Original Article

Effect of the hydroalcoholic extract of pistachio on avoidance learning in male Wistar rats

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Abstract **Received**: December 2015, **Accepted**: January 2016

Background: Pistachio is a plant that has long been cultivated in different parts of Iran. Pistachios are very nutritious and contain vitamins E and B, flavonoids, antioxidants, and carotenoids. Since the pistachio is effective in the treatment of some neurological disorders and is indigenous to Iran and considering the substantial use of this nut, it is necessary to investigate its effects on cognitive functions of the brain. This study aimed to investigate the effect of the hydroalcoholic extract of the pistachio on avoidance learning in rats.

Materials and Methods: In this experimental study, 40 male Wistar rats (200-250 g body weight and 2-3 months of age) were divided into 4 groups. Dimethyl sulfoxide (DMSO) 2.5% (vehicle) and hydroalcoholic extract of the pistachio (10, 50, and 100 mg/kg/day) were administered by gavage for 14 days. Avoidance learning test was performed using the shuttle box.

Results: The results of this study indicated a significant increase in the latency to enter the dark room in the groups receiving different doses of pistachio extract compared to vehicle treated group. Moreover, in the treated groups, time spent in the dark room was decreased compared to the vehicle group. The comparison of different doses of pistachio extract demonstrated that 100 mg/kg was more effective than 10 and 50 mg/kg of the extract.

Conclusions: The results of this study indicated that treatment of rats with pistachio extract, which is rich in vitamins, flavonoids, and antioxidant compounds, can improve learning and memory.

Keywords: Pistachio, Avoidance Learning, Rat

Introduction

Since ancient times, herbs have played a significant role in the treatment of many diseases. According to ancient documents, Iranians are pioneers in the use of herbs for medical purposes. Pistachio is a medicinal herb that records of its consumption as a food date back to 7000 BC (1). Pistachio is a genus of the Anacardiaceae family. There are 15 known species of pistachio, but only some species are found in Iran (1, 2). Only 3 species of pistachio naturally grow in Iran; P. vera L., P. khinjuk Stocks, and P. atlantica Desf (3). P. vera is the only species used for* commercial cultivation,

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and the other species are often used as rootstock for P. vera (3, 4).

The pistachio fruit or nut is the edible part of the pistachio plant and is used as flavoring in food and snacks and has been used for therapeutic purposes. Pistachios have a warm and dry nature and contain a large amount of plant protein, starch, unsaturated fats, vitamins, fiber, and minerals such as potassium and magnesium (5-8). The pistachio is very nutritious and contains vitamins B1, B2, B6, E, and C, calcium, and iron and is effective in boosting physical strength and activity of brain cells (9, 10). Other nutrients found in pistachios consist of carotenoids, phenolic compounds such as phenolic acids, flavonoids and other types of polyphenolic compounds, and resveratrol (10). Different parts of the pistachio plant (such as resins, leaves, fruit, and aerial parts) have been investigated for pharmaceutical activities and have traditionally been used for a wide range of purposes (11).

In folk medicine, pistachios have been used in the treatment of eczema, throat infections, kidney stones, and asthma. Furthermore, it is used as an astringent, anti-inflammatory, antipyretic, anti-bacterial, and anti-virus agent (1, 12-17).

Epidemiological evidence has demonstrated that frequent consumption of nuts is beneficial in reducing risk factors for coronary heart disease (CHD). Moreover, researches have indicated that pistachio consumption in individuals with moderate hypercholesterolemia improved blood lipid disorders (17-20). Some of these beneficial effects may be related to the antioxidants naturally present in pistachios, since pistachio consumption has been shown to reduce oxidative stress markers in healthy subjects (10). Recently, pistachio has been used to treat central nervous system diseases because of its beneficial compounds such as antioxidants and phytochemicals and strong anti-inflammatory properties (21, 22). Its use has been recommended in Alzheimer's disease and epilepsy (21). Since the pistachio is effective in the treatment of some neurological disorders and is indigenous to Iran, and considering the substantial use of this nut, it is necessary to investigate its effects on cognitive functions of the brain. This study aimed to investigate the effect of the hydroalcoholic extract of the pistachio on avoidance learning in rats.

Material and Methods

Animal: In the present study, 40 male Wistar rats (200-250 g body weight) were divided into 4 groups of 10 rats. The rats were housed in standard cages with a 12-hour light-dark cycle. The laboratory temperature was set at 23 ± 2.0 °C. Food and water was available ad libitum.

All experimental procedures were carried out in accordance with the guidelines for the care and use of laboratory animals observed in the Rafsanjan University of Medical Sciences, Iran, and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Plant material: The pistachio nuts (Akbari Pistachio type) were collected from cultivated plants in Rafsanjan botanical gardens, Iran. The species was identified by a specialist at the Botany Research Division, Valiasr University of Rafsanjan.

Plant extraction: The dried pistachio nuts were powdered, then, 500 g of powder was added to distilled water and methanol in a ratio of 1 to 1 for 24 hours. This operation was performed in two stages. Then, the mixture was filtered and the solvent was removed through evaporation under reduced pressure at 50 °C by an incubator apparatus. The extract was reconstituted by dissolving it in dimethyl sulfoxide (DMSO) 2.5% before use.

Animal groups: The animals were randomly divided into 4 groups of 10 rats. The groups included the vehicle group which received DMSO 2.5%, and 4 treatment groups which received 10, 50, and 100 mg/kg/day pistachio extract. The DMSO 2.5% and hydroalcoholic

extract of the pistachio were administered by gavage for 14 days. All drugs (extract and vehicle) were administered between 8-10 hours in the morning.

Avoidance learning test: Avoidance learning test was performed using the shuttle box. The shuttle box apparatus includes two rooms of relatively the same size (20 × 20 × 30 cm). The guillotine doors in the middle of the separation wall can be manually removed. The walls and floor of one chamber were enclosed with opaque Plexiglas and the walls of the other chamber were dark. The floor of the compartments is composed of stainless steel rods (3 mm in diameter and a width of 1 cm). In the grid floor of the dark compartment intermittent electric shocks (50 Hz, 3 seconds, 1.5 mA intensity) were produced by an isolated stimulator.

Process of avoidance learning test

1) Adaptation Phase: The adaptation phase was followed by a single trial in which the rats were put into the illuminated compartment and allowed to enter the dark compartment. When the animal entered the next compartment on all 4 paws, the guillotine door was closed and the rat was drawn out from the chamber. The animal was removed from the study, if it did not enter the dark compartment after 300 seconds.

2) Acquisition phase: This stage was performed 1 hour after the adaptation phase. The animal was placed in the illuminated compartment, and after 30 seconds, the guillotine door was opened and the animal was allowed to enter the dark chamber. The entrance latency (step-through latency, STLa) to the dark compartment was recorded. After the animal had placed all 4 paws in the dark compartment, the guillotine door was closed immediately and a single inescapable scrambled foot shock (0.2 mA, 2 seconds) was delivered through the grid base. After 20 seconds, the rat was withdrawn from the apparatus and temporarily placed in their home cage. The procedure was repeated until the entrance latency to the dark compartment was more than 300 seconds.

3) Retrieval phase: Each animal was placed in the illuminated compartment 24 hours after the acquisition phase and the door was opened after 5 seconds. The entrance latency to the dark compartment (step-through latency, STLr) and time spent in the dark compartment (TDC) was recorded until a maximum of 300 seconds. No foot shock was delivered when in the retrieval phase. Animals that did not enter the dark chamber during the retrieval phase were allotted a latency of 300 seconds.

4) Statistical Analysis: The statistical analysis was performed using the SPSS software (version 20, SPSS Inc., Chicago, IL, USA). The results of the Kolmogorov–Smirnov test confirmed the normality of the data in all groups (all $P > 0.050$). All data are expressed as mean ± SEM, and P-values smaller than 0.05 were considered to indicate statistical significance. Differences between the groups were determined using one-way ANOVA. All post hoc comparisons were made using Tukey's post hoc test.

Results

Step-through passive avoidance test was used to assess learning and memory in rats. ANOVA results indicated that oral administration of pistachio extract (10, 50, and 100 mg/kg/day) had no significant effect on step-through latency (STLa) in the acquisition phase when compared with the vehicle group ($f_{(3,36)} = 0.28$, $P = 0.800$) (Figure 1).

The comparison of different doses of the extract revealed that animals treated with 100 mg/kg of the extract had higher STLr time than the DMSO group ($P = 0.016$) (Figure 2).

In line with STLr, total TDC spent by rats decreased in the extract treated groups compared with the vehicle group ($f_{(3,36)} = 4.22$, $P = 0.010$). In addition, the comparison between different doses of the extract revealed that animals treated

with 100 mg/kg of the extract had lower TDC than the DMSO group (P = 0.006) (Figure 3).

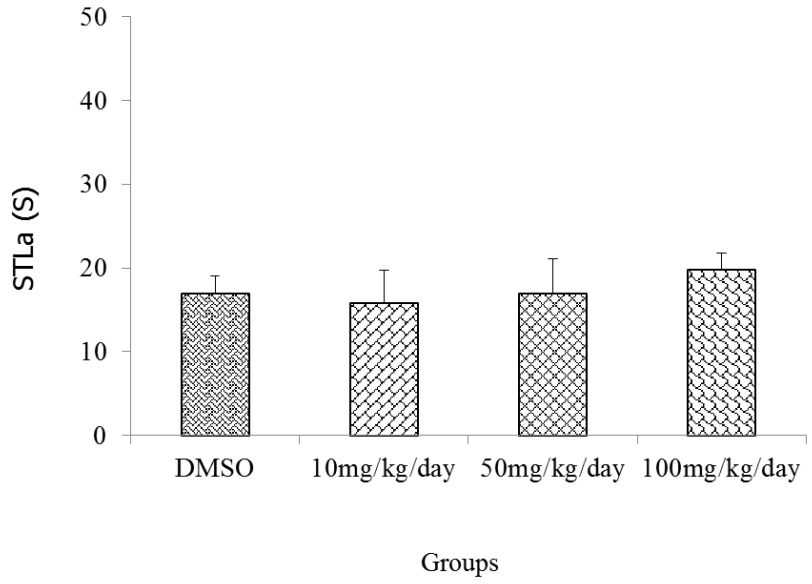

Figure 1. Step-through latency (STLa) of the experimental groups in the acquisition phase In the retrieval phase, the step-through latency (STLr) in rats treated with pistachio extract was significantly higher compared to the vehicle group ($f_{(3,36)}$ = 3.48, P = 0.020).

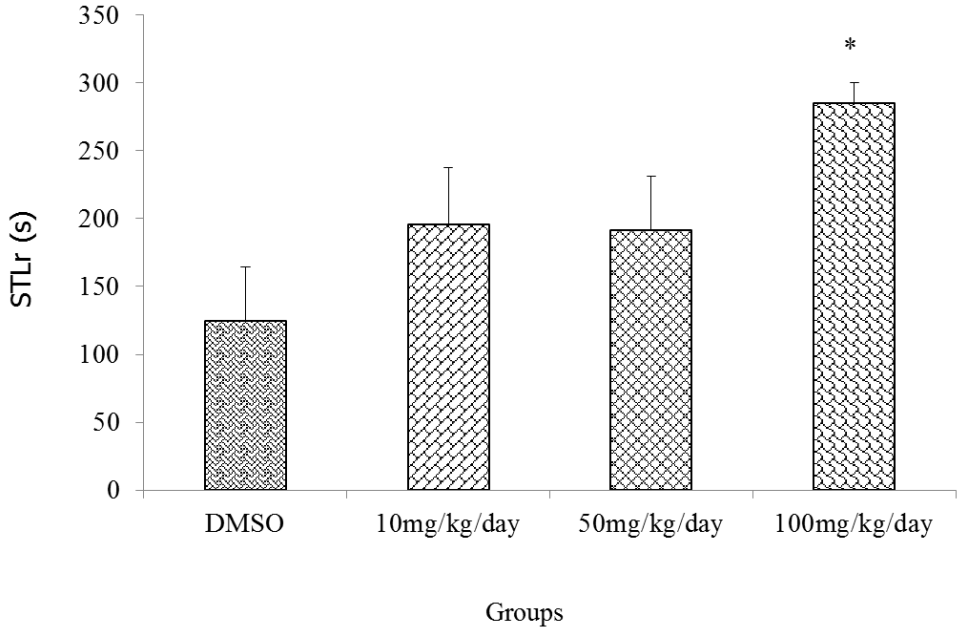

Figure 2. Step-through latency (STLr) of the experimental groups in the retrieval phase
* Significant difference between dimethyl sulfoxide (DMSO) and 100 mg/kg/day group (P = 0.016).

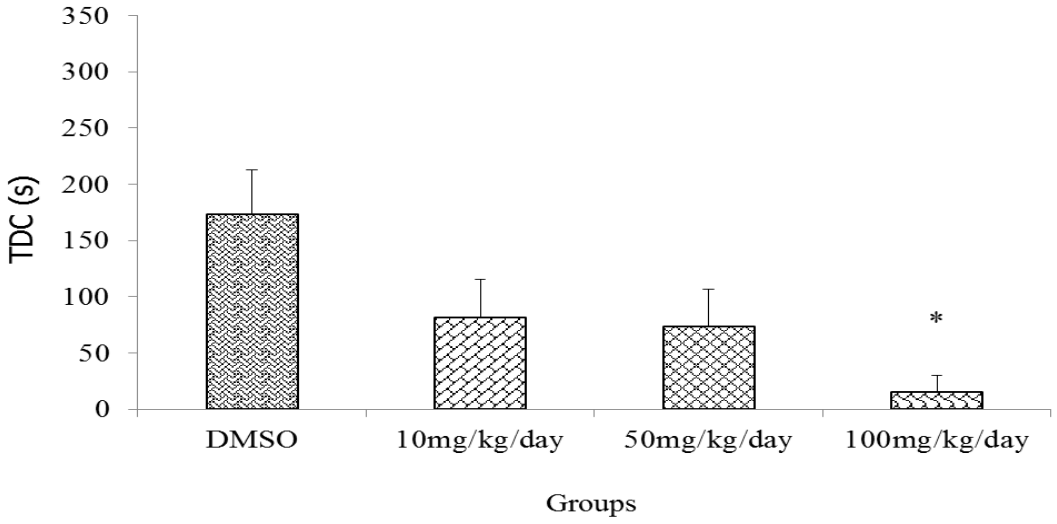

Figure 3. Time spent in the dark compartment by the experimental groups in the retrieval phase

* Significant difference between dimethyl sulfoxide (DMSO) and 100 mg/kg/day group (P = 0.006)

Discussion

In this study, the effects of the hydroalcoholic extract of the pistachio were investigated on avoidance learning in male Wistar rats. The results indicate that treatment of rats with pistachio extract could improve learning and memory.

In traditional Iranian medicine, various species of pistachios have been used as a treatment for various diseases. For example, it was suggested that the kernel of P. vera is a tonic for the heart, liver, stomach, and brain. Fruits of P. atlantica, P. khinjuk, and P. terebinthus were used to treat liver, kidneys, heart, and respiratory system disorders, and their gum for wound healing and treatment of brain and gastrointestinal disorders (23, 24). Furthermore, other beneficial effects of pistachios include antiseptic, antimicrobial, anti-diabetic, anti-inflammatory, anti-nociceptive, anti-hepatotoxic, and anticancer effects (25).

Because of their beneficial ingredients such as antioxidants, flavonoids, vitamins, and minerals, and strong anti-inflammatory properties, pistachios are used to treat central nervous system diseases such as Alzheimer's disease and epilepsy (21, 22). The beneficial effect of the pistachio on the nervous system may be due to its compositions. The impact of some plants on enhancement of memory and relieving of amnesia may be due to polyphenolic antioxidant substances such as flavonoids, vitamin E, and resveratrol (26). Flavonoids are compounds known for their effects on the central nervous system (27). Flavonoids and other polyphenolic compounds inhibit free radicals production and lipid peroxidation. The pistachio extract is rich in natural antioxidants, and thus, can be used as a therapeutic agent for diseases associated with the aging process and free radicals, such as neural degeneration (28, 29). Free radicals play an important role in nerve damage caused by stroke and Alzheimer's disease (30). Hence, pistachios may be used as a beneficial factor for the treatment of these diseases and improvement in learning and memory (21, 22, 28). Various vitamins, particularly vitamins B1, B2, B6, E, and C, in addition to improving physical strength, increase nervous power, and therefore,

are used for the treatment of central nervous system diseases (31).

Research has shown that vitamin E deficiency in cases such as aging, negatively affects learning and memory (32). Vitamin E, through its antioxidant activity in the hippocampus, inhibits memory loss and enhances learning (33-35). It has also been shown that ascorbic acid has positive effects on learning and memory (36, 37). Ascorbic acid regulates the activity of the glutamate and dopamine systems (36, 37). These two neurotransmitters play a major role in learning and memory; therefore, ascorbic acid is effective on learning and memory through these two neurotransmitters and small amounts of ascorbic acid improve learning (37). Since the pistachio contains these vitamins and antioxidant compounds, it can be expected to be effective on the learning and memory processes in animals.

Conclusion

The results of this study demonstrated that gavage of the hydroalcoholic extract pistachio could improve learning and memory in rats. Further studies on patients with learning and memory deficits are suggested to understand whether this nut could be recommended for humans.

Acknowledgement

This article was extracted from the thesis prepared by Masumeh Baloochi to fulfill the requirements for her medical degree. The authors acknowledge the Research Deputy of Rafsanjan University of Medical Sciences for the financial support of this research.

Conflict of interest: None declared

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