The Effect of Hydroalcoholic Extract of Pistachio on Anxiety and Working Memory in Ovariectomized Female Rats

Elham Hakimizadeh (PhD)[1], Iman Fatemi (PhD)[2], Mohammad Allahtavakoli (PhD)[3*]

[1] *Assistant Prof. of Physiology, Physiology-Pharmacology Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

[2] *Assistant Prof. of Pharmacology, Research Centre of Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, Iran,*

[3] *Professor of Physiology, Physiology-Pharmacology Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

Information

Article Type:

Original Article

Article History:

Received: 06.09.2020
Accepted: 13.12.2020

DOI:10.22123/phj.2021.270525.1080

Keywords:

Rat
Ovariectomy
Anxiety
Working Memory
Pistacia Vera

Corresponding Author:

Mohammad Allahtavakoli

Email: allahtavakoli@gmail.com

Tel: +98-343-31315091

Abstract

Introduction: Pistachio has various biological properties, such as antioxidant, anti-inflammatory, and antimicrobial, due to its flavonoids and phenolic compounds. On the other hand, ovarian hormones such as estrogen have a neuroprotective effect, in the absence of which consequences, including inflammation, anxiety, learning, and memory disorders, are more severe. This study aims to evaluate the beneficial therapeutic effects of pistachio extract in ovariectomized rats.

Materials and Methods: In the present study, 5 groups of female rats weighing 300-250 g were used. The groups included control, ovariectomy (OVX), ovariectomy+ DMSO, ovariectomy +10 mg/kg pistachio extract, and ovariectomy+100 mg/kg pistachio extract. In order to prepare the required extract, pistachio nuts were powdered (100 g) and macerated in 1 L of ethanol (80%) for 72 h. Pistachio extract was used orally once a day in ovariectomized rats for sixty days. Anxiety and working memory were evaluated by the Elevated plus-maze (EPM) and Y-maze tests.

Results: The results showed that pistachio extract (more potentially at the dose of 100 mg/kg) decreased anxiety-like behaviors and increased working memory in the ovariectomized rats.

Conclusion: The findings of the current investigation suggest that pistachio extract could be used as a potential strategy for the attenuation of ovariectomy-related manifestation.

►Please cite this article as follows:

Hakimizadeh E, Fatemi I, Allahtavakoli M. The effect of hydroalcoholic extract of pistachio on anxiety and working memory in ovariectomized female rats. Pistachio and Health Journal. 2020; 3 (3): 72-83.

1. Introduction

Menopause is defined as the termination of ovarian activity and reduced sex hormones in women (1, 2). It is associated with depression (3), emotional, mood, and cognitive process disorders (4). Menopausal women suffer from low bone density, decreased muscle mass, sensitive breasts, flushing, cognitive impairment, and anxiety (5, 6). Several experimental studies in rodents and humans have suggested that ovarian hormones affect learning and memory function, including working memory (7, 8). One study showed that induction of ovariectomy could reduce learning and memory performance due to a deficiency of gonadal hormones (9). Estrogen is needed to maintain learning and memory performance due to its neuroprotective, antioxidant, and neurotrophic properties (10).

Moreover, a strong association of decreased estrogen levels with schizophrenia and Alzheimer's has been reported (11, 12). Estrogen replacement therapy is effective in reducing menopausal symptoms (13). However, this therapy benefits in postmenopausal women are usually influenced by serious estrogen side effects such as breast and endometrial cancers (14).

Common pistachio (available on the market), with the scientific name pistacia vera, belongs to Anacardiaceae. The diverse variety of pistachios are generally wild and drought tolerant. It is the only Iranian pistachio with different cultivars varying in shape, color, appearance, size, dimensions, and kernel characteristics (15). Pistachios contain various chemical compounds, including beta-carotene (β-carotene), alpha-tocopherol (α-tocopherol), and lutein (16). Previous studies have also reported that pistachios contain phenolic compounds and triterpenoids (17). Furthermore, pistachios have pharmacological effects, including anti-hyperlipidemia, analgesic, and anti-inflammatory. Pistachio flavonoids also have anti-anxiety, sedative, and anticonvulsant properties (18).

Goli et al. (2005) showed that pistachio green skin contains substantial amounts of phenolic compounds compared to other nuts (19). These compounds have been reported to have antioxidant properties (20). Pistachios have also been shown to have protective effects against oxidative damage to rats' cerebral ischemia (21).

Since pistachios have estrogen-like properties, including antioxidant and anti-inflammatory, they can effectively attenuate complications associated with ovariectomized rats. Given the behavioral manifestations associated with ovariectomy and pistachios' effects on behavioral disorders, it is hypothesized that pistachios might have beneficial effects on behavioral parameters in

ovariectomized rats. As pistachio is rich in antioxidants and has known anti-inflammatory and anti-free radical effects, this study aims to investigate its beneficial therapeutic effects in ovariectomized rats.

2. Materials and Methods

2.1. Laboratory animals and grouping:

In this experimental study, female Wistar rats weighing 200-200 g were used. The animals were kept in separate cages at 22-24 °C with the light cycle for 12 hours of light and 12 hours of darkness. Adequate food and water were provided for the animal throughout the storage period, except during the experiments. In this study, animals were randomly selected and divided into 5 groups of 10 rats each as group 1: control (healthy animals without any laboratory intervention), group 2: ovx, group 3: ovx+ DMSO (animals that received DMSO 10% via gavage once a day for 2 months after induction of ovariectomy (22)), group 4: ovx+ extract10 (animals that received 10 mg/kg of pistachio extract once a day for 2 months after induction of ovariectomy), group 5: ovx+ extract100 (animals that received 100 mg/kg of pistachio extract via gavage once a day for 2 months after induction of ovariectomy). Doses of pistachios were selected based on previous studies (23).

2.2. Pistachio extraction method

In the present study, the kernels of Akbari variety (genetic code: M30), a native of Rafsanjan, were used. The variety was approved with the help of Valiasr University of Rafsanjan experts. After collecting, pistachios were mixed with 300 grams of crushed pistachio powder plus 900 ml of 80% methanol; then, the mixture was kept in an incubator at 80 °C for 12 hours. The mixed residues were extracted three times in the same way. The obtained extracts were mixed and separated by a solvent rotary apparatus. The plant extract was dissolved in 10% (DMSO, Sigma-Aldrich, Germany) and administrated by gavage to animals.

2.3. Ovariectomy (induction of menopause)

To perform an ovariectomy, the animal was first weighed, and 0.1 mg of ketamine anesthetic was injected intraperitoneally for every 100 grams of animal weight. As anesthesia was completed, the animal's abdomen was shaved, and then the surgical site was sterilized. Between the 2nd and 3rd breasts, the mouse's cleft was dissected, and the fallopian tube in the ovary was burned with a cochlear device. Afterward, the ovary (follicular and red tissue connected to the oviduct) was slowly removed. The inner and outer layers were then sutured separately; finally, for every 100 grams of animal

weight, 0.3 mg of penicillin was injected into the mouse thigh muscle, and the animal was returned to the cage to regain consciousness (27).

2.4. Proper evaluation method of ovariectomy

After 3 days, a smear of the animal's vagina was taken for six days (a few drops of normal saline was added to the rat's vagina with a bulb, then was taken out, placed on a slide, and spread in a smear state); a microscope observed it. Ovariectomy is considered successful if the fern pattern and horn cells are not seen in the spread of the smear (27).

2.5. Behavioral tests

2.5.1. The elevated plus-maze (EPM) test

To measure anxiety, a plus-maze behavioral model was used (24). This evaluation was based on a model first proposed by Pellow et al. The tool is made of wood with four arms in the form of a positive sign (+). The dimensions of the open and closed corridor are 100 x5 cm; the sides and the end of the latter have a wall with a height of 40 cm installed on both sides. The end of the open corridor with a 1 cm high is made of glass to prevent rats from falling. The four corridors lead to a central area measuring 40 x30 x 15 cm. In this study, the maze was placed 50 cm above the ground. The rats were placed inside the central area of the maze, facing an open corridor. Proper lighting was provided by a 100-watt bulb 120 cm above the maze center. During the 5 minutes that the animal was moving freely in different parts of the maze, the number of times the animal entered the open corridor, the number of times the animal entered the closed corridor, and the length of time the animal remained in the corridors were measured by video recording.

Entering an open or closed corridor occurred when the animal's four legs were in the corridor. The time spent in these corridors was calculated accordingly. For each animal, the percentage of entry into the open corridor and the time spent there were calculated as follows:

The percentage of open arm entries (% OAE): [(the number of open arm entries/the number of entries of open and closed arms) × 100])

The percentage of open arm time (% OAT): [(time spent in open arms/time spent in open and closed arms) × 100]

A significant increase in each of the two above parameters indicates a decrease in anxiety. However, the OAE% factor is less sensitive in recording animal anxiety or anti-anxiety behavior than the OAT% factor (25).

2.5.2. Behavioral testing (Y-maze)

To evaluate working memory, the Y-maze spontaneous alternation test was used. In this test, animals' performance in terms of working memory was examined by observing and measuring their

spontaneous periodic behavior in a working session. The maze related to this test is made of Plexiglas with dimensions of 40 × 30 × 15 cm on each arm. The arms are connected through a central area. To perform the test, each rat was placed at the end of one arm with free access to all maze areas over 8 minutes. The number of times the animal entered each arm was recorded by observation. The animal entered an arm when its hind legs were entirely inside the arm. Periodic behavior was considered as successful and consecutive entrances into all arms in ternary sets. Thus, the rotation percentage was calculated from the observed rotation ratio to the maximum rotation (total number of arms inserted-2) × 100 (26).

% Correct alternation= [number of alternations / (total number of arm entries – 2)] × 100

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6. The results were expressed as SEM± mean. Normalization of data was performed using the Shapiro-Wilk test. The groups' differences were recognized using a one-way ANOVA test followed by Tukey, Kruskal-Wallis, and Dunn's tests. P<0.05 was considered statistically significant.

3. Results

3.1. Anxiety-like behavioral test (elevated plus-maze)

The results of the elevated plus-maze (EPM) are shown in Figure 1. Ovariectomy decreased the percentage of time spent in the open arm compared to the control group (P< 0.01), and pistachio extract at doses of 10 and 100 mg/kg increased the percentage of time spent in the open arm (P< 0.05 and P< 0.01).

Ovariectomy also decreased the percentage of open arm entry P< 0.05, and pistachio extract in both doses caused an increase in the percentage of open arm entry in ovariectomized rats compared to the ovariectomy group (P< 0.05 and P< 0.01) (Figure. 1 (A and B).

3.2. Behavioral test (Y-maze)

The results of the Y-shaped maze are shown in Figure. 2. The percentage of correct rotations in the ovariectomy group decreased significantly compared to the control group (P< 0.05). However, pistachio extract at doses of 10 and 100 mg/ kg increased this index in ovariectomized rats (P< 0.05 and P< 0.01).

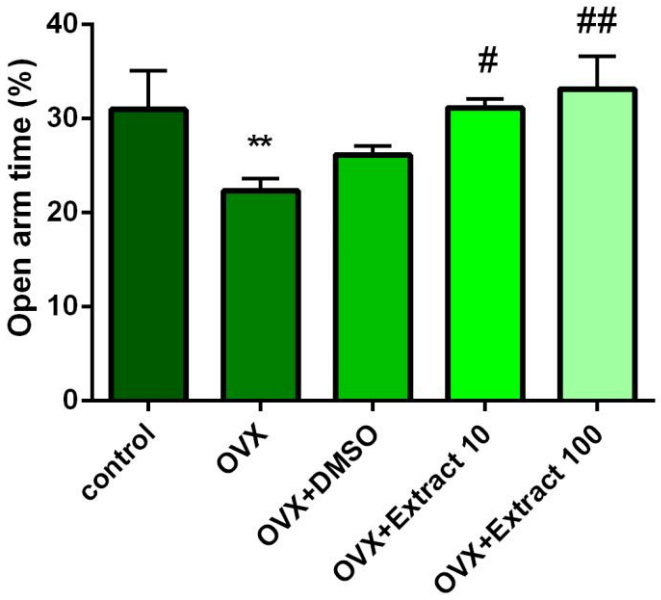

Fig. 1A- The effect of pistachio extract (10 and 100 mg/kg) on the open arm time in ovariectomized mice. Data were presented as SEM± mean. **P< 0.01 compared with the control group; #P< 0.05 and ##P< 0.01 compared to the ovariectomy group.

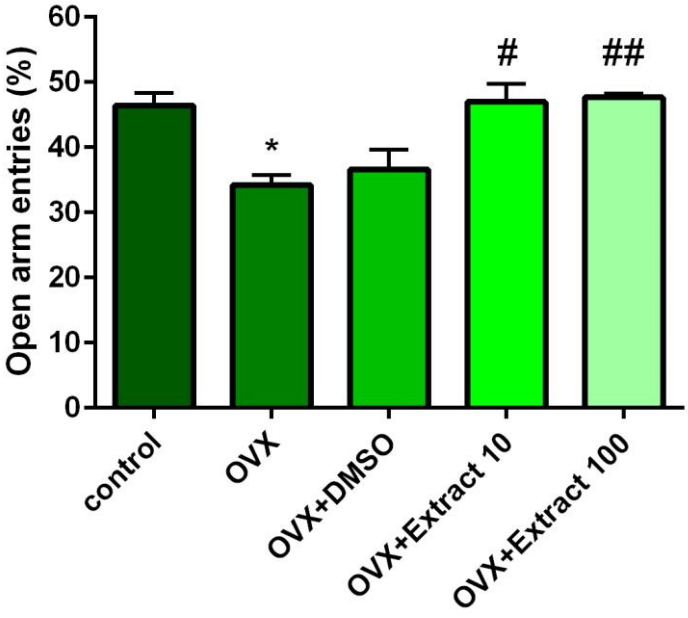

Fig. 1B- The effect of pistachio extract (10 and 100 mg/kg) on the open arm entries in ovariectomized mice. Data were presented as SEM± mean. *P< 0.05 compared with the control group; #P< 0.05 and ##P < 0.01 compared to the ovariectomy group.

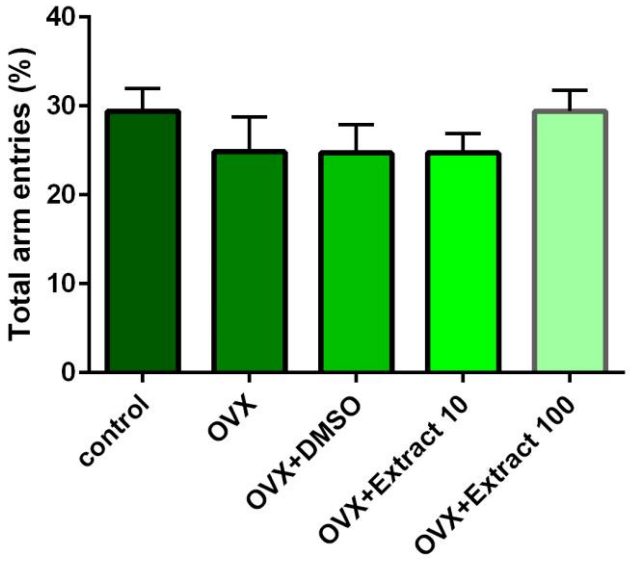

Fig. 1C- The effect of pistachio extract (10 and 100 mg/kg) on the total arm entries in ovariectomized mice. Data were presented as SEM± mean

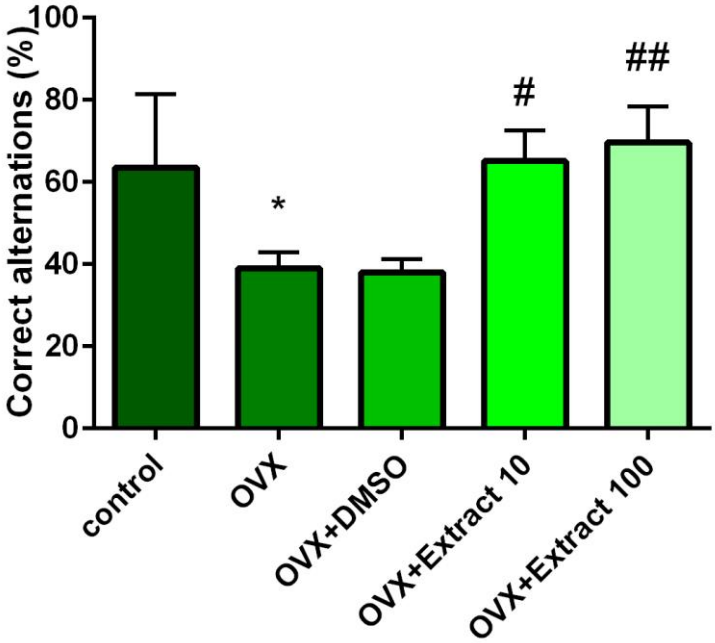

Fig. 2- The effect of pistachio extract (10 and 100 mg/kg) on the percentage of correct rotations in ovariectomized rats (number of animals in each group:n= 10). Data were presented as SEM± mean. *P< 0.05 compared to the control group; #P < 0.05 and ##P < 0.01compared with the ovariectomy group.

4. Discussion

The present study results show that treatment with pistachio extract, in addition to increasing working memory, significantly reduces anxiety-like behaviors. In postmenopausal women, anxiety is one of the most common disorders associated with decreased life satisfaction and dysfunction. Previous reports have shown that animals without ovaries show mood disorders similar to postmenopausal women (27). Moreover, the relationship of age with memory and anxiety has been discussed, indicating that memory's ability decreases with age due to the reduction in the frontal lobe contribution to the initial coding of experience (28).

Anxiety is considered a natural feeling and an adaptive component of the acute stress response that threatens individual cohesion (29). Several factors, including genetics, gender, and hormone levels, cause anxiety (30). According to one study, ovariectomized rats exhibit more anxiety-like behaviors than healthy or treated ones with a specific estrogen dose (31). Ovarian hormones can affect areas of the brain, such as memory and learning. In the meantime, estrogen is essential with its protective role against the decline of cognitive functions that occurs with natural aging. It can also facilitate or increase working memory function (5).

Estrogen has various pre-treatment and post-treatment effects due to its neuroprotective and neurotrophic properties (32, 33). Decreased estrogen by surgical intervention (ovariectomy), pharmacological factors (estrogen receptor antagonists), or aging (reproductive aging) eliminates such a protective effect (34).

Pistachios have significant antioxidant effects due to the presence of anthocyanins, flavonoids, phytosterols, and luteolin (35). Furthermore, they have a high content of composterol, stigmasterol, and β-cytosterol, indicating pistachios potent antioxidant effects (36). Clinical studies have shown that pistachio consumption increases lutein, beta-carotene, and vitamin E (37).

In line with previous studies, the present research results show that anxiety-like behavior increases in ovariectomized animals. The results also suggest that pistachio extract (10 and 100 mg/kg) significantly reduces anxiety-like behavior in ovariectomized rats. However, the dose of 100 mg/kg of pistachio is more effective.

Pilsakova et al. (2010) showed pistachio extract to have anti-anxiety effects based on phytoestrogens' physiological function (38). In 2016, Rostampour et al. reported that the pistachio hydroalcoholic extract had protective effects in a quasi-anxiety experimental model (39). Decreased sex

hormones have been proved to affect cognitive function during menopause (40). Also, previous reports have suggested that ovariectomy reduces learning and memory in rodents (41). In line with previous studies, the current work shows that ovariectomy impairs working memory in rats, and pistachio extract improves this index. Hence, pistachios probably improve cognitive function through their antioxidant properties. Recently, medicinal plants that enhance memory function have attracted the attention of many researchers. Erythrina falcata appears to increase memory and improve spontaneous fear (42). In another study, Sahak et al. showed that Nigella sativa could improve learning and memory in healthy animals and humans (43).

5. Conclusion

This study shows that pistachios have protective effects on anxiety-like behaviors and working memory in ovariectomized rats. Therefore, pistachios can be a potential herbal supplement for relieving anxiety during menopause.

Conflict of Interest

The authors of this article have no conflict of interest.

Acknowledgments

This study was financially supported by the Vice Chancellery for Research and Technology of Rafsanjan University of Medical Sciences (NO: 9/31/3565).

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