of antioxidant activity is exhibited through the link to ferritin and reduction of proxyl and alkoxy radicals (20,21).

Singorelli et al. (2006) reported a significant difference between oxidative stress markers including 4-hydroxy-2-nonenal (4-HNE), MDA, and oxidized lipoprotein among fertile and menopausal women. Moreover, menopausal accompanied the significant decrease of Glutathione peroxidase Antioxidant Enzyme activity (GSH – PX) (22). Signorelli et al. (2001) reported a significant relationship between menopausal period and high MDA level (23).

In the research of Leal (2009) on menopausal women, the reduction of antioxidant capacity, the decrease of sulfhydryl groups and the increase of serum peroxidase activity were reported as the indices of oxidative stress (24).

Researchers suggest that oxidative stress of brain plays an important role in depression and anxiety pathogens in humans and animal models (25,26). Recently, with regard to the role of oxidative stress in the development of anxiety disorder, antioxidant compounds have been suggested as a new solution to the problem. In a study on different doses of F. vulgare extract, estradiol and diazepam caused a significant decrease in MDA and a significant increase in serum and brain antioxidant capacity. Since the estradiol antioxidant effects were reported in previous studies (21). Koppula et al. (2011) reported that F. vulgare extract could decrease lipid peroxidation of liver and brain in stressful rats (27). F. vulgare contains phenol, flavonoid, and phenolic acid compounds. F. vulgare phenolic acids include chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid, and rosmarinic acid. Flavonoids include eriositrin, quercetin, isoquercetin, rutin, hyperacid, nicotiflorin and kaempferol (10).

In the study of Moara et al (2005), anethole and fenchone were found as compounds of F. vulgare extract by gas chromatography (7). Phenolic and flavonoid compounds are natural antioxidants that exist in many fruits and vegetables. These compounds affect the control of the nervous system and decrease the levels of oxidative stress markers, therefore improving the behaviors related to anxiety and stress (28). One study on quercetin demonstrated that the compound could improve anxiety-like and depression-like behaviors due to chronic stress by reducing oxidative stress parameters (29). In the study of Machawal et al. (2011), rutin could cause the anxiety behaviors in rats without kinetic stress; besides that, the researchers reported the effectiveness of rutin on the reduction of oxidative-nitrative stress parameters (28). Moreover, studies have demonstrated the antianxiety and anti-depressant activity of chlorogenic acid and rosmarinic acid (30,31).

Conclusion:

The present study of the treatment of ovariectemized mice with F. vulgare extract demonstrated the improvement of anxiety-like behaviors in the EPM. In ovariectemized mice, the increase of brain and serum MDA and the reduction of brain and serum antioxidant capacity were seen. The treatment with different doses of F. vulgare extract causes the reduction of MDA and the enforcement of antioxidant capacity. It is therefore possible that F. vulgare extract improves the anxiety-like behaviors due to the menopause through the reduction of oxidative stress parameters. This argument, however, deserves further study.

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