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Original Article

Effects of extracts and manna of *Echinops cephalotes* on impaired cognitive function induced by scopolamine in mice

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Abstract

Background and purpose: Alzheimer's disease (AD) is a neurodegenerative disease specified by chronic and irreversible destruction of neurons. This study aimed to evaluate the effects of different extracts (aqueous, hydroalcoholic, hexane, and ethyl acetate) and manna of *Echinops cephalotes* (EC) on impaired cognitive function induced by scopolamine in mice. EC is shown to have anti-cholinesterase-butyrylcholinesterase activities.

Experimental approach: In this study, aqueous and hydroalcoholic extracts, hexane and ethyl acetate fractions of EC (25, 50, 100 mg/kg, i.p.), and the manna (25, 50, 100 mg/kg, gavage) were administered for 14 days alongside scopolamine (0.7 mg/kg, i.p.). Rivastigmine (reference drug) was administered for 2 weeks i.p. Mice were tested for their memory function using two behavioral models, object recognition test (ORT) and passive avoidance test (PAT).

Findings/Results: Administration of scopolamine significantly impaired memory function in both behavioral models. In the PAT model, all extracts at 50 and 100 mg/kg significantly reversed the effect of memory destruction caused by scopolamine. At a lower dose of 25 mg/kg, however, none of the extracts were able to significantly change the step-through latency time. In the ORT model, however, administration of all extracts at 50 and 100 mg/kg, significantly increased the recognition index. Only the manna and the aqueous extract at 25 mg/kg were able to reverse scopolamine-induced memory impairment.

Conclusions and implications: These results suggest that all forms of EC extracts improve memory impairment induced by scopolamine comparably to rivastigmine. Whether the effects are sustained over a longer period remains to be tested in future work.

Keywords: Alzheimer, *Echinops cephalotes*, Memory; Object recognition; Passive avoidance; Scopolamine.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease specified by chronic and irreversible destruction of neurons and also the most common type of dementia (1). In terms of pathology, there are several theories for AD, which include damage of cholinergic neurons, oxidative stress, accumulation of amyloid plaques, tau protein, inflammation, brain atrophy, reduction of steroidal hormones, and neurotoxicity caused by the neurotransmitter glutamate (2-4). Traditionally, medicinal plants have been used to increase memory and reduce the symptoms associated with Alzheimer's disease, the most famous examples are ginkgo, ginger, and *Boswellia_serrata* which have been also introduced to the market as supplements (5-8). Rivastigmine, donepezil, and galantamine are cholinesterase inhibitors currently approved for the treatment of mild, moderate, and severe AD, among them rivastigmine and galantamine are derived from plant sources (9,10). Antagonists of N-methyl-D-aspartate (NMDA) receptors (*e.g.* memantine) are other approved medicines for the treatment of moderate to severe AD (11).

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Echinops cephalotes (EC) belongs to the family of Asteraceae. The plant roots, leaves, fruits, and barks are used in traditional medicine to treat various diseases such as pain, inflammation, and a host of other conditions (12-14). Manna of the plant has been shown to possess antioxidant activities due to its saccharides especially trehalose (15). Aerial parts of the plant contain potentially active secondary metabolites, especially alkaloids such as echinopsine, echinopsidine, echinozolinone, taroxasterol acetate, and echinatisine derivatives; while the roots of the plant are the main source of thiophenes, terpenoid, and flavonoid constituents are generally found in the aerial parts (16). Due to their complex structures containing nitrogen, alkaloids are thought to be the most promising candidates for Alzheimer's treatment (17). Recent studies have shown that various extracts of the plant are potent inhibitors of acetylcholinesterase and butyrylcholinesterase (18).

Manna is a natural product that results from the living and activity of an insect named *Larinus vulpes Oliv.* on the plant. It is mainly composed of cellulose, starch, albuminoid materials, mucilage substances, sialic acid, some chlorophyll, a small amount of fat, and a significant amount of rare sugar, trehalose (around 25%) (15,19). In animal models, the manna of the plant has shown neuroprotective effects, however, its mechanism of action is still unclear. The dominant hypothesis is that trehalose protects neurons by inducing autophagy and clearing protein aggregates (20). Furthermore, due to the antioxidant effects of trehalose, researchers have investigated its role as an anticancer product (15). Scientific research has also shown that sialic acid can improve learning behaviors in animals. Sialic acid improves brain development and memory formation in premature babies, as well as strengthening the formation of synapses and the development of the nervous system, so it may have therapeutic potential for AD treatment (21). Due to the antioxidant and anticholinesterase effects of EC, in this study, we investigated the effect of the plant on impaired cognitive function induced by scopolamine in male mice. Scopolamine is a non-selective acetylcholine receptor antagonist that impairs memory and is administered in animal models to induce Alzheimer's in order to investigate amnesia in humans (22).

MATERIALS AND METHODS

Plant material

The aerial parts of EC were collected from the surrounding area of Khansar, Isfahan province, Iran in October 2020 and authentication was carried out by a pharmacognosy specialist at the Department of Biological Science, University of Isfahan (Voucher No. 4053). The manna of the plant was purchased from the local market of Khansar City and used after authentication by the pharmacognosy department. Different parts of the plant were washed, air-dried at room temperature, and turned into fine powder using an electric mixer.

Plant extraction

The fine powdered aerial part of the plant (1800 g) was macerated using water:ethanol solvent (80:20) three times (each time 48 h) at room temperature. The resulting extract was filtered using a Buchner funnel and was concentrated using a rotary evaporator and sanded with hexane (Hex) and ethyl acetate (EA) solvents. The concentrated extract was decanted with Hex and EA solvents 3 times, and the resulting fractions were rotated and dried again. To obtain the dry powder, the hydroalcoholic (HA) and aqueous (AQ) extracts were lyophilized by a freeze dryer and stored at -20 ℃ in a sterile container (23). To obtain the final concentration, an appropriate amount of the extract was diluted with normal saline (containing 2 drops of Tween 80 in 10 mL).

Qualitative phytochemical analysis

The preliminary phytochemical analysis of the crude extracts of the aerial parts of EC was carried out according to the method described by Harborne, Trease, and Evans. The extract and manna were analyzed for the presence of bioactive secondary metabolites, using standard phytochemical methods including Shinuda and Wilson-Taubook test for flavonoids, foam

production for saponins, Mayer's and Wagner's test for alkaloids, Baljet and Kedde-Reaction for cardiac glycosides, and Borntrager's test for anthraquinones (24,25).

Chemicals

Hyoscine (scopolamine) in the form of 20 mg/mL ampoules, was purchased from Exir Co. (I.R. Iran). Rivastigmine (Exelone, Novartis, Switzerland) was suspended in sterile water and used at 1 mg/kg in all experiments. Scopolamine, rivastigmine, and plant extracts were all dissolved in a normal saline solution (containing 2 drops of Tween 80 per 10 mL) to obtain the final concentration.

Animals

Male Syrian mice (25-30 g) were obtained from the animal house of Isfahan University of Medical Sciences. All animals were kept in polyacrylic cages, in an environment with a temperature of about 25 °C, and under a cycle of 12/12-h light/dark cycle and had free access to standard pellets feed and water. All procedures were approved by the Ethical Committee of Isfahan University of Medical Sciences (Ethical ID IR.MUI.RESEARCH.REC.1400.171) and were carried out according to internationally accepted principles for the use and care of laboratory animals. The animals were acclimatized in the laboratory for 1 week before the start of the behavioral experiments and were trained for both behavioral models.

Experimental design

Mice were randomly divided into 8 groups, each group comprising at least 6 mice as described below:

Group I, control group receiving 0.9% NaCl daily; group II, mice receiving scopolamine (0.7 mg/kg, i.p.) alone; group III, rivastigmine (1 mg/kg, i.p.) and scopolamine (0.7 mg/kg, i.p.); group IV, AQ extract of EC (25, 50, 100 mg/kg, i.p.) and scopolamine (0.7 mg/kg, i.p.); group V, HA extract of EC (25, 50, 100 mg/kg, i.p.) and scopolamine (0.7 mg/kg, i.p.); group VI, Hex fraction of EC (25, 50, 100 mg/kg, i.p.) and scopolamine (0.7 mg/kg, i.p.); group VII, the EA fraction of EC (25, 50, 100 mg/kg, i.p.) and scopolamine (0.7 mg/kg, i.p.); group VIII, the manna of EC (25, 50, 100 mg/kg, gavage) and scopolamine (0.7 mg/kg, i.p.). Rivastigmine, EC extracts, and the manna were administrated 1 h after scopolamine injection. The extracts and the manna or rivastigmine were administered once daily during the experimental period (14 days). Scopolamine administration was completed for 14 days. The cognitive and memory functions of mice were evaluated 30 min after the administration of scopolamine. All solutions were prepared fresh on the day of the experiment and administered i.p. except for the manna that was gavaged, in a volume of 0.1 mL/10 g mice body weight. The doses of EC were based on our preliminary work in mice.

Passive avoidance test

The test was done using the method introduced by Safavi *et al*. with minor changes (26).

Passive avoidance test (PAT) is a performance style in which, in the presence of a fear stimulus, the animal avoids performing that action. This test is used to evaluate long-term and short-term memory. According to this test, the animals learn to avoid entering a chamber without light in which they were previously punished. This test was carried out in a device called a shuttle box manufactured by Technik-Azma, Iran. The device consists of two chambers of equal size (25 × 25 × 20 cm) separated by a guillotine gate (6 × 7 cm) (Fig. 1). One of the chambers has no light and the other has LED. The floors of the dark and light rooms were made of stainless-steel rods (diameter 3 mm) spaced 1 cm apart. The rods of the dark chamber are connected to the electronic source.

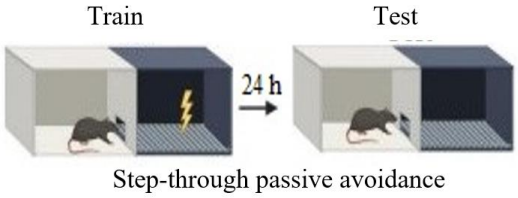

Fig. 1. Passive avoidance test design. Shuttle box is a flexible system for passive avoidance experiments. It comes with two independent grid floors that allow for flexible adverse stimuli. The cage contains a visual stimulus (light) that functions separately for each compartment. Rodents in the cage are detected by a highly sensitive sensor.

The position of the animal is detected by a high-sensitivity photoelectric transducer. The shuttle box is controlled by a microprocessor-based controller (SB100) with a touch screen. In the train phase, the animal is placed in a light chamber and after 5 s the guillotine gate is opened and the rodent passes through the gate out of curiosity and enters the dark chamber. The time spent during the detection and entry of the mouse into the dark chamber was recorded as latency time (LT). At this time, the door sensor detected the mouse passing and closed the door. Then, after 3 s, the mice were given a shock (1 mA for 5 s). Then, the mouse returned to the cage until the test phase. Rodents that did not enter the dark chamber after 180 s were excluded from the study. The test phase was performed 24 h after the training phase. In the test phase, the rodent was placed in the light chamber with a closed door. After 5 s, the door was opened and the first time the rodent entered the dark chamber was recorded as the LT. The number of crossings between the chambers and the total time the rodents spent in the dark and light chambers were recorded. The experiment continued for 5 min. After each practice and experiment, the chambers were cleaned with 70% ethanol.

Object recognition test

The object recognition test (ORT) was performed based on Leuptow's study (27), with some changes. In this experiment, the animal's ability to recognize between new and old objects is checked. Also, there is no intervening stimulus, and the test is performed simply by placing the mouse in the arena without introducing any external stimulus. The experiment was conducted in a circular field with a diameter of 32 cm and a height of 20 cm (Fig. 2). Easy evaluation was achieved by using a video tracking camera. Twenty-four h before the training trial, the mice were allowed to acclimatize to the space for 10 min and then returned to the cage. In the training trial, two identical objects were placed in the arena at a distance of 10 cm from each other and 5 cm from the wall. The objects were made of glass (height 4 cm). Then, we added the mouse to explore the experimental space where the objects were located. Exploration and identification had criteria such as touching and smelling the object, and it is also necessary to mention that considering the object a part of the space is not counted as a criterion of identification. In the training trial, the mice must explore for at least 5 s. The animals were removed and then returned to the cage, and after 1 h, the test trial was performed by presenting a familiar object and a new object (different in size and shape from the familiar object). The new object was made of plastic. As soon as the mice explored the two objects for 5 min, the experiment was completed. Among several identical objects, a pair of objects is randomly selected at any time and we use probability and mathematics to measure the final effects of the drugs. After each trial, all the objects and the field were cleaned with ethanol 70%. Finally, the discrimination index (DI) and the recognition index (RI) were recorded. Percentages of DI and RI were calculated as follows where N and F are the exploration time of the novel and familiar objects, respectively.

$$DI = \frac{N - F}{N + F} \times 100 \quad (1)$$

$$RI = \frac{N}{N+F} \times 100 \quad (2)$$

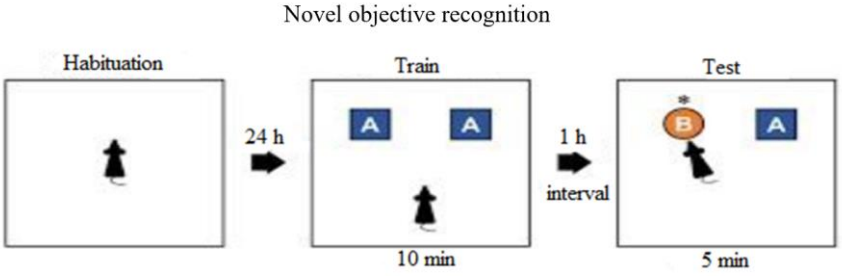

Fig. 2. Object recognition test design. The ORT takes place over 2 days. For habituation, a mouse was allowed to explore the open field for 10 min, followed by a training trial 24 h after the habituation session using 2 identical objects. Testing was performed 1 h after training, in which mice were allowed to explore the arena with one of the familiar objects and one novel object.

Statistical analyses

Statistical analysis for multiple comparisons was performed using a one-way ANOVA test followed by a Tukey post hoc test to measure two behavioral models using GraphPad Prism software. All data are expressed as mean ± SEM. *P*-values < 0.05 were considered statistically significant. Also, the Student's t-test was used to compare two groups.

RESULTS

Qualitative phytochemical analysis

The presence of flavonoids was verified by revealing fluorescence and intense red color in the plant extract, while the formation of a cloudy and clear precipitate with the reagents indicated the presence of alkaloids in manna and plant extract. According to the results, the plant extract and manna did not contain saponins, cardiac glycosides, and anthraquinones.

Effects of EC extracts on step-through LT during training session in PAT

The effects of administration of manna, AQ, HA, Hex, and EA extracts of EC at 25, 50, and 100 mg/kg on LT during the training phase (on day 14) is shown in Fig. 3. Administration of different fractions of EC at higher doses than 100 mg/kg proved to be toxic in animals, therefore in the following experiments does of 25, 50, and 100 mg/kg were used. As explained in the methods section, 30 min after administration of test compounds, the training session was started by placing the animals in the lightened chamber. Injection of scopolamine led to a significant decrease in LT ($P < 0.05$, compared to the control group), which was completely reversed by rivastigmine ($P < 0.001$). All plant extracts (except Hex at 25 mg/kg) as well as the manna of EC at doses of 25, 50, and 100 mg/kg, significantly enhanced LT during the training phase ($P < 0.001$ compared to the scopolamine). The AQ extract and the manna of the plant were only effective at 100 mg/kg in increasing the LT values when compared to the control group ($P < 0.05$ and $P < 0.01$, respectively).

Effects of EC extracts on step-through LT in PAT

The test was carried out 24 h after the training phase. As shown in Fig. 4, the LTs during the testing phase were significantly reduced by injection of scopolamine compared to control values ($P < 0.001$), rivastigmine fully reversed this lag in memory ($P < 0.001$). In a similar fashion to rivastigmine, chronic administration of all extracts and manna of EC at 50 and 100 mg/kg reversed the memory impairment induced by scopolamine. The extracts and the manna at 25 mg/kg, on the other hand, were ineffective in reversing the scopolamine-induced memory impairment. The LT values in the presence of extracts and the manna at 50 mg/kg were somehow very close to rivastigmine values and in some cases even greater (Fig. 4).

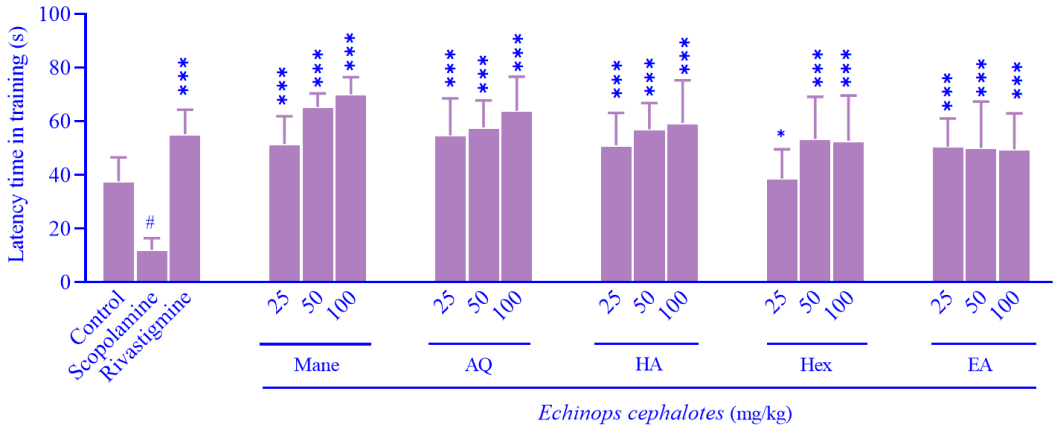

Fig. 3. The effect of manna and different extracts of *Echinops cephalotes* on latency time during the training phase in passive avoidance test. Data were measured by the latency time (s) required for the animal to enter the dark chamber. Data represent mean ± SEM, n = 6. Scopolamine was injected (0.7 mg/kg) before the extracts for 14 days. $^{\#}P < 0.05$ Indicates a significant difference compared to the control group; $^{*}P < 0.05$ and $^{***}P < 0.001$ versus scopolamine group. AQ, Aqueous extract; HA, hydroalcoholic extract; Hex, hexane extract; EA, ethyl acetate extract.

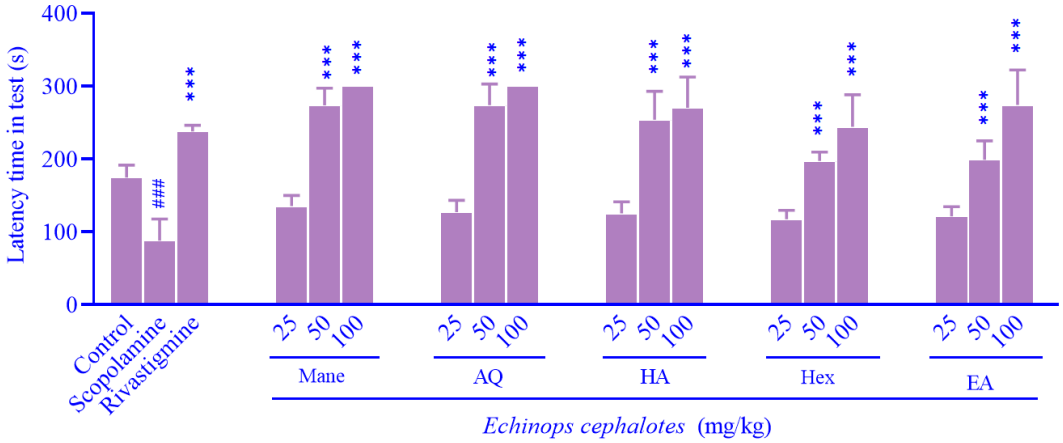

Fig. 4. The effect of manna and different extracts of *Echinops cephalotes* on latency time (test) in the passive avoidance test. Data were measured by the latency time (s) required to enter the dark chamber. Data represent the mean ± SEM, n = 6. Scopolamine was injected (0.7 mg/kg) before the extracts for 14 days. $^{###}P < 0.001$ Indicates a significant difference compared to the control group; $^{***}P < 0.001$ versus scopolamine group. AQ, Aqueous extract; HA, hydroalcoholic extract; Hex, hexane extract; EA, ethyl acetate extract.

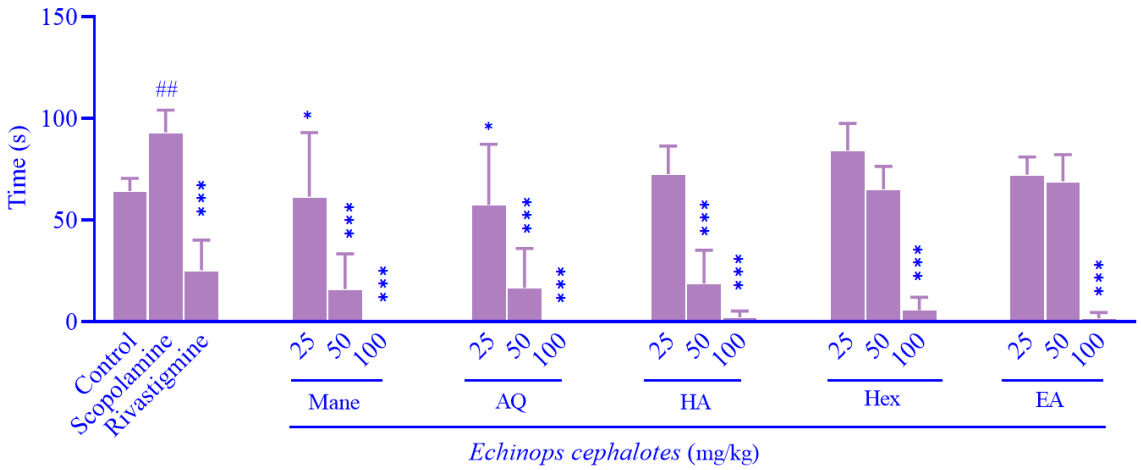

Fig. 5. The effect of manna and different extracts of *Echinops cephalotes* on the time spent in the dark zone of the passive avoidance test. Data represent the mean ± SEM, n = 6. Scopolamine was injected (0.7 mg/kg) before the extracts for 14 days. $^{###}P < 0.001$ Indicates a significant difference compared to the control group; $^{***}P < 0.001$ versus scopolamine group. AQ, Aqueous extract; HA, hydroalcoholic extract; Hex, hexane extract; EA, ethyl acetate extract.

Effects of EC extracts on the time spent in the dark chamber in PAT

Figure 5 demonstrates the time that mice spent in the dark chamber in PAT. An increase in the time spent by mice in the dark chamber of the equipment is another indication of memory impairment. Administration of scopolamine resulted in a 45% increase in the time that the animal spent in the dark chamber ($P < 0.01$, compared to the control group). Rivastigmine at 1 mg/kg significantly reversed this time to a value lower than the control group ($P < 0.001$). All extracts as well as the manna of EC at 100 mg/kg (the maximum experimental dose) significantly reduced the time spent in the dark chamber ($P < 0.001$ compared to the scopolamine-treated group). At lower doses of 25 and 50 mg/kg, only AQ extract and the manna of EC were effective in significantly reducing the time spent in the dark chamber compared with the scopolamine-treated group ($P < 0.05$).

Effects of EC extracts on the time spent in the light chamber in PAT

Figure 6 shows the effect of extracts and manna of EC at three doses on the time spent in the light chamber. The reduction in this time is another indication of loss in memory by animals. In comparison to the control group,

scopolamine reduced the time in the light compartment by 13% ($P < 0.01$). Rivastigmine at 1 mg/kg increased the time spent in the light chamber by 33% ($P < 0.001$, compared with the scopolamine group). All the extracts and manna of EC at 100 mg/kg significantly increased the time spent by animals in the light chamber ($P < 0.001$, compared to the scopolamine group). At a lower dose of 50 mg/kg, however, all extracts except the EA of the plant significantly reversed the scopolamine-induced reduction in time in the light chamber to various degrees ($P < 0.05$). None of the extracts or manna at 25 mg/kg, however, was effective in reversing the scopolamine-induced time spent in the light chamber.

Effects of EC extracts on RI in ORT

RI which assesses the level of discrimination against the familiar object during the test phase was carried out next. As shown in Fig. 7, scopolamine significantly decreased (37%) the discrimination against the familiar object ($P < 0.05$, compared to the control group). The manna and all the extracts of the plant at 100 mg/kg significantly increased the percentage of time spent with the novel object and somehow reversed the loss of memory induced by scopolamine ($P < 0.001$, in comparison with the scopolamine). At a lower dose of 50 mg/kg, only the manna and AQ were effective in reversing the scopolamine-induced reduction in RI ($P < 0.001$ and $P < 0.01$, respectively, in comparison with the scopolamine).

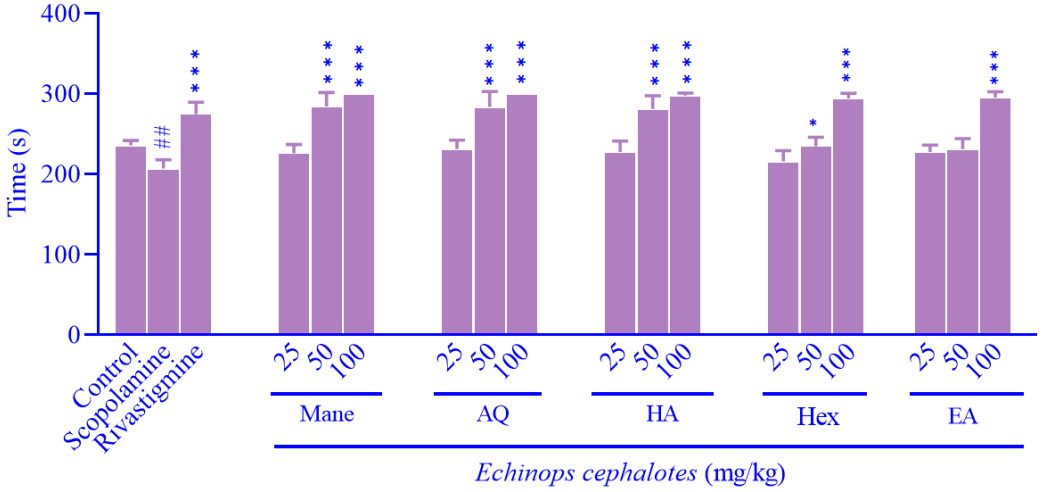

Fig. 6. The effect of manna and different extracts of *Echinops cephalotes* on the time spent in the light zone during the passive avoidance test. Data represent the mean ± SEM, n = 6. Scopolamine was injected (0.7 mg/kg) before the extracts for 14 days. $^{\#\#}P < 0.01$ Indicates a significant difference compared to the control group; $^{*}P < 0.05$ and $^{***}P < 0.001$ versus scopolamine group. AQ, Aqueous extract; HA, hydroalcoholic extract; Hex, hexane extract; EA, ethyl acetate extract.

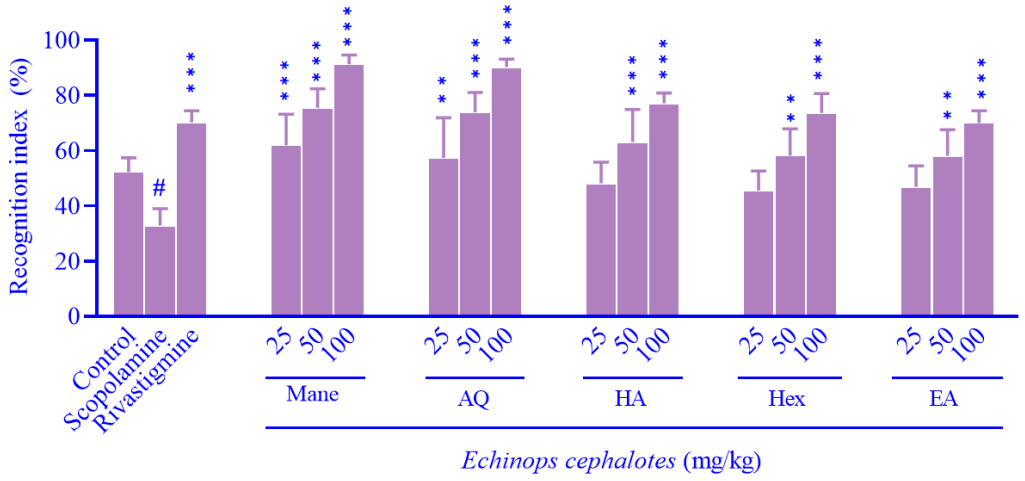

Fig. 7. The effect of manna and different extracts of *Echinops cephalotes* on the recognition index in the object recognition test. Data represent mean ± SEM, n = 6. Scopolamine was injected (0.7 mg/kg) before the extracts for 14 days. $^{\#}P < 0.05$ Indicates a significant difference compared to the control group; $^{**}P < 0.01$ and $^{***}P < 0.001$ versus scopolamine group. AQ, Aqueous extract; HA, hydroalcoholic extract; Hex, hexane extract; EA, ethyl acetate extract.

DISCUSSION

Among the various hypotheses for the pathophysiology of AD, the three hypotheses of amyloid beta, tau protein, and cholinergic are more prominent (2-4). Plants provide a rich source of bioactive compounds that can be considered for the treatment of neurological disorders such as AD (28,29). EC is one of these plants that is shown to contain about 151 compounds with different biological activities. Rich source of nitrogen-containing alkaloids in extracts of leaves stems, flowers, and seeds of this genus with potent anticholinesterase and antibutyrylcholinesterase enzyme activities encouraged us to study its action on memory-impaired mice. The manna of the plant with alleged antioxidant activity was also included in this study (14,15,18).

In parallel with previous reports, a single dose injection of scopolamine in this study (Figs. 3-7) caused a cognitive deficit in both models of memory performance, including PAT and ORT (30-35). Scopolamine is a nonselective muscarinic antagonist that decreases cholinergic transmission in the central nervous system and impairs cognitive function, including long-term memory loss in various paradigms: radial maze, ORT, and spatial tasks, Morris water maze, and PAT (36-41). In PAT, the scopolamine group showed the same performance as we expected, as the mice had a shorter time to enter the dark chamber (lower latency). The same result was obtained for the ORT; since RI was significantly affected by scopolamine. Scopolamine damages cholinergic neurons in the cerebral cortex, leading to learning and memory impairment in rodents. This results in damaging the hippocampus nerves and eventually leads to memory loss and learning problems (42). Also, according to previous documents, the effect of scopolamine on memory impairment can be related to the high level of lipid peroxidation and the low amount of antioxidants in the brain of mice (36). Rivastigmine improved learning in PAT and ORT in mice treated with scopolamine. Administration of rivastigmine completely reversed the significant reduction in RI values observed in scopolamine-treated animals in the ORT model. It is well known that rivastigmine enhances cholinergic function by prolonging the activity of endogenously released acetylcholine. An increase in acetylcholine in turn, is thought to modulate glutamatergic function as well as acting on muscarinic receptors and therefore enhancing the NMDA-mediated component of excitatory postsynaptic potential. Furthermore, acetylcholine is thought to play a prominent role in neocortical excitability through complex interactions with glutamatergic pathways (43).

To study the neuroprotective effects of EC, different extracts and manna were administered for 14 days before conducting two behavioral tests after scopolamine injection. The PAT is used to investigate non-spatial long-term memory after an unpleasant experience (44). The ORT relies on rodents' natural propensity for novel exploration and is very similar to that used in the study of human cognition, enhancing the ecological validity of this test over many other rodent memory tests (27). In both behavioral paradigms, AQ extract and the manna were most effective in reversing scopolamine-induced memory impairment in comparison with other components of the plants. The memory-ameliorating effect of EC could be attributed to various components of the plant. In this genus, there are compounds such as quinazoline alkaloids with a structure similar to acetylcholine that could mimic the effects of acetylcholine by inhibiting the cholinesterase enzymes. The total flavonoid content of EC in the stem and the leaf extract is thought to be very high. Flavonoid content of the plant has strong antioxidants, anti-inflammatory, and anticancer properties (18).

The polar fraction of the plant also carries effective concentrations of alkaloid compounds. Analysis of different parts (leaf, stem, flower, and seed) of the plant shows potential activity for acetyl- and butyryl-cholinesterase inhibition. Both enzymes are effective in acetylcholine degradation, so we expect that dual inhibitors, which inhibit both enzymes, can increase acetylcholine levels and ultimately provide greater clinical efficacy (18). In the present study, mice were used as an animal model to test memory performance, while most animal studies related to memory performance were conducted on rats. Snyder *et*

al. reported that during learning, more neurons are involved in the rat brain than in the mouse brain, and their nerve cells mature faster (45). According to the research conducted by Ellenbroek *et al*., rats are better options for cognitive tests than mice since they have more stable performance over time and are less affected by distractions, which can be a more suitable model than mice in understanding how memory and learning work (46). In the current study, the administration of extracts and manna at 25 mg/kg proved effective in improving memory performance only ORT model. Contrary to this, a 25 mg/kg dose of extracts did not change the memory performance in the PAT model. Therefore, apart from the composition of extracts and manna which are crucial, the type of testing paradigms is also important.

CONCLUSION

The current study showed that the effect of EC on memory in mice is somehow dependent on the type of model that is used. In the ORT model, EC as low as 25 mg/kg provided a modest improvement in memory performance, an effect that is not seen in the PAT paradigm. This study suggests that acute administration of this typical daily dose may have a significant effect in the long run and be used in clinical studies.

Acknowledgments

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

M. Rabbani supervised the pharmacological experiments and prepared the manuscript. M. Sadeghi supervised the pharmacognosy studies and G. Sadeghi carried out all the experimental work. All authors read and approved the finalized article.

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