Effects of Pistachio Oil on Anxiety-Like Behavior in Ovariectomized Rats

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Abstract

Background: Menopause reduces sexual hormones in female rats and is accompanied by depression and emotional disorders. Flavonoids of pistachios have anxiolytic, sedative, and anticonvulsant properties.

Objectives: The current study aims to investigate effects of pistachio oil on behavioral disorders of ovariectomized rats.

Material and Methods: As many as 35 female Wistar rats (150-200g) were randomly divided into five groups (n=7 in each group). In the sham group, the animals received standard drinking water after surgery. In the OVX.C group, the ovariectomized rats received standard drinking water. The ovariectomized rats in the OVX.P group received Premarin (64.5µg/kg) for 28 days. In the h1 300 and h1 1200 groups, the ovariectomized rats received 300 and 1200mg/kg/day of pistachio oil orally for 28 days, respectively. Besides, anxiety was evaluated by the elevated plus-maze (EPM) and open field tests (OFT).

Results: According to the results, oral administration of pistachios significantly increased the percentage of the open arm entries (OAE) test approximately three folds in the h1 300 and hi 1200 groups ($P < 0.05$). In addition, the percentage of the OAE test was raised in the OVX.P group compared to the OVX.C group ($P < 0.05$). Besides, the percentage of the open arm time was higher in the OVX.C group than in the sham group ($P < 0.01$). Anxiety-line behaviors in the ovariectomized rats showed a smaller inner time parameter as observed in the OVX.C group compared to other groups. Besides, it was reduced significantly compared to the sham group ($P < 0.01$). Furthermore, there were no significant differences in the velocity parameter in all treated groups compared to the sham and OVX.C groups.

Conclusions: The results of this study implied that pistachios had protective impacts on anxiety-like behaviors in ovariectomized rats; therefore, pistachio oil could be used as an effective remedy for alleviating anxiety in menopausal women.

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1. Introduction

Menopause is a biologic and physiologic stage in women's life, which is defined as natural termination of menstruation that commonly happens from the age of 45 to 60 [1]. Physiologically, it is associated with a reduction in the level of estrogen. Depression, stress, hot flashes, sleep problems, headaches, mood changes, and hormonal defects are the main psychological symptoms of menopause. These defects can affect the quality and major aspects of women's life [2].

Hormonal or non-hormonal methods are used to reduce menopause symptoms. Hormonal therapy is beneficial for menopause. However, these methods are used in breast cancer and have the risk of thromboembolic disease [3]. Complementary medicine is one of the main non-hormonal approaches to eliminating and reducing these symptoms. Research shows that women aged 40 or older are the greatest receivers of complementary therapies [4]. Herbal medicine is one of the most popular methods in complementary medicine. In the United States and the United Kingdom, 80% of postmenopausal women use herbal remedies. Besides, 60 to 70% of them believe that these supplements solve their problems and are safe with no side effects [5]. The World Health Organization proposed complementary and alternative medicines as therapeutic methods for improving menopausal symptoms and changing women's lifestyle during this period [6].

Pistachio oil contains compounds that chemically include saturated fatty acids, such as myristic, palmitic and stearic acids, as well as unsaturated acids, such as linoleic and oleic, plant sterols, and elements, such as selenium, zinc, calcium, potassium, iron, and magnesium [7]. Research shows that compounds in pistachio oil inhibit production of nitric oxide (NO) [8, 9]. Since this compound is able to inhibit steroidogenesis, pistachios and their oil were used in the past as a medicine to treat sex-related diseases, such as improving sexual potency. On the other hand, plants containing linoleic acid have been reported to have been used in treating impotence [10]. Unsaturated fatty acids can inhibit the function of the enzyme 5-alpha-reductase. On the other side, saturated fatty acids increase the cholesterol level in the human body [11]. Evidence shows effects of phytosterols on the sexual cycle and sex hormones [12]. Beta-sitosterol is the most common phytosterol in nuts. Pistachios have been reported to contain high levels of beta-sitosterol [13]. Rostam pour *et al* (2016) reported that the dose of 10mg/kg of the pistachio hydroalcoholic extract reduced anxiety in female rats [14]. Saeb *et al* (2008) reported that female rats were given 5, 10, and 20% of wild pistachio oil daily for 60 days. The results of their study showed that with an increase in the percentage of pistachio oil, the serum level of leptin decreased [15].

This study aims to investigate effects of pistachio oil on menopausal complications, such as anxiety and spatial memory in ovariectomized rats.

2. Materials and Methods

2.1. Plant material collection and oil extraction

Fresh fruits of Akbari pistachios were collected from Rafsanjan, Kerman province, Iran. Confirmation of the species with voucher specimens was approved by an expert in the Department of Botany, Vali-e-Asr University of Rafsanjan, Iran (Genetic code: M30). Different parts of the fruits (the green hull and the kernel) were separated, and oil was extracted using the cold press method after drying. The oil was filtered and centrifuged at 5000 rpm for 10 min; next, it was stored in a refrigerator [16].

2.2. Animals and ethics

A total of 35 female Wistar rats (150-200g) were kept under standard laboratory conditions of a 12-hour light/dark cycle, temperature of 22 ± 2 °C, and humidity of 55 ± 5%. Animals had free access to food and water, with each of them used only once during the study. Experiments were performed at the same time of the day (11 to 12am). The animals were cared and treated according to Institutional Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised in 1978) upon the approval of Research and Medical Ethics Committees of Rafsanjan University under ethics number IR.RUMS.REC.1398.032.

2.3. Ovariectomy surgery protocol

After a two-week acclimation period, the animals were anesthetized by ketamine/xylazine (60/4mg/kg) intraperitoneally, and the ovaries were removed completely by bilateral incision through the skin [17]. The rats received penicillin (22,000IU/kg) for two days after ovariectomy. To confirm menopausal induction, we observed vaginal smears three to eight days after surgery using a light microscope. Besides, the animals entered our study according to the vaginal cell type. We used animals that no epithelial cells in their vaginal smear [18]. The rats in the sham group underwent the same procedure without removal of their ovaries. The animals were weighed every seven days to the end of the study.

2.4. Experimental design

The female rats were randomly divided into five groups (n=7 in each group). The sham group included animals that received standard drinking water (0.25ml/kg) after surgery for 28 days. In the OVX.C group, the ovariectomized rats received standard drinking water (0.25 ml/kg) for 28 days. The OVX.P group included the ovariectomized rats that received Premarin (64.5µg/kg) orally for 28 days. In addition, the h1 300 group included the ovariectomized rats that received 300mg/kg/day of pistachio oil orally for 28 days. On the other side, the h1 1200 group included ovariectomized rats that received 1200 mg/kg/day of pistachio oil orally for 28 days. Besides, 24h after the last application, behavioral tests were performed at the same time of the day.

2.5. Elevated plus maze (EPM) test

The EPM test was performed to examine anxiety-like behaviors in rodents. The method was highly similar to our previous research [19]. The maze included two opened arms (50×10cm) and two closed arms (50×10×40cm), with each animal having been placed at the center of the apparatus facing an opened arm for 5 min. The percentages of opened arm entries (OAE%) and the opened arm time (OAT%) were reported as standard indices of anxiety-like behaviors. A significant reduction in OAE% and/or OAT% would indicate an increase in the level of anxiety. Total arm entries were reported as an index of locomotor activity [20].

2.6. Open field test (OFT)

The OFT was used to examine anxiety-like behaviors and locomotor activity in rodents. The animals were placed individually in a 50 × 50 × 50cm white box for a 5-min period. The bottom surface of the box was divided into two inner and outer zones. The rats' activity in the box was digitally monitored and analyzed using Ethovision software (version 7.1, Noldus Information Technology, Ethovision, Wageningen, the Netherlands). At the beginning of the session, each rat was placed at the center of the arena, with its activity recorded for 5 min. Next, behavioral parameters, including the total distance moved (cm), velocity (cm/sec), total duration in the inner zone (sec), and total duration in the outer zone (sec) were scored. At the end of each session, the rats were removed from the open field, and the experimental chamber was thoroughly cleaned with diluted ethanol (10%) and dried.

2.7. Statistical analysis

Data analysis was performed using the data analysis program of Graphpad Prism, version 6 (Graphpad Software, San Diego, CA, USA). Besides, one-way analysis of variance (ANOVA) was used followed by the Tukey's multiple comparison test. The results were expressed as the mean ± standard error (SEM), with p-value less than 0.05 considered statistically significant.

3. Results

According to the results, there was a heavier weight in the OVX.C group than in other experimental groups after 28 days. Besides, the weight in other experimental groups did not decrease significantly compared to the control group $p > 0.05$ (Fig. 1). The percentage of the OAE test significantly increased approximately three folds in the h1 300 and h1 1200 groups ($P < 0.05$). Besides, the percentage of the OAE test was higher in the OVX.P group than in the OVX.C group after 28 days ($P < 0.05$). In addition, it was demonstrated that the percentage of the OAE test decreased in the OVX.C group compared to the sham group ($P < 0.05$) (Fig. 2).

The percentage of OAT was higher in the OVX.C group than in the sham group ($P < 0.01$) (Fig. 3). In addition, the percentage of OAT showed a remarkable increase in the OVX.P group after Premarin administration for 28 days ($P < 0.001$, Fig. 3). OAT% was

approximately 5 folds in the h1 1200 group, yet there were no significant changes in the h1 300 and OVX.C groups (Fig. 3). Besides, there were no significant changes in the distance moved and the total arm entries among all groups after oral administration of pistachios in menopausal rats (Figs. 4 and 5).

The open field test in ovariectomized rats showed a smaller inner time parameter in the OVX.C group than in other groups, which significantly decreased compared to the sham group ($P < 0.01$, Fig. 6). Furthermore, there were no significant differences in the velocity parameter in all treated groups compared to the sham and OVX.C groups (Fig. 7).

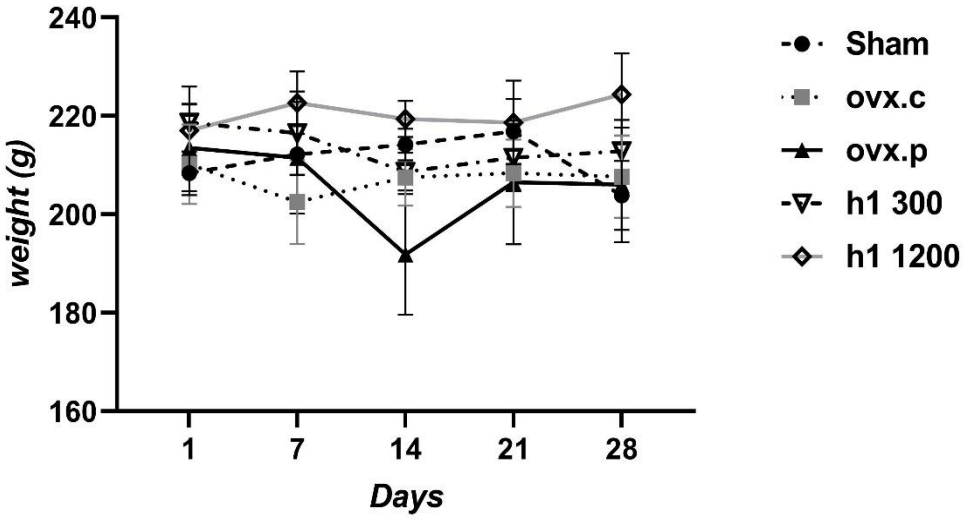

Fig. 1. Weight alterations in ovariectomized rats; Sham group: rats that received standard drinking water after surgery; OVX.C group: ovariectomized rats that received standard drinking water; OVX.P group: ovariectomized rats that received Permarin (64.5µg/kg), h1 300 group: ovariectomized rats that received 300mg/kg/day of pistachio oil orally; h1 1200 group: ovariectomized rats that received 1200mg/kg/day of pistachio oil orally for 28 days. The data are presented as Mean ± SEM.

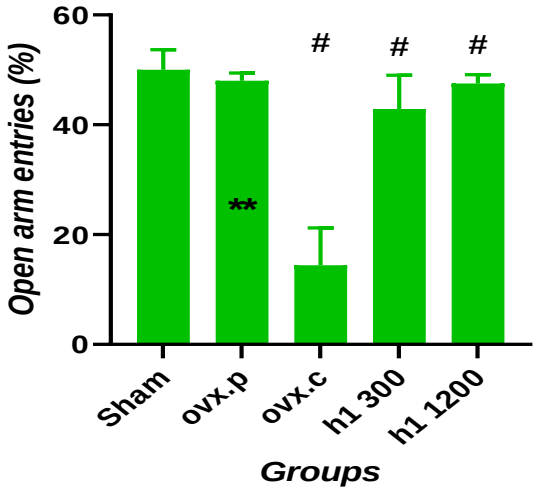

Fig. 2. Comparison of the open arm entries test in ovariectomized rats; Sham group: rats that received standard drinking water after surgery; OVX.C group: ovariectomized rats that received standard drinking water; OVX.P group: ovariectomized rats that received Permarin (64.5µg/kg); h1 300 group: ovariectomized rats that received 300 mg/kg/day of pistachio oil orally; h1 1200 group: ovariectomized rats that received 1200 mg/kg/day of pistachio oil orally for 28 days. The data are presented as Mean ± SEM. Analyses were considered statistically significant at **P < 0.01 compared to the sham group and at # P < 0.05 compared to the OVX.C group.

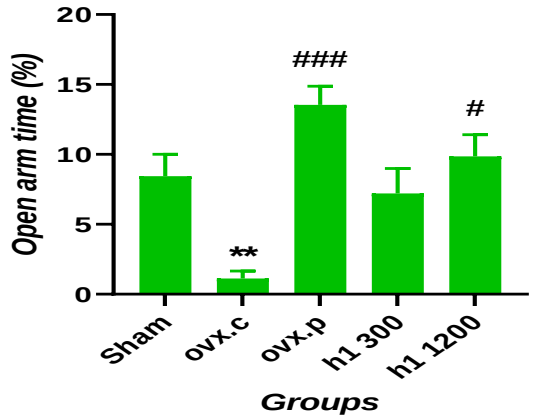

Fig. 3. Comparison of the open arm entries test in ovariectomized rats; Sham group: rats that received standard drinking water after surgery; OVX.C: ovariectomized rats that received standard drinking water; OVX.P: ovariectomized rats that received Permarin (64.5µg/kg); h1 300 group: ovariectomized rats that received 300mg/kg/day of pistachio oil orally; h1 1200 group: ovariectomized rats that received 1200mg/kg/day of pistachio oil orally for 28 days. The data are presented as Mean ± SEM. Analyses were considered statistically significant at **P < 0.01 compared to the sham group, # P < 0.05, and ### P < 0.001 compared to the OVX.C group.

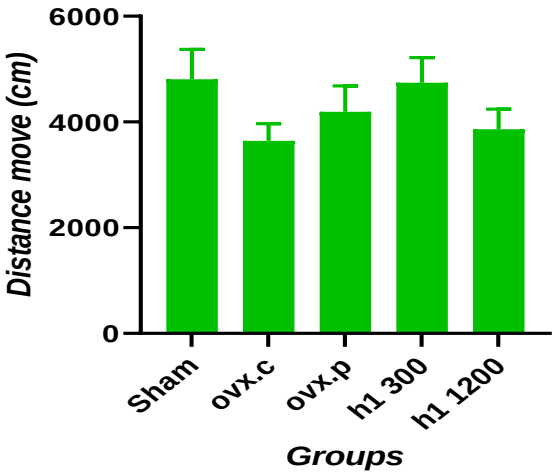

Fig. 4. Alternation of the open arm entries test in the ovariectomized rats; Sham group: rats that received standard drinking water after surgery; OVX.C: ovariectomized rats that received standard drinking water; OVX.P: ovariectomized rats that received Permarin (64.5µg/kg); h1 300 group: ovariectomized rats that received 300mg/kg/day of pistachio oil orally; h1 1200 group: ovariectomized rats that received 1200mg/kg/day of pistachio oil orally for 28 days. The data are presented as Mean ± SEM.

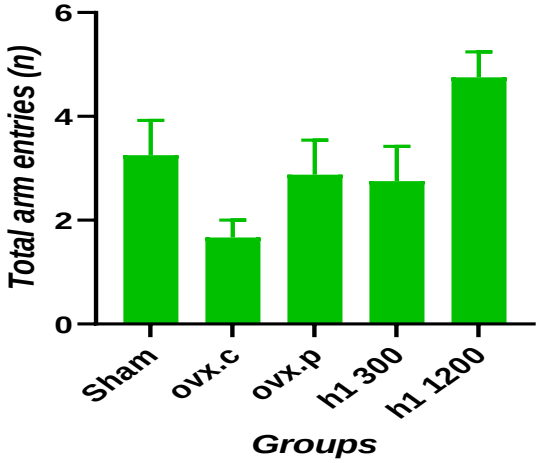

Fig. 5. Comparison of the total arm entries test in ovariectomized rats; Sham group: rats that received standard drinking water after surgery; OVX.C: ovariectomized rats that received standard drinking water; OVX.P: ovariectomized rats that received Permarin (64.5 µg/kg); h1 300 group: ovariectomized rats that received 300mg/kg/day of pistachio oil orally; h1 1200 group: ovariectomized rats that received 1200 mg/kg/day of pistachio oil orally for 28 days. The data are presented as Mean ± SEM.

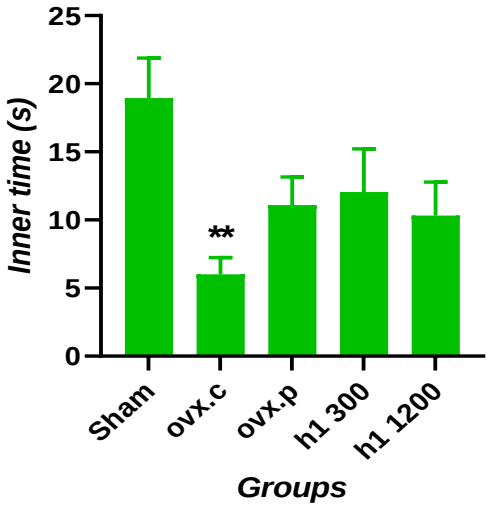

Fig. 6. Effects of oral pistachio administration on the inner time parameter of anxiety-like behaviors in ovariectomized rats; Sham group: rats that received standard drinking water after surgery; OVX.C: ovariectomized rats that received standard drinking water; OVX.P: ovariectomized rats that received Permarin (64.5µg/kg); h1 300 group: ovariectomized rats that received 300mg/kg/day of pistachio oil orally; h1 1200 group: ovariectomized rats that received 1200mg/kg/day of pistachio oil orally for 28 days. The data are presented as Mean ± SEM. Analyses were considered statistically significant at **P < 0.01 compared to the sham group.

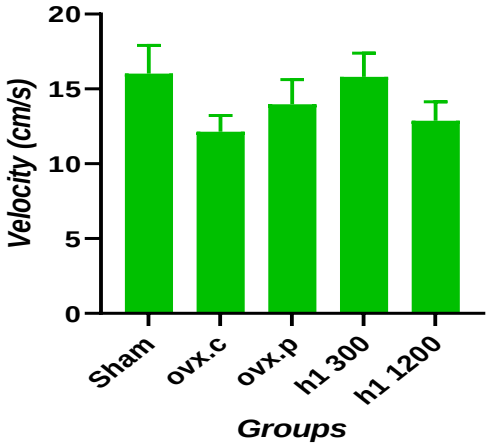

Fig. 7. Comparison of the velocity parameter in ovariectomized rats; Sham group: rats that received standard drinking water after surgery; OVX.C: ovariectomized rats that received standard drinking water; OVX.P: ovariectomized rats that received Permarin (64.5µg/kg); h1 300 group: ovariectomized rats that

received 300mg/kg/day of pistachio oil orally; h1 1200 group: ovariectomized rats that received 1200mg/kg/day of pistachio oil orally for 28 days. The data are presented as Mean ± SEM.

4. Discussion

Estrogens have various pharmacological properties, such as neuro-protective and neurotrophic properties [21]. Decreased levels of estrogen in menopausal periods induced different psychological abnormalities, such as anxiety [22]. Similarly, our results demonstrated that induction of ovarectomy in female rats led to anxiety in EPM and OFT as standard experimental tests for evaluation of anxiety-like behaviors in rodents. Different groups showed no impairments in the locomotor function. Moreover, Premarin (64.5µg/kg for 28 days), being a kind of estrogen, reduced anxiety-like behaviors.

Treatment of OVX animals with pistachio oil (especially 1200mg/kg/day for 28 days) reduced anxiety-like behaviors in ovariectomized animals. Anxiolytic effects of pistachios have been shown under different conditions. Hakimizadeh *et al* demonstrated that the hydroalcoholic extract of pistachios reduced anxiety in ovariectomized rats [23]. Moreover, Mohammadi-Nasab *et al* demonstrated that pistachio oil decreased anxiety-like behaviors in rats with polycystic ovary syndrome [24].

On the other hand, *Pistacia* species contain various active constituents, such as different kinds of phytosterols and flavonoids, lutein, β-carotene, vitamin E, β-sitosterol, stigmasterol, and campesterol [25-27]. These active constituents are the main causes of pharmacological properties in pistachios, including neuroprotective, anti-inflammatory, and antioxidant effects [9]. It is well established that these compounds exert anxiolytic effects under normal and pathological conditions through the mentioned properties. For example, vitamin E reduces anxiety-like behaviors in an animal model of autism [28] and zebrafish with high caffeine intake [29]. Moreover, it has been shown that lutein supplementation reduces anxiety in young adults [30]. Furthermore, phytosterols can interact with estrogen receptors, such as intrinsic estrogens and induce anxiolytic effects [31-33]. In another study, anxiolytic effects of the hydroalcoholic extract of pistachios were exhibited in an experimental model of anxiety, which were antagonized by tamoxifen through blockade of estrogen receptors [34].

5. Conclusions

To conclude, the results of this study demonstrated that pistachio oil reduced anxiety-like behaviors in ovariectomized rats without significant effects on the animals' weight; therefore, pistachio oil could be used as a promising remedy for reducing anxiety in menopausal women.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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