The effect of *Salvia officinalis* hydroalcoholic extract on scopolamine-induced memory impairment in adult male mice

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Abstract

Introduction: The role of medicinal plants in enhancement of memory and improvement of Alzheimer disease symptoms has attracted researchers' attention. Genus Salvia (sage) is the largest and most valuable type of herbal medicine from Lamiaceae family, and its therapeutic effects have long been considered. This study investigated the effect of hydroalcoholic extract of *Salvia officinalis* (*S. officinalis*) leaves on scopolamine-induced amnesia in adult male mice.

Materials and methods: A step-through inhibitory avoidance task was used for memory assessment. Animals received hydroalcoholic extract of *Salvia* (10, 20 or 40 mg/kg) 30 minutes after administration of scopolamine (1 mg/kg, intraperitoneally) after training and before testing, (based on experimental design). Animals were tested 24 h after the training session, and step-through latency in entering the dark compartment was recorded as passive avoidance memory. The data were analyzed by one-way ANOVA, followed by Tukey test. Statistical significance level was set at $P<0.05$.

Results: The results indicated that administration of scopolamine impaired both consolidation and retrieval of passive avoidance memory. Administration of 40 mg/kg hydroalcoholic extract of *Salvia* after training, or 20 and 40 mg/kg on the day of experiment ameliorated the effect of scopolamine.

Conclusion: The hydroalcoholic extract of *Salvia* can inhibit scopolamine-induced impairment of passive avoidance memory in mice.

Keywords: *Salvia officinalis*, Scopolamine, Mice, Memory impairment

Introduction

Alzheimer is a progressive neurological disorder that is followed by progressive degeneration of memory and neuronal death. It causes deep disorders in cognitive functions and memory, especially among the elderly (1). Central cholinergic system damage is one of the symptoms of Alzheimer, and reduction in cholinergic system function seems to be the major cause of cognitive disorders in this disease. On the other hand, in people with Alzheimer and other types of amnesia, the cholinergic neurons of the anterior part of brain undergo sever collapse (2). Scopolamine is a nonselective antagonist of muscarinic acetylcholine receptors that impairs memory and induces a kind of amnesia similar to Alzheimer (3). Scopolamine is administered to animal models to cause Alzheimer in order to

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investigate similar human amnesia (4, 5).
Most drugs used for Alzheimer focus on cholinergic system, but they are accompanied by side effects (6). That is why medicinal plants have been considered greatly for treatment of Alzheimer. Among these plants, *Salvia officinalis* (*S. officinalis*) with Anti-cholinesterase properties has been greatly taken into account (7, 8).
S. officinalis is a perennial herbaceous plant from Lamiaceae family with about 900 species in the world. It is the largest and most valuable pharmaceutical plant of Lamiaceae family (9), and its therapeutic effects have long been considered by humans. The leaves of Lamiaceae family, owing to widespread pharmaceutical properties, have been used in various studies. *S. officinalis* has been reported to have numerous pharmaceutical properties such as antimicrobial (10), wound healing (11) and anti-cancer (12, 13) properties, making it a good treatment of choice for diseases. In addition, experimental evidence is indicative of the effect of *S. officinalis* on learning. It has been shown that hydroalcoholic extract of this plant affects the learning process and memory in mice (14, 15). The extract of *S. officinalis* leaves has anti-cholinesterase properties, which can play a pivotal role in learning and memory (16, 17). Moreover, the antioxidant effects of *S. officinalis* have been reported in many studies, and many of the beneficial effects of this plant have been attributed to its antioxidant properties (18, 19).
Given the significance of Alzheimer disease in the society, recommendations on the use of traditional medicine and herbal treatments, anti-cholinesterase role of *S. officinalis* and abundant antioxidants in this plant, and that this plant is native to Iran, it is easy to prepare its extract and few studies have investigated its effects on memory and learning, the present research was conducted to evaluate the effect of hydroalcoholic extract of *S. officinalis* on scopolamine-induced amnesia in passive avoidance model.

Materials and methods

Ethics: All animal experimentations were conducted in accordance with institutional guidelines for animal care and use, which adhered to the international principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985).
In this experimental study, 140 male mice, with the weight range of 30-35 gr, were purchased from Tehran Pasteur Institute. The animals were transferred to the research animal house and kept in cages, eight animals in each cage, at controlled temperature of 23±2 °C under 12:12 dark/light cycle. Except for the time of experiment, they had free access to food and water. The experiments were performed in the light period from about 9:00 am to 14:00 pm, and each animal was used only in one period of experiment.
The drugs used in this study included scopolamine (Sigma, Germany) and hydroalcoholic extract of *S. officinalis* (Barij Essence Pharmaceutical Co). The drugs were prepared daily and a little before the start of experiment. Both drugs were dissolved in normal saline and administered intraperitoneally at 1 mg/kg concentration.
To measure retrieval in passive avoidance memory in this study, a shuttle box was used. It was composed of two chambers with 30×20×20 cm dimensions separated by a guillotine door through which the animal could pass freely when it was open. The walls and floor of one of the chambers (light chamber) was white and those of another one (dark chamber) was black. The floor of the dark chamber had parallel metal bars with 1 cm distance, which were connected to a stimulator by which electric shock was delivered to the animals.
Behavioral tests: In this method, analysis of memory was done in three stages as follow:
A) Adaptation: first, the animal was put in the light chamber and the guillotine

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door was opened slowly after 5 seconds, and the animal was allowed to move freely in the dark and light chambers for three minutes to adapt to the environment. If an animal did not enter the dark chamber after 100 seconds, it was excluded from the study.

B) Training: thirty minutes after adaptation stage, the animal was put in the light chamber, and as soon as the animal entered the dark chamber, the guillotine door was closed slowly and 0.2 mA electric shock was applied to the animal's feet for 3 seconds. After 20 seconds, the animal was removed from the dark chamber. Then, the animal was put in the light chamber after 2 minutes, and guillotine door was opened slowly. If the animal did not enter the dark chamber after 120 seconds, training was found to be successful; otherwise, the electric shock was repeated again as mentioned above. Administration of drugs was done on this day and immediately after training to evaluate the memory consolidation process.

C) Retrieval: to determine memory consolidation level, the animal was put in the light chamber 24 hours after the training session, the guillotine door was opened slowly after 10 seconds, and the time delay the animal entered the dark chamber for the first time was recorded as step through latency (STL). The cut-off point in this experiment was 600 seconds. The animals on which memory retrieval process was performed received scopolamine on the day of experiment and hydroalcoholic extract of *S. officinalis* after 30 minutes, following which memory measurement was carried out.

Experimental groups: To carry out the experiments, the mice were divided into the following groups, with eight mice in each group:

Experimental group 1) the effects of administration of different doses of hydroalcoholic extract of *S. officinalis* after training on consolidation of inhibitory avoidance memory were investigated in this group. One group of animals received normal saline twice at intervals of 30 minutes after training, and three groups first received normal saline and then hydroalcoholic extract of *S. officinalis* (10, 20 and 40 mg/kg) after 30 minutes.

Experimental group 2) the effects of intraperitoneal administration of scopolamine and different doses of hydroalcoholic extract of *S. officinalis* on consolidation of inhibitory avoidance memory were evaluated in this group. One of the animals received 1 mg/kg scopolamine immediately after training and normal saline after 30 minutes, and three groups received 1 mg/kg scopolamine after training and hydroalcoholic extract of *S. officinalis* (10, 20 and 40 mg/kg) after 30 minutes.

Experimental group 3) the effects of administration of different doses of hydroalcoholic extract of *S. officinalis* on consolidation of inhibitory avoidance memory before the experiment were investigated in this group. At the day of experiment, one of animals receive normal saline twice at intervals of 30 minutes, and three groups first received normal saline and *S. officinalis* (10, 20 and 40 mg/kg) after 30 minutes.

Experimental group 4) the effects of intraperitoneal administration of different doses of hydroalcoholic extract of *S. officinalis* and scopolamine before the experiment on consolidation of inhibitory avoidance memory were assessed in this group. One group of animals first received 1 mg/kg scopolamine and then normal saline after 3 minutes before the experiment, and three groups first received 1 mg/kg scopolamine and then *S. officinalis* (10, 20 and 40 mg/kg) after 30 minutes.

Statistical analysis

The differences among groups were analyzed by one-way ANOVA, followed by Tukey test. $P<0.05$ was considered significant for all tests. Statistical analyses

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were done by SPSS software, and Excel software was used to draw the graphs.

Result

As mentioned before, the time delay the animals entered the dark chamber was recorded as STL. Figure 1 shows the effects of administration of different doses of hydroalcoholic extract of *S. officinalis* on mean STL on the day of experiment. As indicated, administration of different doses of hydroalcoholic extract of *S. officinalis* alone could not significantly affect this index [F (1, 3) =0.519].

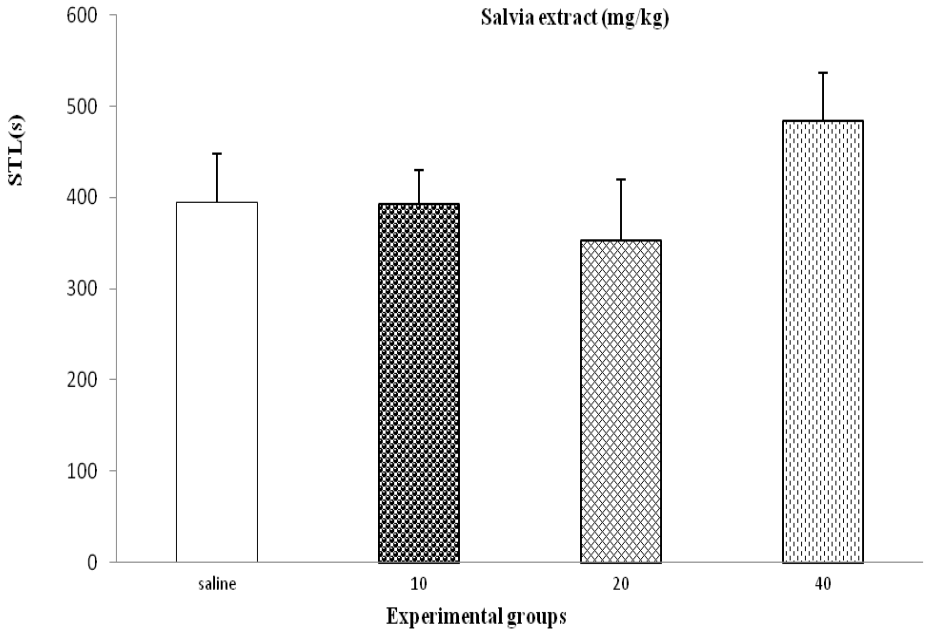

Figure 1. Effect of administration of hydroalcoholic extract of *S. officinalis* (10, 20 and 40 mg/kg, ip) on Step Through Latency (STL) in retrieval stage after training (n=8).

Figure 2 indicates the performance of animals in control group and groups receiving scopolamine with and without administration of hydroalcoholic extract of *S. officinalis* in the shuttle box. The statistical analysis showed a significant difference among the experimental groups [F (1, 4) =7.47, P<0.001]. The results of post-hoc test indicated administration of scopolamine changed the inhibitory passive avoidance memory, as it significantly reduced STL 24 hours later, i.e. it decreased memory consolidation in scopolamine group compared to saline group. Further, administration of hydroalcoholic extract of *S. officinalis* 30 minutes after scopolamine improved scopolamine-induced amnesia, as 40 mg/kg *S. officinalis* significantly increased the memory retrieval.

Figure 3 shows the effect of administration different doses of hydroalcoholic extract of S. officinalis on mean STL before experiment. As shown, administration of different doses of hydroalcoholic extract of S. officinalis did not significantly change memory retrieval despite increase in STL [F (1, 3) =1.34].

Figure 4 presents the performance of animals in control group and groups receiving scopolamine with and without administration of hydroalcoholic extract of *S. officinalis* in the shuttle box before experiment. The statistical analysis showed a significant difference among experimental groups [F (1, 4) =7.99,

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P<0.001]. The findings of post-hoc test showed administration of scopolamine altered the inhibitory avoidance memory, causing a significant decrease in STL 24 hours later. On the other hand, it significantly reduced memory in scopolamine group in comparison to saline group. Moreover, administration of hydroalcoholic extract of *S. officinalis* 30 minutes after scopolamine improved scopolamine-induced amnesia in a dose-dependent manner, and 20 and 40 mg/kg *S. officinalis* significantly increased memory retrieval.

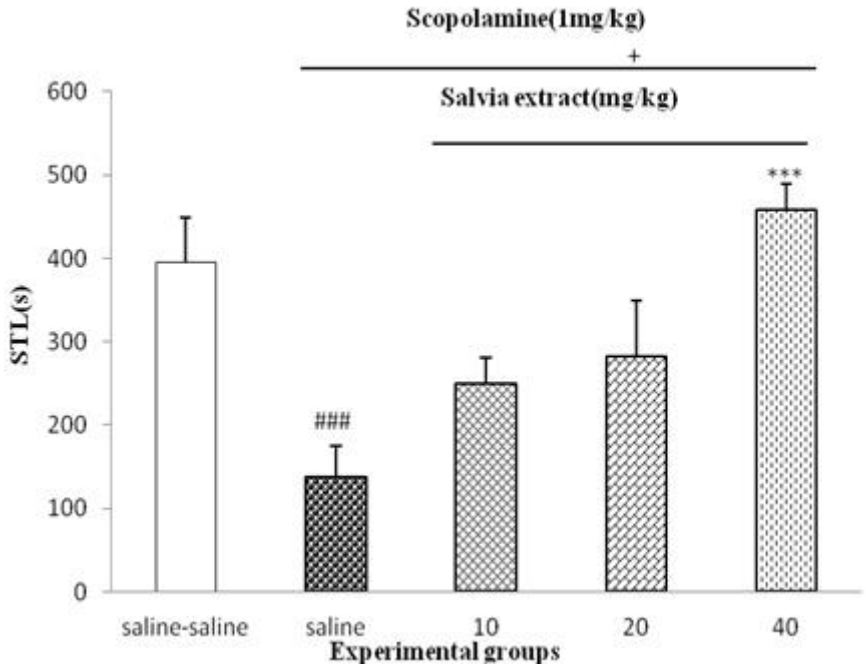

Figure 2. Effect of administration of 1 mg/kg scopolamine alone and in combination with *S. officinalis* (10, 20 and 40 mg/kg, ip) on mean Step Through Latency (STL) in retrieval stage after training (n=8). ***P<0.001: compared to scopolamine group, ###P<0.001: compared to saline group.

Discussion

Studies on memory have shown that administration of drugs immediately after training affect memory consolidation (20), but administration of drugs before memory test (24 hours after training) can influence the memory retrieval on the day of memory testing (21). Therefore, the present study evaluated the therapeutical effects of hydroalcoholic extract of *S. officinalis* on scopolamine-induced amnesia using a shuttle box after training and before the experiment separately. The same as previous studies (22, 23), intraperitoneal administration of scopolamine, as an antagonist of cholinergic receptors, impaired passive avoidance learning so that scopolamine reduced SLT on the day of experiment, which is indicative of impaired passive avoidance learning in the mice. On the other hand, administration of 40 mg/kg hydroalcoholic extract of *S. officinalis* on the training day increased STL compared to saline group. Also, administration of 20 and 40 mg/kg hydroalcoholic extract of *S. officinalis* in the testing day significantly improved the scopolamine-induced impaired learning so that mean STL was significantly increased in comparison with scopolamine-saline group.

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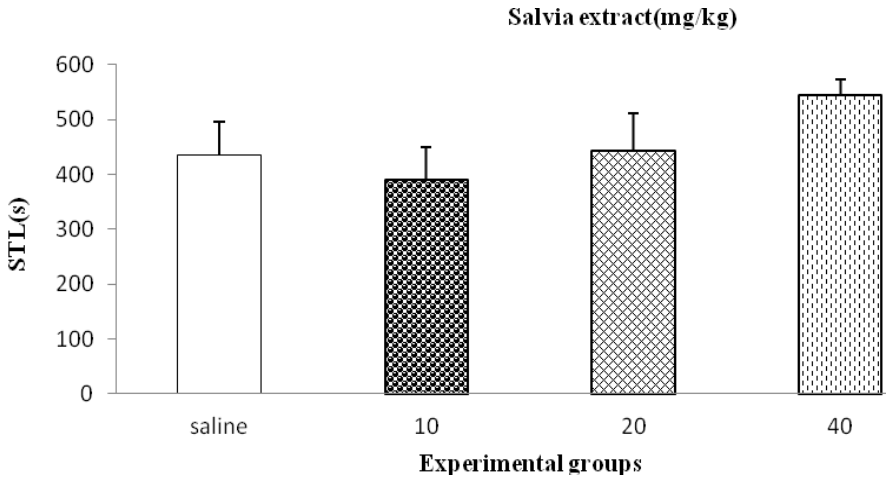

Figure 3. Effect of administration of hydroalcoholic extract of *S. officinalis* (10, 20 and 40 mg/kg, ip) on Step Through Latency (STL) in retrieval stage before training (n=8).

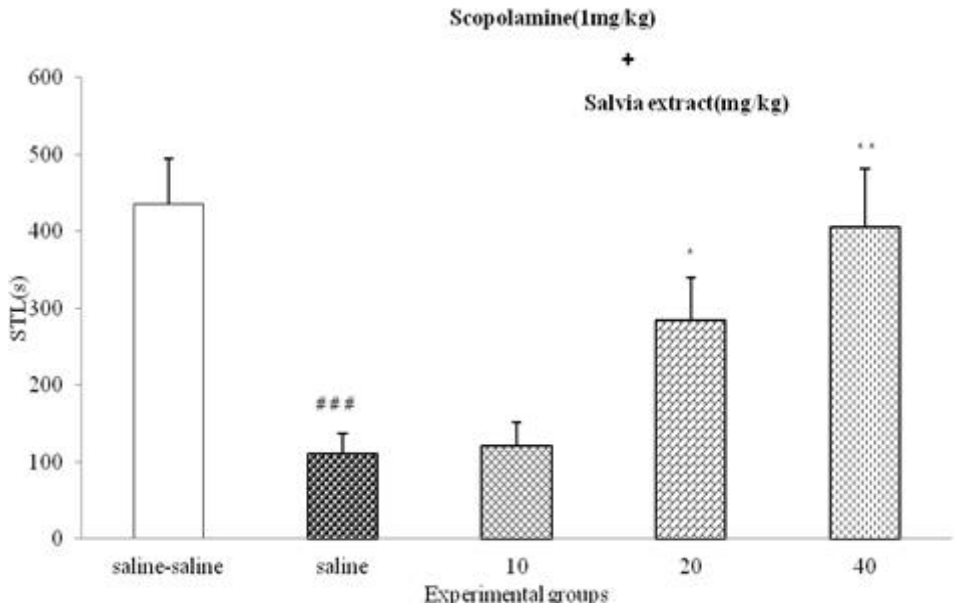

Figure 4. Effect of administration of 1 mg/kg scopolamine alone and in combination with *S. officinalis* (10, 20 and 40 mg/kg, ip) on mean Step Through Latency (STL) in retrieval stage after training (n=8), **P<0.01 and *P<0.05: compared to scopolamine group, ###P<0.001: compared to saline group

Nowadays, a special attention has been drawn to plant resources for treatment of diseases all around the world. Lamiaceae plants have long been used in traditional medicine and are currently being used extensively (24). Numerous studies have reported different species of Salvia from lamiaceae family, including Salvia Miltiorrhiz, Salvia Leriifolia Benth, Salvia Lavandulaefolia and *S. officinalis* have beneficial effects on memory and cognitive disorders (25, 26). It has also been shown that hydroalcoholic extract of *S. officinalis* reinforces the memory (14). The results of some studies have indicated that some species of Salvia such as Salvia Miltiorrhiza (25) and Salvia Leriifolia Benth reduce scopolamine-induced amnesia in mice. *S. officinalis* contains various compounds that play a pivotal role

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in its therapeutic properties (9). In vivo studies performed on animals have shown that hydroalcoholic extract of *S. officinalis* reduces acetylcholinesterase (27, 28, 17), thereby affecting memory and learning.

Tanshinones are a group of diterpenoids that are abundantly found in *S. officinalis* and are used for the treatment of many diseases. Kim et al. in 2007 showed that Tanshinones in species of Salvia cold improved scopolamined-induced memory impairment (25). Flavonoids are also of the numerous compounds of aerial parts of Lamiaceae family that have ameliorating effects on memory and learning (29, 30). Thus, a part of anti-amnesia effects of hydroalcoholic extract of Salvia in this study can be attributed to the presence of such important and beneficial compounds.

On the other hand, evidence indicates that free radicals are associated with cognitive disorders (31-34), and neurodegenerative diseases like Alzheimer and Parkinson are induced due to cell damages caused by the activity of free radicals (35). That is why treatment with antioxidants has been proposed as a therapeutic method for these diseases. Since hydroalcoholic extract of Salvia has strong anti-oxidant properties that can protect the nerve cells against impairments induced by aoxidative stress, it seems that this property can influence the effects of Salvia on memory.

However, further stduies are needed to determine the mechanisms of the effects of this compound. In brief, the findings of the present stduy indicated that hydroalcoholic extract of Salvia could imporve scopolamine-induced impaired learning and memory in a dose-dependent manner.

Conclusion

In general, the findings of this study showed that Salvia treated scopolamine-induced amnesia in passive avoidance model in a dose-dependent manner. Therefore, this plant can be used as a medication for reinforcement and improvement of memory and cognitive disorders owing to its important properties and beneficial effects on memory and learning.

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