

Chronically Pre-treatment of Pistachio Alters the Morphology of CA1 Hippocampus Neurons Following Transient Focal Cerebral Ischemia in Male *Wistar* Rats

Hossein Pourmobini¹, Mohammad Reza Afarinesh^{2,3}, Ali Shamsara¹, Seyed Hassan Eftekhbar-Vaghefi^{1,4}, Majid Asadi-Shekaari¹

¹Anatomical Sciences Department, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran; ²Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman; ³Student Research Committee, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran; ⁴Anatomical Sciences Department, Kerman Branch, Islamic Azad University, Kerman, Iran

Abstract. *Background:* Consumption of antioxidants is effective on reducing the damage caused by cerebral ischemia. *Objectives:* We investigated the effect of *Pistacia vera* (pistachio) pretreatment on the morphology of the cornu ammonis (CA1) region of hippocampus neurons of the rats' hippocampus following transient focal cerebral occlusion of the middle cerebral artery (MCA). *Methods:* In this study, 30 male *Wistar* rats were divided into 3 groups of control, ischemia, and pretreatment with pistachio (fed with pistachio at 6% of the diet for a five-week duration before the right MCA occlusion). Neurological scores of the rats were assessed using Baderson rating. Thereafter, the animals' balance and muscle power were assessed by Rotarod and forelimb wire-grip strength tests, respectively. Finally, histopathological and morphometrical characteristics of hippocampal neurons were studied using Hematoxylin-Eosin method. *Results:* Neurological scores of the ischemia group significantly decreased compared to the control group ($p < 0.05$), while pretreatment with pistachio significantly improved Baderson rating scores compared to the ischemia group ($p < 0.05$). Although stroke significantly decreased the balance and muscular strength in the studied rats compared to the normal rats ($p < 0.05$), pistachio's exposure significantly increased the balance and muscular strength compared to the ischemia group ($p < 0.05$). Additionally, a significant decrease was observed in the volume of stroke and neuronal degradation in the pistachio-treated rats compared with the ischemia group ($p < 0.05$). *Conclusions:* Pistachio consumption reduces the volume of infarction and neuronal damage and improves neurological disorders after ischemia. Therefore, pretreatment with pistachio would have a protective effect against stroke. (www.actabiomedica.it)

Key words: *Pistacia vera*, Stroke, Antioxidant, Anti-inflammatory, Cerebral ischemia

1. Background

Today, stroke is known as the third leading cause of death after heart disease and cancer, as well as the leading cause of disability by annually affecting millions of people in developed countries worldwide. A stroke occurs in humans as a result of some factors such as diabetes, hypertension, old age, heart disease

(1). Strokes can generally be divided into the following two types: ischemic and hemorrhagic. Accordingly, the ischemic type is much more common that accounts for about 87% of all strokes (2). The current treatment for this dangerous complication is limited to the use of the enzyme recombinant tissue plasminogen activator, which consequently causes the clot to dissolve; however, this drug is effective when administered within

the first 3 hours after the onset of stroke. The high number of patients, the high cost of their care and rehabilitation, and the lack of appropriate treatment for this disease necessitate conducting extensive and comprehensive research in this field (3).

Cerebral ischemia in rats is induced in two ways as follows: 1) occlusion of a cerebral artery, called a stroke, causes irreversible damage to the central region as well as a reversible injury to the surrounding area, and 2) Cardiac arrest or coronary artery occlusion that disrupts blood flow to the brain and consequently causes cell death in neuronal populations such as pyramidal neurons in the cornu ammonis (CA1) region of hippocampus (4). The Middle Cerebral Artery (MCA) is one of the terminal branches of the internal carotid artery that supplies blood to parts of the cortex, basal ganglia, and hippocampus (5). The obstruction of this artery is considered as one of the main causes of stroke (6). The hippocampus is also considered as the main area during the processes of memory and learning. CA1 of the hippocampus is one of the most sensitive areas to ischemic damage (7). Many factors that follow ischemia, including cytotoxicity, ionic imbalance, depolarization around the stroke, oxidative stress, inflammation, and apoptosis lead to neuronal damage (2). Therefore, to control ischemia, oxidative stress and ischemia must be reduced by any way (8).

Nowadays, the use of herbal medicines as a substitute for chemical drugs, which is moving away from chemical treatment and approaching herbal medicine, requires more attention to medicinal plants (9, 10). *Pistacia vera* (pistachio) belongs to the family Anacardiaceae, which has been known for its medicinal properties since ancient times (9). Pistachio seed is a nut with different compounds such as β -carotene, α -tocopherol, and lutein (11). Moreover, previous studies reported that *P. vera* contains phenolic compounds and triterpenoids (12). Pistachio has a high amount of phenolic compounds like anthocyanins whose antioxidant and anticarcinogenic properties have been previously proven (13). In addition, it has been shown that *P. vera* has many pharmacological effects such as antimicrobial (12) and anti-hyperlipidemia (14) activities. Pistachio is a popular nutrient in the European Union, the United States, Southeast Asia, and Japan. Of note,

Pistachio in Iran has the second rank of non-oil exports after carpet (15). Gentil et al. (2007) reported that pistachio is among the top 50 foods with rich anti-inflammatory properties worldwide (16). Mansouri et al. (2005) studied the neuroprotective effects of pistachio extract on cerebral ischemia in rats and showed that pistachio extract could reduce the brain level of the Malondialdehyde enzyme (17). Due to the presence of some pistachio compounds such as vitamin E and beta-carotene, its consumption may affect the spatial learning in rats. In addition, the abundant antioxidant properties of pistachio can affect balance, forelimb strength, and hippocampal tissue damage due to cerebral ischemia in adult male rats that were investigated in the present study.

2. Materials and methods

2.1. Animals

In the present study, 30 male *Wistar* rats weighing between 250 and 200g were used. The rats were placed under laboratory conditions for 12 hours of light and 12 hours of darkness at a set temperature of $25 \pm 2^\circ\text{C}$. Thereafter, they were fed in Plexiglas cages with free access to water and food. The Ethical Committee of Kerman University of Medical Sciences has approved the experimental procedures (Code: IR.KMU.AH.REC.1396.115). Accordingly, the animal handling and experiments were performed in standard manner to minimize the pain and stress. The animals were divided into 3 groups ($n=10$):

1. The control group: the rats were fed with normal food for 5 weeks. Afterward, the skin and muscles of the neck were cut and sutured, but the MCA artery occlusion was not performed.
2. The ischemia group: the rats were fed with normal food for 5 weeks and MCA artery was then blocked in them after surgery.
3. The pistachio group: the rats were fed with pistachio for 5 weeks at the rate of 6% of the diet. Normally, the daily diet was considered as 25g/rat (18). After surgery, the MCA artery was blocked in them.

2.2. Focal cerebral ischemia procedure

Focal cerebral ischemia injury was induced by middle cerebral artery occlusion (MCA) in terms of the method developed by Longa et al. (19). The rats were then anesthetized with an intraperitoneal injection of ketamine/xylazine (80/10 mg/kg). Subsequently, the left common carotid artery, external carotid artery (ECA), and internal carotid artery (ICA) were isolated via blunt dissection following a ventral midline incision of the neck. Next, the distal end by electric coagulation. A monofilament nylon suture (0.26 mm) was inserted 18–20 mm into the ICA from the ECA, in order to occlude the origin of the MCA. The suture was finally fixed and the incision was closed. Following 30min of ischemia, the branches of the ECA were cut off, and the whole of the ECA was ligated and then dissociated at, the nylon suture was withdrawn to allow reperfusion. Next, the sham-operated rats in the control group underwent identical surgery, with the exception that the nylon suture was not inserted to this group. The incision was sutured and the animals were allowed to regain their consciousness. After surgery and recovery, the animals were returned to the cage and provided with adequate water and food.

2.3. Stroke confirmation

Neurological impairment in the stroked animals was examined by the modification of the method published by Bederson et al. (1986) (20) during 24 h after ischemia induction. Briefly, the animals were suspended from their tails and their behaviors were scored on a 0–5 scale for 1 minute as follows: no disturbance (0), bending of front limb (1), bending of front limb plus reduction of lateral pushing resistance (2), turning to one side (3), turning to one side plus decreasing the level of consciousness (4), and lack of consciousness and mobility (5).

2.4. Behavioral evaluations

The possible Neurologic deficits such as motor and balance defects were evaluated using Hanging and Rotarod tests in all rats (n=10) per each one of the above-mentioned groups.

2.4.1. Hanging test. By passing 24 hours from ischemia, the power of the forelimbs of the rats was evaluated by the hanging test. Each rat was hanged from forelimbs on a horizontal steel cable (80 cm length and 7 mm in distance), which was linked between two slicks. Although the rats were catching the cable, they were put up in a perpendicular location. The animals were brought back to their home cages during inter-trial intervals for 5 min. Of note, latency to the dropping slump was recorded using a chronometer. The average of three times hanging the animal from the wire was finally reported (21).

2.4.2. Rotarod test. Rotarod test was performed to investigate the animal's equilibrium balance by passing 24 hours from ischemia. To acclimatize the rats, each rat was placed on a rotating rod of apparatus (Rotarod 3375-4R, Germany) for 5 min at 4 rpm rotating speed. On the next day, in 3 consecutive trials, the speed of the rotating rod has increased from 4 to 40 rpm during 5 min. The cutoff point was 5 min, and each rat was located in its home cage for 5 min. The average duration of the animals' staying on the rotating rod of apparatus was recorded (22).

2.5. Histology

2.5.1. Triphenyl Tetrazolium Chloride staining. By passing 48 hours from ischemia Triphenyl Tetrazolium Chloride (TTC) staining were performed to assess the infarct volume of the experimental group. The 6 of 10 rats in each groups which were subjected to behavioral tests, were anesthetized with ketamine/xylazine (80/10 mg/kg). The fixative solution containing 10% formaldehyde and normal saline, was then injected into the left ventricle of the heart and the brain was finally fixed. Subsequently, the brain was removed and prepared for sampling (23). Coronal thick blocks (2 mm) were placed into dishes containing a 1.5% solution of TTC for 30 min at 37°C until normal tissue stained a plum red color. When the stain had developed, the tissue blocks were removed into 10% formalin for analysis. TTC reacted with dehydrogenase enzymes inside normal cells and stained red. Thereafter, normal cells were seen as red, but dead neurons did not change color (due to the lack of dehydrogenases). After fixing the slices with 10% formalin, they were imaged using

a scanner and then measured with image processing software. Infarct volume was also calculated using the formula: $(\text{Left hemisphere volume} - \text{right hemisphere volume} - \text{TTC infarct volume measured}) \times 100 / \text{left hemisphere volume}$ (22, 24).

2.5.2. Hematoxylin-Eosin staining. By passing 48 hours from ischemia, Hematoxylin-Eosin (H&E) stain was performed on 5 μm sections of tissue cut from the formalin-fixed, paraffin-embedded blocks ($n=4$ of 10 rats/groups). Afterward, coronal brain sections were made using a sliding microtome (5 μm). For H&E staining, the samples were submerged in the hematoxylin and then rinsed in distilled water. Next, the slides were submerged in eosin and stepwise dehydrated in graded alcohol (70%, 85%, 95%, and 100%) each for 30 seconds. Finally, the samples were cleared with 4 rinses in xylene. The tissue's preparations were examined for morphologic evidence of cell death.

2.6. Data analysis

Differences in infarct volume, sensory-motor impairment, and paresis were analyzed by one-way ANOVA followed by Tukey post-test using Prism Graphpad software. The obtained data were reported as $\text{SEM} \pm \text{Mean}$. Neurological disorders were also compared with the Kruskal-Wallis parametric bread test and reported as median and 25th and 75th percentiles. The statistical significance level was considered as $p < 0.05$.

3. Results

3.1. Neurological Disorders Test: Focal cerebral ischemia significantly ($p < 0.001$) increased neurological disorders in the ischemia group compared to the control group. Moreover, pretreatment with pistachio significantly ($p < 0.05$) reduced neurological disorders in the pistachio group compared to the ischemia group (Fig. 1A).

3.2. Hanging Test: Cerebral ischemia significantly reduced the time of hanging animals from the wire compared to the control group ($p < 0.001$). Pistachio pretreatment in the treatment of the animals significantly ($p < 0.05$) increased the duration of wire hanging

in the pistachio group compared to the ischemia group (Fig. 1 B).

3.3. Rotarod Test: a significant decreased in duration time was observed in animals with ischemia when the animal was kept on the rotating bar of rotarod compared to the control group ($p < 0.001$). Exposure to chronic Pistachio in the treatment group significantly ($p < 0.001$) improved the duration time on the rotating rod compared to the ischemic group (Fig. 1C).

3.4. Infarct volume: As shown in the Fig. 1D, an infraction was occurred in the ischemia (15%) and the treatment groups following ischemia (9%). Pistachio pretreatment significantly reduced the infarct volume in the treatment group compared to the ischemia group ($p < 0.05$).

Neuronal degeneration: After H&E staining, the parameters, including cytoplasmic condensation and the total number of pyknotic cells in the CA1 region of the hippocampus, were evaluated in all the studied groups. As shown in Fig. 2, in the control group, the cells were normal and the cell cytoplasm was not changed (Fig. A2, A1). However, in the ischemia (Fig.2 B2, B1) and the treatment groups (Fig.2 C2 C1), the nuclei were shrunk and the cytoplasm became dense.

Focal ischemia induction was resulted from severe neurodegeneration in the neurons of the CA1 region of the hippocampus, which increased the ratio of degenerated to all neurons in the ischemic group up to 73.23%. It was found that pistachio pretreatment could reduce the number of degenerated neurons and also significantly reduce ($p < 0.05$) the ratio of degenerated to all neurons in this group by 10% compared to the ischemic group (table 1).

4. Discussion

In this study, the effects of pistachio pretreatment on the morphology of neurons in the CA1 region of the hippocampus following temporary occlusion of the middle cerebral artery in male rats, were studied in order to find some ways for reducing the complications of stroke and subsequent neurological disorders. The effect of pistachio pretreatment on neurological disorders following stroke was evaluated using the

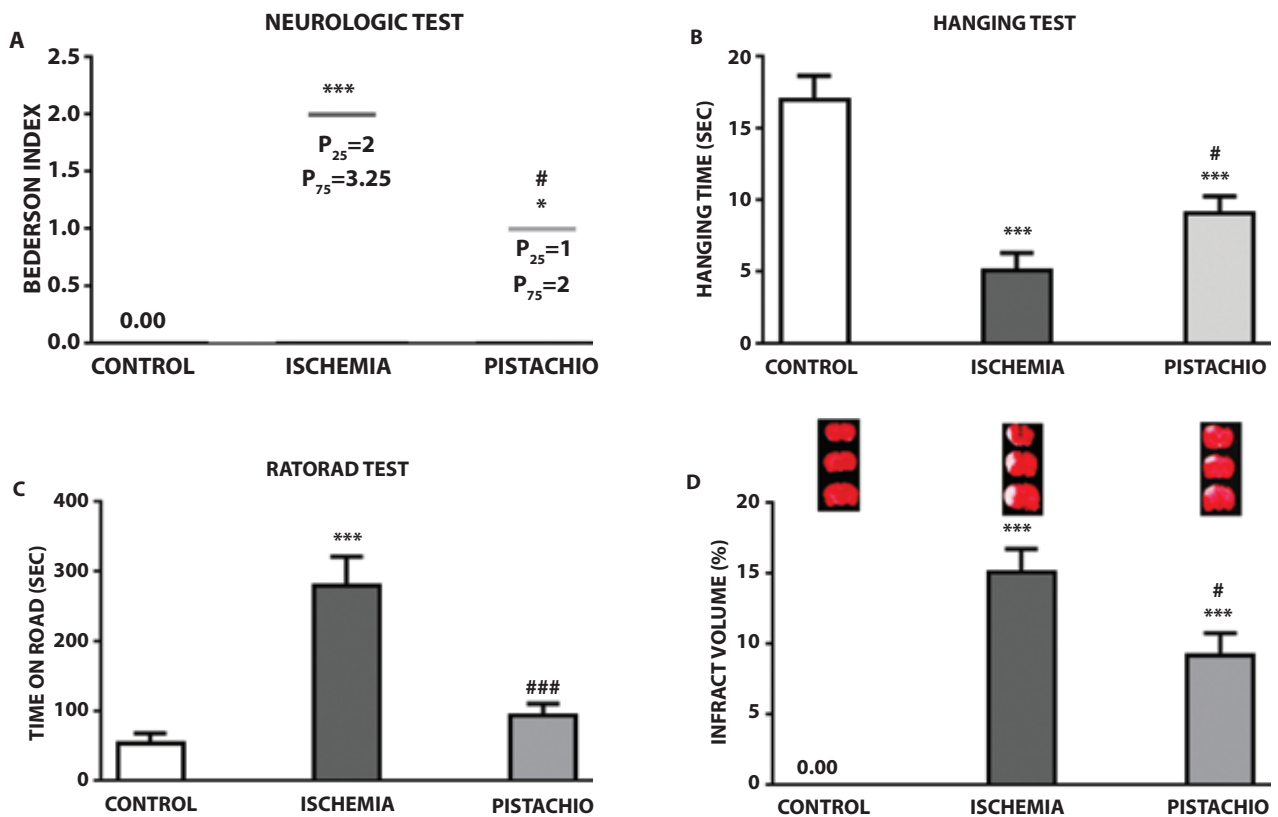


Figure 1. Effect of pistachio pretreatment on Baderson index in neurological test, hanging behavioral test, balancing on rod of rotarod test (A, B, and C respectively, n=10/groups), and rat heart infarction volume (D, n=6 of 10 rats/groups). In panel (A), data is shown as the median, 25th, and 75th percentiles. In panels (B–D), data is displayed as means \pm SEM. The sign (*) indicates a significant difference compared to the control group. (***)p<0.001 and sign (#) show a significant difference compared to the ischemia group (#p<0.05 and ### p<0.001).

Baderson neurological test as well as using behavioral tests by hanging and rotarod tests. Pistachio pretreatment was found to increase the time that it takes for animals to hang from wires, the time that animals keep on the rod of rotarod station as well as reducing neurological disorders that are weak as a result of a stroke. It was shown that some active constituents of pistachio such as naringenin, epicatechin, quercetin, and apigenin have significant effects on physical power (25). For example, it has been demonstrated that quercetin improves cycling time trial performance in humans via antioxidative properties (26). In another study, it has been reported that epicatechin inhibits adaptations in relative peak aerobic power and skeletal muscle compared with the placebo (27). Moreover, it was found that naringenin also has potential anti-fatigue

effects on female rats by reducing the oxidative stress and matrix metalloproteinases-9 level (28). Additionally, in the field of neurological disorders, Wang et al. (2002) reported that a combination of pistachios and several other plant nutrients can improve brain function, which is consistent with the present study's results (29). In this regard, Sinha et al. (2002) reported that pistachio nutrition increases stroke coordination and balance in stroke patients (14).

Inconsistent with behavioral experiments, the present study showed that focal cerebral ischemia causes neuronal degeneration in the CA1 region of the hippocampus, which was also confirmed by previous studies (14, 30). This type of ischemia model has been shown to have severe effects on pyramidal cells in the CA1 region of the hippocampus, causing

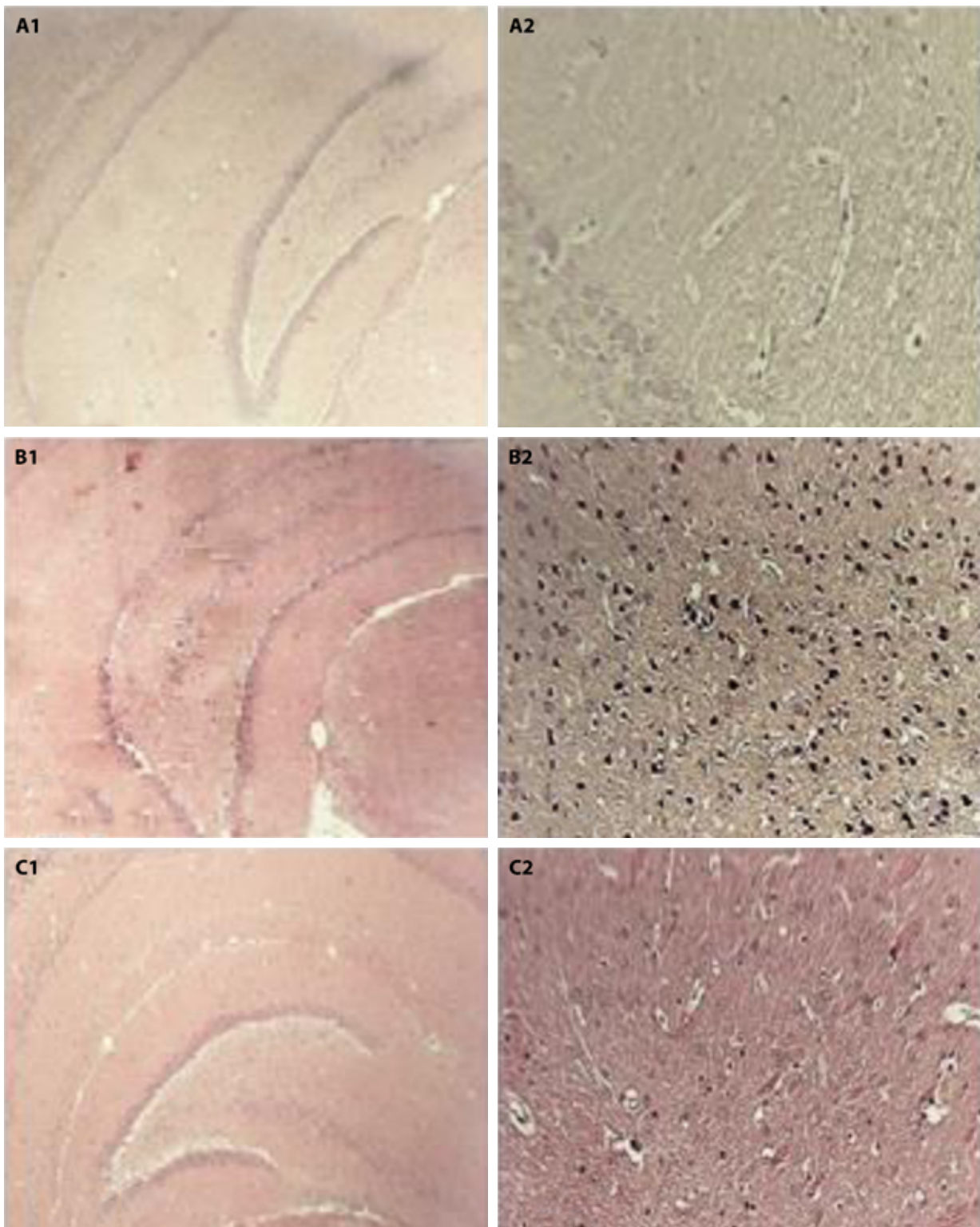


Figure 2. Effect of temporary occlusion of the middle cerebral artery on the morphology of neurons in the CA1 region of the hippocampus that stained by eosin-hematoxylin staining. As can be seen, in the control group the cells were normal and the cytoplasm staining did not change much (panels A2 and A1), but in the ischemia group (panels B2 and B1) and pistachios (panels C2 and C1) the cells were necrotic.

Table 1. Comparison between the number of total neurons and degenerate neurons among control, ischemia and pistachio groups (n=4 of 10 rats/groups)

Groups	Total number of neurons (All)	Number of Degenerated Cells (DC)	DC/All	DC/All%
Control	327.2	24.8	0.0762	7.62
Ischemia	342.6	***250.1	0.732	73.23
Pistachio	338.1	***#211.1	0.626	62.63

The data is represented as Mean \pm SEM. The sign (*) indicates a significant difference compared to the control group. (***) $p < 0.001$ and sign (#) show a significant difference compared to the ischemia group ($\#p < 0.05$).

neuronal destruction, nuclear shrinkage, and discoloration of the cytoplasm (30, 31). The results of the present study also show that pretreatment with pistachio could significantly prevent the destruction of neurons in the CA1 of the hippocampus. Therefore, the present study is in line with previous studies reporting that pistachio has neuroprotective properties and could protect hippocampal neurons from damage caused by cerebral ischemia (17). It has been reported that the pistachio administration improved the neurocognitive behaviors in rats (32). Furthermore, it has been reported that Pistacialentiscus oil (one of the species of Pistacia) improved memory dysfunction. In another study, Ammari et al. (2018) showed that Pistacialentiscus oil reduced lipopolysaccharide-induced memory impairments in rats. They found that Pistacialentiscus oil could improve the activities of superoxide dismutase and catalase in the brain tissue (33). The present study also reported that pistachio pretreatment significantly reduced the volume of infarction in mice with cerebral ischemia compared to the control and ischemia groups (34). Histological studies have shown that ischemia causes some changes in the nucleus and cytoplasm of hippocampus neurons (35).

Ischemic neuronal damage is a biochemical process that, as a result of glucose and oxygen deficiencies, destroys the membrane surface gradient of ions and their action potential. Subsequently, potassium is released from the cells, which leads glutamate to be released from the glial cells through glutamate transporters. This glutamate consequently causes sodium to enter the postsynaptic cells, resulting in depolarization and acute edema. Acidosis helps calcium to accumulate inside cells. Calcium accumulation activates calcium-dependent enzymes (proteases, lipases, and

nucleases), which eventually becomes cell death (36, 37). Neuroprotective substances such as antioxidants and free radical scavengers play important roles in protecting neurons from ischemic damage (38). Plants and natural substances are medicinal substances that have been used for the treatment of human diseases for many years (39).

Pistachios have various active constituents, including α -pinene, β -pinene, phytosterol, flavonoids, and α -Tocopherol (11, 40, 41). It is well-established that pistachios have significant antioxidant properties due to the presence of anthocyanins, flavonoids, phytosterols, and luteolin in them (13). Besides, several clinical studies have demonstrated that the consumption of pistachios increases the levels of lutein, β -Carotene, and vitamin E in blood (42). Therefore, it can be said that pistachio has a significant antioxidant activity due to the presence of some compounds such as anthocyanins (13), vitamin E (42), campesterol, stigmasterol, and β -sitosterol (43) whose antioxidant and anticarcinogenic properties have been previously proven. Considering the abundant antioxidant properties of pistachio and the possibility of its positive effects on preventing ischemic brain lesions, the protective effects of this plant on ischemic complications as well as on behavioral and neurological disorders following ischemia were justified in the present study.

Conclusion

This study showed that pistachio pretreatment in patients with cerebral ischemia not only reduced neuronal damage following transient ischemia significantly, but it also reduced infarct volume, neurological

disorders, and motor learning disabilities. According to the above-mentioned reasons, it can be concluded that pistachio has neuroprotective effects, so it can reduce the complications resulted from stroke. As a result of this reduction, it can be hoped that life expectancy in patients with cerebral ischemia will increase.

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Abbreviations: CA1: cornu ammonis region of hippocampus; MCA: Middle Cerebral Artery; TTC: Triphenyl Tetrazolium Chloride; H&E: Hematoxylin-Eosin; KMU: Kerman University of Medical Science; KNRC: Kerman Neuroscience Research Center.

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References

1. Fisher M, Feuerstein G, Howells D W, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke* 2009. 40; 2244–2250.
2. Doyle K P, Simon R P, Stenzel-Poore M P. Mechanisms of ischemic brain damage. *Neuropharmacology* 2008. 55; 310–318.
3. Yasuhara T, Hara K, Maki M, et al. Dietary supplementation exerts neuroprotective effects in ischemic stroke model. *Rejuvenation Res* 2008. 11; 201–214.
4. Kokaia Z, Lindvall O. Neurogenesis after ischaemic brain insults. *Curr Opin Neurobiol* 2003. 13; 127–132.
5. Kanemitsu H, Nakagomi T, Tamura A, Tsuchiya T, Kono G, Sano K. Differences in the extent of primary ischemic damage between middle cerebral artery coagulation and intraluminal occlusion models. *J Cereb Blood Flow Metab* 2002. 22; 1196–1204.
6. Liu Y, Belayev L, Zhao W, Busto R, Belayev A, Ginsberg M D. Neuroprotective effect of treatment with human albumin in permanent focal cerebral ischemia: histopathology and cortical perfusion studies. *Eur J Pharmacol* 2001. 428; 193–201.
7. Alzaga A G, Cerdan M, Varon J, et al. Trauma and rehabilitation. *Brain Inj* 2006. 20; 889–894.
8. Zhang X, Shan P, Otterbein L E, et al. Carbon monoxide inhibition of apoptosis during ischemia-reperfusion lung injury is dependent on the p38 mitogen-activated protein kinase pathway and involves caspase 3. *Journal of Biological Chemistry* 2003. 278; 1248–1258.
9. Tsokou A, Georgopoulou K, Melliou E, Magiatis P, Tsitsa E. Composition and enantiomeric analysis of the essential oil of the fruits and the leaves of *Pistacia vera* from Greece. *Molecules* 2007. 12; 1233–1239.
10. Pahlavan Y, Sepehri G, Sheibani V, et al. Study the antinociceptive effect of intracerebroventricular injection of aqueous extract of *origanum vulgare* leaves in rat: possible involvement of opioid system. *Iran J Basic Med Sci* 2013. 16; 1109–13.
11. Tokuşoglu Ö, Ünal M K, Yemiş F. Determination of the phytoalexin resveratrol (3, 5, 4'-trihydroxystilbene) in peanuts and pistachios by high-performance liquid chromatographic diode array (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS). *J Agric Food Chem* 2005. 53; 5003–5009.
12. Rajaei A, Barzegar M, Mobarez A M, Sahari M A, Esfahani Z H. Antioxidant, anti-microbial and antimutagenicity activities of pistachio (*Pistacia vera*) green hull extract. *Food Chem Toxicol* 2010. 48; 107–112.
13. Tomaino A, Martorana M, Arcoraci T, Monteleone D, Giovino C, Saija A. Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie* 2010. 92; 1115–1122.
14. Sinha K, Chaudhary G, Gupta Y K. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life Sci* 2002. 71; 655–665.
15. Mobli H, Oghabi H, Alimardani R. Study of the strength properties of pistachio nuts and cluster stem joints for the design and development of a harvesting machine. *Int Agrophys* 2004. 18.
16. Gentile C, Tesoriere L, Butera D, et al. Antioxidant activity of Sicilian pistachio (*Pistacia vera* L. var. Bronte) nut extract and its bioactive components. *J Agric Food Chem* 2007. 55; 643–648.
17. Mansouri S M T, Naghizadeh B, Hosseinzadeh H. The effect of *Pistacia vera* L. gum extract on oxidative damage during experimental cerebral ischemia-reperfusion in rats. *Iran Biomed J* 2005. 9; 181–185.
18. Aschoff J, von Goetz C, Kl. H. Restricted Feeding in Rats: Effects of Varying Feeding Cycles 1, 2. *Z Tierpsychol* 1983. 63; 91–111.
19. Longa E Z, Weinstein P R, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989. 20; 84–91.
20. Bederson J B, Pitts L H, Tsuji M, Nishimura M C, Davis R L, Bartkowski H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. *Stroke* 1986. 17; 472–476.
21. Balkaya M, Krober J M, Rex A, Endres M. Assessing post-stroke behavior in mouse models of focal ischemia. *J Cereb Blood Flow Metab* 2013. 33; 330–8.
22. Nia A M, Kalantaripour T P, Basiri M, et al. Nepeta Dschuparensis Bornm Extract Moderates COX-2 and IL-1beta Proteins in a Rat Model of Cerebral Ischemia. *Iran J Med Sci* 2017. 42; 179–186.
23. Belayev L, Busto R, Zhao W, Ginsberg M D. Quantitative evaluation of blood-brain barrier permeability following

- middle cerebral artery occlusion in rats. *Brain Res* 1996. 739; 88–96.
24. Rizk N N, Rafols J, Dunbar J C. Cerebral ischemia induced apoptosis and necrosis in normal and diabetic rats. *Brain Res* 2005. 1053; 1–9.
 25. Bozorgi M, Memariani Z, Mobli M, Salehi Surmaghi M H, Shams-Ardekani M R, Rahimi R. Five *Pistacia* species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. *Sci World J* 2013. 2013.
 26. MacRae H S H, Mefferd K M. Dietary antioxidant supplementation combined with quercetin improves cycling time trial performance. *Int J Sport Nutr Exerc Metab* 2006. 16; 405–419.
 27. Schwarz N A, Blahnik Z J, Prahadeeswaran S, McKinley-Barnard S K, Holden S L, Waldhelm A. (-)-Epicatechin Supplementation Inhibits Aerobic Adaptations to Cycling Exercise in Humans. *Front Nutr* 2018. 5; 132.
 28. Zamanian M, Hajizadeh M, Shamsizadeh A, et al. Effects of naringin on physical fatigue and serum MMP-9 concentration in female rats. *Pharm Biol* 2017. 55; 423–427.
 29. Wang Q, Xu J, Rottinghaus G E, et al. Resveratrol protects against global cerebral ischemic injury in gerbils. *Brain Res* 2002. 958; 439–447.
 30. Ohyama H, Hosomi N, Takahashi T, Mizushige K, Kohno M. Thrombin inhibition attenuates neurodegeneration and cerebral edema formation following transient forebrain ischemia. *Brain Res* 2001. 902; 264–271.
 31. Ozben T, Balkan E, Balkan S, Serteser M, Gümüslü S. Effects of MK-801 on nitrite and cGMP levels during focal cerebral ischemia in rats. *Nitric Oxide* 2005. 13; 210–215.
 32. Hakimizadeh E, Jandaghi F, Hajmohammadi M, Fatemi I, Kaeidi A, Shamsizadeh A. Pistachio extract improves neurocognitive behaviors in ovariectomized mice. *Res J Pharmacogn* 2019. 6; 45–51.
 33. Ammari M, Othman H, Hajri A, Sakly M, Abdelmelek H. *Pistacia lentiscus* oil attenuates memory dysfunction and decreases levels of biomarkers of oxidative stress induced by lipopolysaccharide in rats. *Brain Res Bull* 2018. 140; 140–147.
 34. Ramassamy C. Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. *Eur J Pharmacol* 2006. 545; 51–64.
 35. Lipton P. Ischemic cell death in brain neurons. *Physiol Rev* 1999. 79; 1431–1568.
 36. Elston G N, Elston A, Freire M A M, et al. Specialization of pyramidal cell structure in the visual areas V1, V2 and V3 of the South American rodent, *Dasyprocta prymnolopha*. *Brain Res* 2006. 1106; 99–110.
 37. Nazifi S, Saeb M, Sepehrmanesh M, Poorgonabadi S. The effects of wild pistachio oil on serum leptin, thyroid hormones, and lipid profile in female rats with experimental hypothyroidism. *Comp Clin Path* 2012. 21; 851–857.
 38. Wang Y, Chang C-F, Chou J, et al. Dietary supplementation with blueberries, spinach, or spirulina reduces ischemic brain damage. *Exp Neurol* 2005. 193; 75–84.
 39. Salah S M, Jäger A K. Screening of traditionally used Lebanese herbs for neurological activities. *J Ethnopharmacol* 2005. 97; 145–149.
 40. Yousfi M, Nedjmi B, Bellal R, Bertal D B, Palla G. Fatty acids and sterols of *Pistacia atlantica* fruit oil. *J Am Oil Chem Soc* 2002. 10; 1049–1050.
 41. Orhan I, Küpeli E, Aslan M, Kartal M, Yesilada E. Bioassay-guided evaluation of anti-inflammatory and antinociceptive activities of pistachio, *Pistacia vera* L. *J Ethnopharmacol* 2006. 105; 235–240.
 42. Kay C D, Gebauer S K, West S G, Kris-Etherton P M. Pistachios increase serum antioxidants and lower serum oxidized-LDL in hypercholesterolemic adults. *J Nutr* 2010. 140; 1093–1098.
 43. Ros E. Health benefits of nut consumption. *Nutrients* 2010. 2; 652–682.

Correspondence:

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Kerman Neuroscience Research Center, Kerman University of

Medical Sciences, Kerman, P.O. Box: 76198-13159

Mohammad Reza Afarinesh

E-mail: r.afarinesh@kmu.ac.ir; ORCID:

0000-0002-5122-9900

Seyed Hassan Eftekhari-Vaghefi

E-mail: sheftekhari-vaghefi@kmu.ac.ir; ORCID:

0000-0002-5083-9563