

Effect of Afghan Senjed (*Elaeagnus Angustifolia L.*) Leaves Aqueous Extract on Memory of Male Rats

Research Article

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Abstract

This study is aimed to evaluate the effect of *Elaeagnus Angustifolia* leaves extract (EALE) on the memory of male rats. Rats were divided into 7 groups: 4 groups in the first stage (Control, EALE 100, 200 and 400 mg/kg) to determine the effective-dose of EALE on memory; and 3 groups in the second stage (Normal, Scopolamine and EALE effective-dose) to evaluate the effect of EALE on scopolamine-induced memory impairment. Rats received EALE by i.p. administration for 14 days and the memory function of all groups was evaluated by the Y-maze test on days 8 and 15. Only scopolamine and effective dose of EALE groups were received scopolamine 30 min before Y-maze test. The total cholesterol and triglyceride levels of stage 2 rats were also measured. On day 8, the percentage of spontaneous alternation (%SA) was significantly increased in EALE 400 mg/kg group, as compared with the control group. On day 15, there was a significant difference in %SA only between EALE 100 mg/kg group and the control group. The %SA was significantly increased in the EALE effective-dose group only on day 15, as compared with scopolamine group ($P < 0.05$). The effective-dose of EALE was also significantly decreased the total cholesterol ($P < 0.01$) and triglyceride ($P < 0.001$) levels in comparison with scopolamine group. In conclusion, a high dose of EALE only in a short-term administration period and its low dose in a long-term administration period had memory-enhancing effects. The low dose of EALE as an effective-dose of EALE could reverse the scopolamine-induced memory impairment.

Key Words: Afghanistan, Cholesterol, *Elaeagnus Angustifolia*, Leaves, Memory, Rat.

Introduction

Learning and memory are two fundamental abilities of human beings. Most of the knowledge about the world and the majority of the skills of humans are learned rather than innate. Thus, the behavior is greatly shaped on whatever one learns, memorizes and even forgets (1). Memory disorders can have effects on cognitive capabilities. There are a wide group of pathologies that may involve memory-related brain areas and thus impair the memory function (2). Alzheimer's disease is one of common neurodegenerative diseases, which characterized by progressive impairment of memory and cognition (3).

By increase of human population, the prevalence of Alzheimer's disease had increased (3). Despite the rapid progression of this disorder, scientists could not develop a highly ideal drug for this disease. Available synthetic drugs are relatively effective, but

they have a long list of adverse side effects (4, 5). Fortunately, medicinal plants have fewer side effects and lower the rate of death and hospitalization (5, 6). For this reason, most researchers have a great interest in the use of medicinal plants to treat multiple diseases, including diabetes and Alzheimer's disease (7, 8).

Elaeagnus Angustifolia (*E. Angustifolia*), one of useful medicinal plants, is famous because of its therapeutic effects (9, 10). All parts of *E. Angustifolia* including leaves, flower, bark and fruit are known as their pharmacological and biological activities and have different effects (11). The leaves of *E. Angustifolia* have anti-microbial, anti-oxidative, anti-mutagenic activity (12), antidiabetic (13), and protective effects on the cardiovascular system (14).

E. Angustifolia leaves contain most useful constituents such as flavonoids, alkaloids, steroids, terpenoids, as well as beta-sitosterol (15). It has been shown that the amount of flavonoids in *E. Angustifolia* leaves is more than its flower (16).

Despite these useful properties and the presence of beneficial constituents in *E. Angustifolia* leaves, there is no evidence of any study on its memory-enhancing effect. This study is going to investigate the effect of aqueous extract of Afghan *E. Angustifolia* leaves on the memory of rats, using behavioral method.

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Materials and Methods

Animals

Fourthly-two adult wistar male rats weighing between 180 and 220 g, randomly selected from Khatam Al-Nabieen University Research and Technology Center (KNURTC), were housed in Plexy-glass cages with free access to food and water. Animals were kept under stable room temperature ($23\pm 2^{\circ}\text{C}$) and a 12 hours light/dark cycle (the light period started at 7 a.m.). The experimental protocol related to animal's use has complied with all the relevant national regulations and institutional policies, so approved by the ethic research board of Khatam Al-Nabieen University and were conducted following the ethical guidelines set by the 8th edition of National Institute of Health (NIH) guide for the care and use of laboratory animals. Rats were carefully handled to minimize unwanted stress during housing and experiments.

Preparation of extract

Fresh *E. angustifolia* leaves were collected from Kabul, Afghanistan. The leaves were washed in distilled water and dried in the shade. Thirty grams of leaves were grinded and extracted with 70°C distilled water thrice in an incubator at 85°C for 3 h. Then, the water extract has filtered and concentrated in a vacuum rotary (Vargha Tajhiz, Iran) at 60°C . Consequently, *E. angustifolia* leaves extract (EALE) was obtained (14).

Experimental design

This study was conducted in two stages:

First stage

In this stage, rats were randomly divided into 4 following groups ($n=6$) to determine the effective-dose of EALE on memory:

Group I (Control), Rats of this group received Normal saline (1 ml, i.p.) for 14 days;

Group II-IV (EALE 100, 200, 400 mg/kg), Rats of these groups received EALE (100, 200 and 400 mg/kg, respectively, i.p.) for 14 days.

The memory function of rats was evaluated by the Y-maze test on days 8 and 15.

Second stage

In this stage, rats were randomly divided into 3 following groups ($n=6$) to evaluate the effect of EALE effective-dose on scopolamine-induced memory impairment:

Group I (Normal), Rats of this group received Normal saline (1 ml, i.p.) for 14 days, and 30 min before Y-maze test;

Group II (Scopolamine), Rats of this group received Normal saline (1 ml, i.p.) for 14 days, and Scopolamine hydrobromide (Sigma Aldrich, USA) (2 mg/kg, i.p.) 30 min before Y-maze test (17);

Group III (EALE effective dose), Rats of this group received EALE (100 mg/kg, i.p.) as effective-dose for 14 days, and Scopolamine hydrobromide (2 mg/kg, i.p.) 30 min before Y-maze test.

The memory function of all groups was evaluated by the Y-maze test on days 8 and 15. Also, after last behavioral test of the second stage groups, blood was withdrawn from their retroorbital plexus after

mild anesthesia and postprandial serum total cholesterol and triglyceride levels were estimated by kits (Human, Germany).

Y-maze

A black plexy-glass maze ($50\times 30\times 11$) constructed in research and technology center of Khatam Al-Nabieen (KNURTC), used in this study to evaluate the spatial working memory. A rat was placed in the Y-maze and allowed to move freely through the maze for 8 min, without any rewards (e.g. food and water). If a rat entered three different arms consecutively, a spontaneous alternation (%SA) was counted, as an index of spatial working memory. The number of entries into arms was considered as an index of locomotor activity (18, 19).

Statistical analysis

The statistical analysis was done with Graph pad prism (6.07) software. Parameters without considering the time were analyzed by One-way ANOVA test, and Two-way ANOVA analyzed the parameters with consideration of time. The difference amongst means was considered statistically significant if the $P<0.05$. The results are expressed as mean \pm SD.

Results

Effect of EALE on %SA

On day 8 of the first stage, the %SA was significantly ($P<0.05$) increased in EALE 400 mg/kg group (70.45 ± 15.61), but not in EALE 100 (60.15 ± 14.58) and 200 mg/kg (64.17 ± 17.50) groups, as compared with the control group (50.55 ± 6.14). However, on day 15 of the first stage, %SA was significantly ($P<0.05$) increased in EALE 100 mg/kg group (72.05 ± 9.41), but not in EALE 200 (49.25 ± 18.39) and 400 mg/kg (63.00 ± 13.57) groups, as compared with the control group (49.08 ± 9.48) (Table 1, Figure 1). At the second stage, there was a significant difference between %SA of normal and scopolamine groups on days 8 and 15 ($P<0.05$). However, there was a significant difference between %SA of EALE effective dose (67.43 ± 9.06) and scopolamine (50.52 ± 14.37) groups only on day 15 ($P<0.05$) (Table 2, Figure 2).

Effect of EALE on number of arm entries

At the first stage, there was not a significant difference in the number of arm entries between each EALE 100, 200 and 400 mg/kg groups and the control groups on days 8 and 15 ($P>0.05$) (Table 1, Figure 3). Also, at the second stage, there was not a significant difference in the number of arm entries between each normal and EALE effective-dose groups and the scopolamine group on days 8 and 15 ($P>0.05$) (Table 2, Figure 4).

Effect of EALE on total cholesterol and Triglyceride

There was a significant difference in total cholesterol level between normal and EALE effective dose and the scopolamine group ($P<0.01$). Also, there was a significant difference in Triglyceride level between normal and EALE effective dose and the scopolamine group ($P<0.001$) (Table 3).

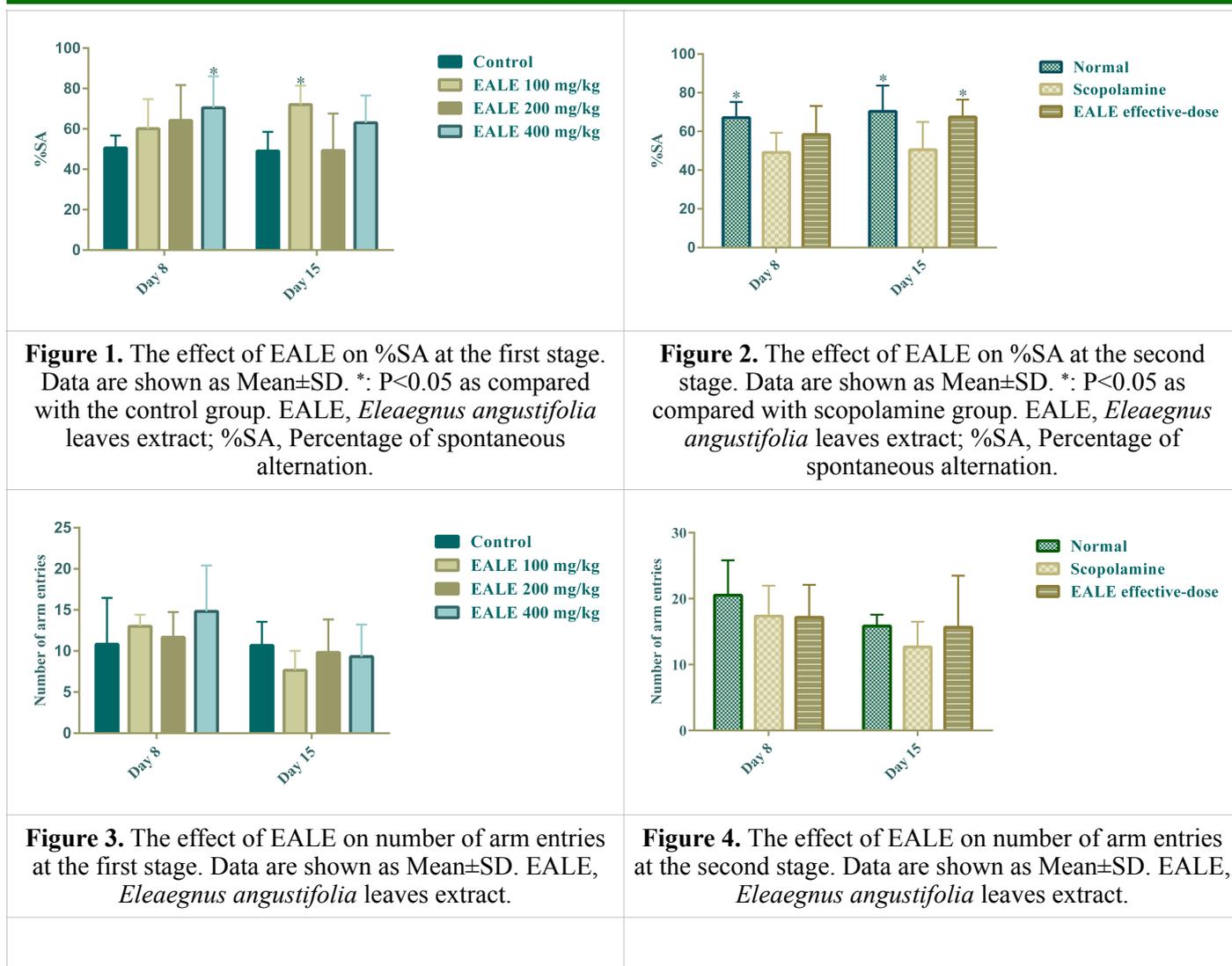


Figure 1. The effect of EALE on %SA at the first stage. Data are shown as Mean±SD. *: P<0.05 as compared with the control group. EALE, *Eleaegnus angustifolia* leaves extract; %SA, Percentage of spontaneous alternation.

Figure 2. The effect of EALE on %SA at the second stage. Data are shown as Mean±SD. *: P<0.05 as compared with scopolamine group. EALE, *Eleaegnus angustifolia* leaves extract; %SA, Percentage of spontaneous alternation.

Figure 3. The effect of EALE on number of arm entries at the first stage. Data are shown as Mean±SD. EALE, *Eleaegnus angustifolia* leaves extract.

Figure 4. The effect of EALE on number of arm entries at the second stage. Data are shown as Mean±SD. EALE, *Eleaegnus angustifolia* leaves extract.

Table 1. The effect of EALE on %SA and number of arm entries at the first stage

Groups	%SA		Number of arm entries	
	Day 8	Day 15	Day 8	Day 15
Control	50.55±6.14	49.08±9.48	10.83±5.64	10.67±2.87
EALE 100 mg/kg	60.15±14.58	72.05±9.41 *	13.00±1.41	7.67±2.34
EALE 200 mg/kg	64.17±17.50	49.25±18.39	11.67±3.08	9.83±4.02
EALE 400 mg/kg	70.45±15.61*	63.00±13.57	14.83±5.56	9.33±3.88
Two-way ANOVA	F = 5.361 df = 3 P = 0.0071		F = 0.3469 df = 3 P = 0.7918	

Data are shown as Mean±SD. *: P<0.05 as compared with control group. EALE, *Eleaegnus angustifolia* leaves extract; %SA, Percentage of spontaneous alternation; df, degree of freedom.

Table 2. The effect of EALE on %SA and number of arm entries at the second stage

Groups	%SA		Number of arm entries	
	Day 8	Day 15	Day 8	Day 15
Normal	67.14±8.10*	70.35±13.38*	20.50±5.32	15.83±1.72
Scopolamine	49.16±10.11	50.52±14.37	17.33±4.63	12.67±3.83
EALE effective dose	58.38±14.76	67.43±9.06*	17.17±4.92	15.67±7.81
Two-way ANOVA	F = 7.912 df = 2 P = 0.0045		F = 0.8692 df = 2 P = 0.4394	

Data are shown as Mean±SD. *: P<0.05 as compared with scopolamine group. EALE, *Eleaegnus angustifolia* leaves extract; %SA, Percentage of spontaneous alternation; df, degree of freedom.

Table 3. The Effect of EALE on total cholesterol and triglyceride

Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)
Normal	99.76±5.60**	105.1±11.21***
Scopolamine	131.3±27.05	166.2±11.87
EALE effective-dose	98.27±7.59**	107.0±4.73***
One-way ANOVA	F = 7.654 df = 2 P = 0.0051	F = 19.62 df = 2 P = <0.0001

Data are shown as Mean±SD. **: P<0.01, ***: P<0.001 as compared with the scopolamine group. EALE, *Elaeagnus angustifolia* leaves extract; df, degree of freedom.

Discussion

In this study, the effect of EALE on the spatial working memory of male rats was evaluated. The results of the first stage experiments showed that the high-dose (400 mg/kg) of EALE increased the %SA after short-term administration period (7 days). However, this high-dose of EALE could not increase the %SA after long-term administration period (14 days). It means that the memory-enhancing effect of the high-dose of EALE is temporary, so it cannot improve the spatial working memory in long-term administration period. On the other hand, the low-dose (100 mg/kg) of EALE could not significantly increase the %SA after short-term administration period. However, this low-dose of EALE significantly increased the %SA after long-term administration period. It means that the low-dose of *E. angustifolia* leaves extract can improve spatial working memory in long-term administration period. Based on these results, we choose the 100 mg/kg dose as EALE effective-dose and evaluated its effect on scopolamine-induced memory impairment.

Scopolamine is an anticholinergic drug, which produces reversible memory impairment in both rodents and humans. There are similarities in memory deficits between Alzheimer patients and scopolamine-treated rodents. Scopolamine is commonly used for evaluation of the anti-amnesic property of herbs or drugs in rodents (20, 21).

Results of second stage experiments showed that the %SA was significantly decreased in scopolamine group, as compared with a normal group in both days 8 and 15. Thus, one can conclude that the administration of scopolamine in rats could develop memory impairment model. In addition, the EALE effective-dose could significantly increase the %SA only after a long-administration period and reversed the scopolamine-induced memory impairment.

Besides, the number of arm entries was evaluated to exclude false-positive results. The experimental groups showed no significant differences regarding the number of entries into the arms, either after short-term or long-term administration period.

As there was not any study on the evaluation of the effect of EALE on memory, we cannot compare the results of this study with others.

Because the main constituents of *E. angustifolia* leaves are the flavonoid compounds and the beta-sitosterol terpene (22), this behavioral change may probably have resulted from the effect of these constituents on memory processes. Various studies have

been shown that flavonoids may improve memory, by activation of neuronal receptors, signaling proteins and gene expression (23, 24). In a study that had been done on the effect of dietary flavonoids on the memory of young rats, suggested that flavonoids may induce changes in synapses and thus in the behavior, by various molecular mechanisms. Especially, the behavioral improvement is may related to an increase in the levels of polysialyted form of the neural adhesion molecule (PSA-NCAM) in the dentate gyrus of the hippocampus (23).

In addition, it is shown that scopolamine can increase acetylcholinesterase activity and reduce the acetylcholine level in the hippocampus, which leads to memory impairment. Therefore, many compounds with anti-acetylcholinesterase activity can reverse its effect and thus improve memory (21). There are reports that most of the plant-derived flavonoids possessed the anti-acetylcholinesterase activity and showed anti-alzheimeric properties (25). In addition, the beta-sitosterol terpene, which is known as an antihypercholesterolemic compound, also showed anti-alzheimeric effects, due to its anti-acetylcholinesterase and antioxidant properties (26).

Despite these beneficial properties of the constituents of *E. angustifolia* leaves on memory, the present study showed that the high dose of EALE could not improve the memory during the long-term administration period. The ineffectiveness of high doses of EALE may have resulted from its sedative effects. There are some reports on sedative effects of *E. angustifolia*, which has been proposed that its flavonoid contents have a relative agonistic effect on benzodiazepine receptors and exerts sedative effect (10). Similarly, a previous study showed that low doses of *Glycyrrhiza glabra* can improve memory, but its high doses do not have a memory-enhancing effect, due to its sedative effects. *Glycyrrhiza glabra* also has flavonoid compounds (27).

Moreover, the administration of scopolamine in this study had increased the total cholesterol and triglyceride levels in rats and the EALE could decrease their levels significantly. There are multiple reports about the relation between high cholesterol level and Alzheimer's disease. Studies have been shown that hypercholesterolemia may increase the predisposition to Alzheimer's disease. The new therapeutic strategies are aimed at lowering blood cholesterol level is gathering momentum for the management of Alzheimer's disease (28). Hence, the memory-enhancing effect of EALE can

be attributed to anti-acetylcholinesterase and also cholesterol-lowering activities of its constituents.

Conclusion

One can conclude that low dose (100 mg/kg) of Afghan EALE during a long-term administration period has memory-enhancing effect, reversed scopolamine-induced memory impairment in young rats and showed cholesterol-lowering properties. More studies seem to be needed to investigate the possible mechanisms responsible for the memory-enhancing property of this plant.

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