The effects of pistachio hydroalcoholic extract on stroke in permanent middle cerebral artery occlusion in ovariectomized female rats

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Abstract

Background: Phytoestrogens, found in abundance in the extracts of oilseeds such as pistachios, have estrogen-like and anti-inflammatory effects. This study investigated the effects of pistachio kernel extract on stroke in ovariectomized rats.

Materials and Methods: This study was conducted on four groups of female rats weighing 200-250 g. The groups included the control group and three groups treated with pistachio hydroalcoholic extracts (100, 250, and 500 mg/kg) (n = 10 per group). One week after ovariectomy and its confirmation (via vaginal smear examination), the extracts were gavaged for four weeks and at the end of the fourth week, stroke was induced by closing the middle cerebral artery. Infarct volume and cerebral edema were measured 72 hours after brain extraction and staining with tetrazolium chloride. Neurological disorders were measured using the Bederson ranking system and sensory-motor disorders were measured via two behavioral tests including the sticky test and hanging test (hanging the animal from a thin wire string). Furthermore, cerebral edema was measured by determining the percentage of water in the affected hemisphere compared to the intact hemisphere.

Results: Pistachio extract (especially at a dose of 500 mg/kg) reduced the risk of infarct volume ($p < 0.0001$) and cerebral edema ($p < 0.0001$) in the treatment groups compared to the animals in the control group. Pistachio extract also improved neurological disorders and sensorimotor function.

Conclusion: According to its anti-inflammatory, antimicrobial, antioxidant, and estrogen-like properties, pistachio extract improves the stroke complications in ovariectomized female rats.

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1. Introduction

Stroke has been a major cause of death and disability worldwide [1]. Several mechanisms including inflammation, oxidative damage, cerebral edema, programmed cell death, and excitatory toxicity are responsible for brain damage after stroke [2]. Inflammation that begins after an ischemic attack and lasts for several days contributes to neuronal cell death after stroke [3]. The inflammatory response resulting from the infiltration of activated neutrophils and microglia into the ischemic brain begins 24 to 72 hours after brain damage and accounts for a wide range of inflammatory mediators such as cytokines and chemokines that promote ischemic brain damage and neurological outcomes [4]. Intrinsic immunity has been reported to play a vital role in the pathogenesis of inflammatory disorders including cerebral ischemia [5].

Stroke is caused by risk factors such as diabetes, high blood pressure, old age, heart disease (such as atrial fibrillation), and the continued use of certain medications. In addition to the mentioned factors, gender differences may also have a significant effect on the stroke mechanism and response to treatment [6]. Previous reports have shown that a decrease in ovarian steroid hormones leads to an increase in stroke risk and related disabilities and mortality [7]. Exogenous estrogen administration has been shown to improve complications of various stroke models in rodents [7]. Furthermore, estrogen replacement therapy has been shown to reduce ischemic brain damage in animal models [8]. Estrogen replacement therapy, despite neuroprotection in human and rodent stroke models, is associated with serious side effects such as breast and endometrial cancers as well as cardiovascular disease [9].

Like estrogen, phytoestrogens (abundant in some plants) have estrogenic and anti-inflammatory effects. The main sources of phytoestrogens are isoflavones such as isoflavones, coumestrol, and lignans [10]. These compounds show less estrogenic effect than estrogens and act through estrogen receptors [11]. Many studies have provided evidence of the neuroprotective effects of phytoestrogens in various diseases, including stroke [12]. Moreover, some studies have reported that phytoestrogens can dilate cerebral arteries and thus improve cerebral blood flow [13]. This finding may at least partially suggest that the neuroprotective function of phytoestrogen diets in animal models of ischemic stroke may be associated with increased cerebral blood flow [14]. In recent years, there has been a growing tendency to use herbal medicines for the treatment and prevention of diseases. One of these diseases is ischemic brain injury, which is one of the leading causes of death in the world. Research has shown that some plants, such as pistachio extract, have been shown to improve cerebral ischemia or damage caused by hypoxia [15].

Pistachio grows geographically in Khorasan, Semnan, and Kerman provinces (Iran). Research on other species of this plant has been shown to have medicinal effects such as lowering blood pressure, anti-inflammatory effects, and antimicrobial action [16, 17]. Recently, the natural antioxidant properties of flavonoids and phenolic compounds extracted from plants have received much attention [18]. Plants rich in these compounds are an ideal source of natural antioxidants. The components of gallstones isolated from the leaf extract of Pistacia weinmannifolia have been shown to eliminate

free radicals and have protective effects on oxidative nerve cell damage [19]. Since nuts contain omega-3 and omega-6 fatty acids, they play a potential role in counteracting brain disorders. Thus, compared to other nuts, pistachios are a rich source of antioxidants and phenolic compounds. [20]. Recent studies have shown that oxygen-free radicals are an important mediator of tissue damage, especially brain tissue in cerebral ischemia. In addition, cerebral ischemia increases the lipid peroxidation response and increases the production of free radicals that contribute to secondary nerve tissue damage [21]. Given the anti-inflammatory, antimicrobial, and antioxidant effects of pistachio, the present study aimed to investigate the beneficial therapeutic effects of pistachio in the model of permanent middle cerebral artery occlusion in ovariectomized female rats.

2. Materials and Methods

Groups and stroke induction

In this experimental study, female Wistar rats weighing 250-200 g were examined. The animals were placed in four groups: The control group (ovariectomized rats), the second group (ovariectomized rats receiving 100 mg/kg pistachio extract), the third group (ovariectomized rats receiving 250 mg of pistachio extract), and the fourth group (ovariectomized rats receiving 500 mg/kg pistachio extract). The number of rats in each group at the beginning of the study was equal to 10 animals. The animals in all groups underwent permanent middle cerebral artery occlusion and the drugs were administered to the animals for 4 weeks before the induction of the stroke. To induce a stroke, after anesthetizing with ketamine and xylazine, the animal was placed on its side and a 2 cm diagonal incision was made in the skin in the middle of the left orbit and external auditory canal. The next steps were performed using surgical techniques in which the skin around the incision was removed and the parotid gland was seen in the dorsal-lower quarter of the visual field. The vascular source was burned and divided at its anterior-upper pole, and the gland was moved backward. Then, an incision was made around the upper and posterior edges of the temporalis muscle, and it was pushed aside from the side of the skull with a muscle lift and turned forward. The main part of the muscle was then cut with a vertical incision toward its anterior edge and from its connection to the tip of the coronoid appendage. This bone and the dorsal half of the cheekbone were removed, and the middle cerebral artery was burned, the soft tissues were turned back, and then the skin was sutured.

Extract preparation

Akbari cultivar pistachios, which are native to Rafsanjan, were used. Species were approved by experts from the Valiasr University of Rafsanjan. After collecting pistachios, they were powdered, and 300 g of chopped pistachios was mixed with 900 ml of 80% methanol and the mixture was kept in an incubator at 80 °C for 12 hours. The residues of the mixture were extracted in the same way three times. The obtained extracts were mixed, and their solvent was separated by a rotary device (made in Iran). The plant extract was dissolved in 2.5% DMSO and gavaged in animals.

Ovariectomy procedure

First, the rats were weighed and for every 100 grams of animal weight, 0.1 mg of ketamine anesthetic was injected intraperitoneally. After complete anesthesia, the abdominal area was incised, and the surgical site was disinfected. The two sides of the abdominal area and the

three sides of the thigh muscle were dissected, and the ovary was found. Afterward, the ovarian attachment tube (the follicular and red tissue connected to the oviduct) was slowly removed with a cochlear device. Then, the inner and outer layers were sutured separately, and finally, for every 100 grams of animal weight, 0.3 mg of penicillin was injected into the rat thigh muscle and the animal was returned to the cage to regain consciousness [22].

Ovariectomy evaluation

After 3 days, the animal's vagina smear was taken for 6 days (and a few drops of normal saline was added to the vagina with a bulb). Then, it was taken out and placed on the slide and spread in a smear state, and was observed under a microscope. If a fern-like pattern was not observed, then ovariectomy was performed correctly [22].

Infarct volume and cerebral edema measurement

Infarct volume and cerebral edema were measured 72 hours after stroke. To determine the volume of infarction after removing the brains of animals and cutting them (2 mm coronal incisions), they were stained with tetrazolium chloride (TTC). For this purpose, after preparing a 2% TTC solution, the sections of the brain of each animal were placed in the solution and then incubated for 30 minutes at 37 ° C. TTC reacts with dehydrogenase enzymes inside living cells and turns red. Thus, living cells turn red but dead neurons do not change color (due to the absence of dehydrogenases). Then, after fixing the slices with 10% formalin, they were photographed by a digital camera and measured with image processing software. The sections obtained from each incision were multiplied by its thickness and the infarct volume was measured for a total of 6 incisions in the left and right hemispheres using the following formula. Cerebral edema was measured by determining the percentage of brain water.

Infarct volume = [The left hemisphere volume - (the right hemisphere volume - infarct volume measured with TTC)] × 100/left hemisphere volume

Cerebral edema was measured by determining the percentage of brain water using the following formula.

[(The right hemisphere volume – the left hemisphere volume)/left hemisphere volume] × 100

Behavioral tests

Neurological disorders

Neurological disorders were scored on the second and third days after surgery with the Bederson ranking system (0 = Absence of any disorder, 1 = Bending of the front limb, 2 = Bending of the front limb in addition to reduced resistance to lateral pushing, 3 = Rotation to one side, 4 = Rotation to one side in addition to a decreased level of consciousness, and 5 = Death or absence of awareness and mobility) [17].

Sensory-motor disorders were also measured using the sticky test and hanging test:

Sticky test

The sticky test was performed using standard adhesive label. For this purpose, during the 3 days before the induction of the stroke, the animals were trained to use their teeth to separate the paper label attached to the palm opposite to the hemisphere that was to be damaged. The time it took for the animal to touch and remove the sticker was considered as the amount of sensorimotor activity, and the longer it took to

remove the label, the greater was the sensorimotor disorder.

Hanging test

The hanging test measured motor disorders by determining how long the animal was able to hold itself hanging with both hands from a narrow string. Obviously, the shorter the handing, the greater the motor disorder.

Data analysis

The data in this study were analyzed using SPSS V.21 software. Differences in infarct volume, cerebral edema, and sensorimotor disorder were compared with one-way ANOVA and Tukey posthoc test and reported as mean ± SEM. Neurological disorders were compared with Kruskal-Wallis non-parametric test. All values with P <0.05 were considered statistically significant.

3.Results

The impact of pistachio extract on infarct volume

The percentage of infarct volume is shown in Figure 1. Compared to the control group, pistachio extract at a dose of 500 mg/kg (p <0.0001) and pistachio extract at a dose of 250 mg/kg (p <0.005) reduced the infarct volume. Besides, pistachio extracts at doses of 500 and 250 mg/kg reduced the infarct volume compared to 100 mg/kg of pistachio extract (p <0.005 vs. p <0.05).

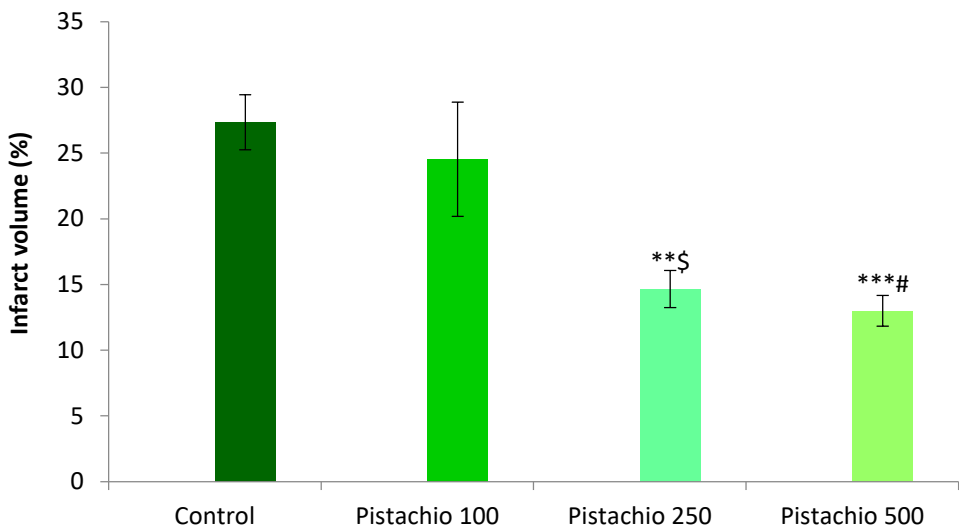

Figure 1: The effect of hydroalcoholic pistachio extract (100, 250, and 500 mg/kg) on infarct volume. (n = 10). Data were presented as SEM ± mean. *** p <0.0001 and ** p <0.005 compared to the control group, #p <0.005 and $ p <0.05 compared to the dose of 100 mg/kg of pistachio extracts.

The impact of pistachio extract on cerebral edema

The percentage of cerebral edema is shown in Figure 2. Compared to the control group and the dose of 100 mg/kg of pistachio extract, pistachio extract at doses of 250 and 500 mg/kg reduced the rate of cerebral edema ($p < 0.0001$):

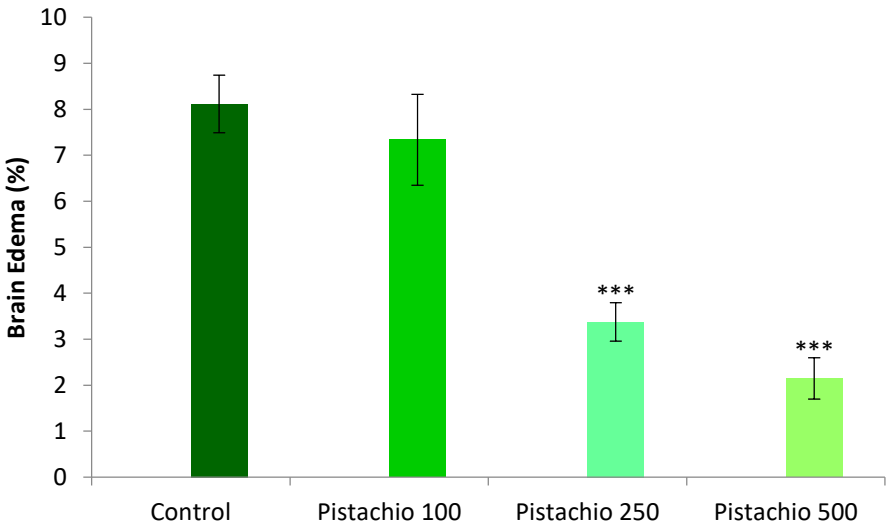

Figure 2: The effect of hydroalcoholic pistachio extract (100, 250, and 500 mg/kg) on cerebral edema. Data were presented as SEM ± mean. *** $p < 0.0001$ compared to the control group and dose of 100 mg/kg of pistachio extract (n = 10).

The effect of pistachio extract on sticky test

The delay time in touching and removing the adhesive label (1 cm × 1 cm) from the animal's forepaw is shown in Figure 3. The delay time in touching and removing the adhesive label from the animal's forepaw opposite the stroke area in the group treated with pistachio extract in two doses of 250 and 500 mg/kg in 48 hours ($p < 0.001$) and 72 hours ($p < 0.001$) after stroke decreased significantly compared to the control group. Besides, the delay time in touching and removing the adhesive label from the forepaw in the group treated with 500 mg/kg of pistachio extract was reduced compared to the group treated at the dose of 100 pistachio extract 72 hours after stroke ($p < 0.05$).

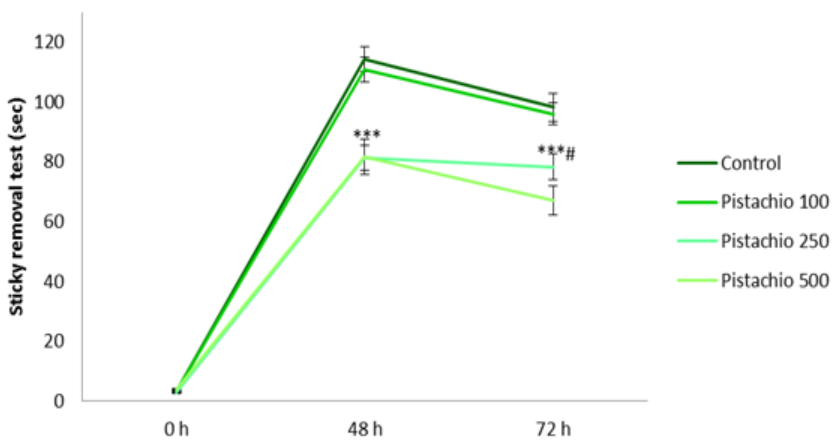

Figure 3: The effect of hydroalcoholic pistachio extract (100, 250, and 500 mg/kg) on the sticky test. Data were presented as SEM ± mean. *** p <0.0001 compared to the control group. # p <0.05 compared with 100 mg/kg dose of pistachio extract (n = 10).

The impact of pistachio extract on the hanging test

The length of time the animal hanging from a thin wire is shown in Figure (4). The hanging time of the animal from a thin wire in the group treated with 500 mg/kg of pistachio extract in 48 hours after stroke was significantly increased compared to the control group (p <0.005). In the groups treated with 250 mg/kg and 500 mg/kg of pistachio extract, the duration of hanging the animal from the thin wire at 72 hours after stroke increased compared to the control group (P = 0.000). The hanging time of the animals in the 100 mg/kg pistachio extract group decreased compared to the 500 mg/kg pistachio extract group 72 hours after stroke (p <0.001).

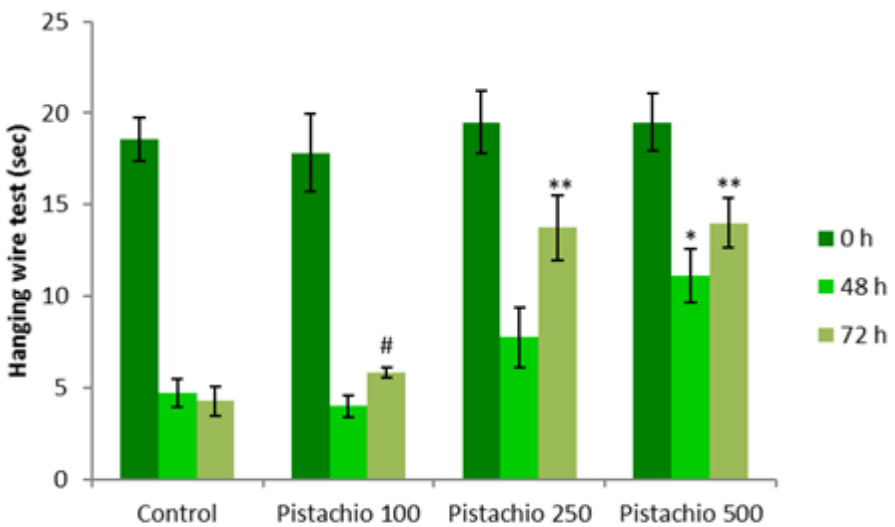

Figure 4: The effect of hydroalcoholic pistachio extract (100, 250 and 500 mg/kg) on the hanging animal. Data were presented as SEM ± mean. * p <0.005 compared to the control group. ** p = 0.000 compared to the control group. # p <0.001 compared to 500 mg/kg pistachio extract (n = 10).

The effect of pistachio extract on neurological disorders

The neurological disorders observed in the four groups were scored 48 and 72 hours after the induction of stroke. The results showed that 48 and 72 hours after stroke, no significant differences were observed between the control group and the group treated with100 mg/kg of

pistachio extract. However, pistachio extracts in doses of 250 and 500 mg/kg significantly reduced neurological disorders 48 and 72 hours after stroke compared to the control group and the group treated with 100 mg/kg of pistachio extracts ($p < 0.01$).

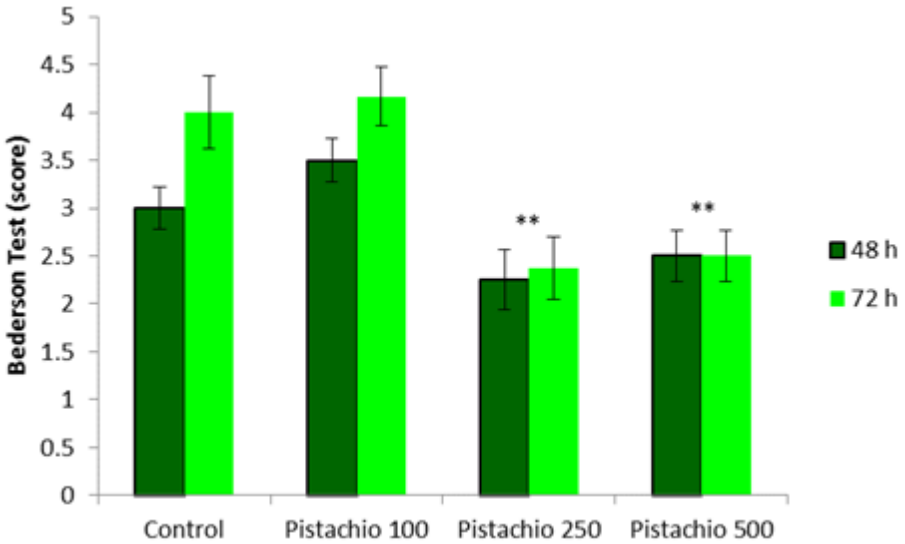

Figure 5: The effect of hydroalcoholic pistachio extract (100, 250, and 500 mg/kg) on neurological disorders. Data were presented as SEM ± mean. $p < 0.01$ ** compared control group and 100 mg/kg pistachio extract group (n = 10)

4. Discussion

The present study examined the effects of pistachio extract on stroke intensity, cerebral edema, neurological disorders, and sensory-motor disorders by developing a laboratory model of stroke. The results indicated that administration of pistachio extract after stroke in ovariectomized female mice reduced the intensity of stroke and cerebral edema and reduced sensory and motor disorders and improved the complications of stroke.

Postmenopausal complications occurring to a decrease in ovarian hormones are treated with alternative therapies with estrogen. However, continuous use of estrogenic drugs increases the risk of breast cancer. Besides, there is ample evidence pointing to a significant increase in stroke and gallbladder disease following hormone therapy with estrogen alone as well as combination therapy. Accordingly, herbs are used increasingly in developing countries and about 80-65% of the population use herbal products. Among non-hormonal alternative therapies, herbs, and among plants, a variety of estrogen-like compounds (phytoestrogens) such as pistachios have a special place in treating menopausal symptoms [23, 24]

Phytoestrogens are hormonal modulators that act as estrogen agonists when estrogen levels are low, producing estrogen-like effects and estrogen antagonists when estrogen levels are high [25, 26]. Accordingly, the present study examined the

effect of pistachio extract on stroke in ovariectomized female rats. The findings showed that pistachio extract at doses of 250 mg/kg and 500 mg/kg significantly reduced infarct volume. These effects were equal to estrogen, especially at high doses of pistachio extract. Our data were consistent with the results of previous studies that showed the protective effects of phytoestrogens in different models of stroke. For instance, Schreihofer et al. found that the rate of infarction in stroke was significantly reduced in animals fed a soy-rich diet for only two weeks compared with animals fed a low-soy diet [27]. Moreover, Aras et al. showed that treatment with phytoestrogens in cerebral ischemia reduces oxidative stress and nerve cell destruction [28]. Wang et al. also showed that Biochanin A, a natural isoflavonoid classified as phytoestrogens, has neuroprotective effects on the ischemic model in rats [29].

The data in the present study also suggested that impaired sensory-motor coordination (sticky test) following a stroke. However, the animals in the treatment group spent less time removing the labels from their hands. On the other hand, the index of hanging the animal from a thin wire string was disturbed after the stroke, so that the duration of hanging the animals in the group treated with pistachio extract was longer than that of the animals in the control group. In line with the findings of this study, Huang et al. suggested that phytoestrogens improve neural function in temporary occlusion of the middle cerebral artery [30]. There are also extensive studies showing that stroke leads to an increase in the time for removing labels from the animal's forepaw, which confirms our results [31, 32].

Since free radical scavenging improves stroke, researchers have found ample evidence showing the inactivation of free radicals produced after a stroke. As an example, Edaravon and NXY-056, which have antioxidant properties, have been used in the clinic to treat patients. Research has shown that they can reduce the volume of cerebral infarction, improve neurological function, and reduce neurological disorders [33].

Pistachio has some components with high antioxidant activity such as polyphenols, tocopherols, lutein, phytosterols, vitamin B6, gallic acid, and carotenoids [34]. Kocyigit et al. showed that pistachio consumption significantly reduces oxidative stress and improves plasma fat [35]. Shahraki et al. also reported the liver protection effects of pistachio extract against the formation of reactive oxygen species and lipid peroxidation. They have shown that methanolic extract of pistachio destroys the active species of oxygen and carbonyl and inhibits lipid peroxidation [36]. In addition, in a clinical study, Gentile et al. found that pistachios significantly improved the oxidative status and reduced inflammatory biomarkers [37]. These observations confirm the hypothesis that the neuroprotective effects of pistachio extract may be due to the reduction of reactive oxygen species (antioxidant activity) and the strengthening of the antioxidant system. Therefore, in line with previous studies, the present study showed the antioxidant properties of pistachio extract improve neurological disorders.

5. Conclusion

The present study showed that pistachio extract reduced the volume of infarction, cerebral edema, neurological disorders, and sensory-motor disorders in ovariectomized rats. Thus, pistachio extract can be considered as a complementary therapeutic combination in the treatment of menopausal stroke.

Conflict of interest: The authors declared no conflict of interest.

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