Original Article

Evaluation of the effect of *Cyperus rotundus* L. in scopolamine-induced learning deficit in mice

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Abstract

Background: *Cyperus rotundus* L. was used in traditional Iranian medicine to treat memory and cognition disorders. The present study was aimed at investigating the effect of the extract and essential oil of *C. rotundus* on memory dysfunction.

Materials and Methods: Cognition was evaluated using the object recognition task that was composed of a square wooden open field box with different shape objects. The test was consisted of three sections: 15 min exploration, first trial for 12 min and second one for 5 min. In the second trial the difference in exploration between a previously seen object and novel one, was considered as an index of memory performance (recognition index). Memory deficit was induced by scopolamine (0.5 mg/kg) before injection of plant extracts and essential oil.

Results: Rivastigmine at 0.6 mg/kg reversed the scopolamine induced memory dysfunction in mice ($P < 0.05$). On the contrary, neither the hydroalcholic extracts (100, 200, 400 mg/kg) nor the polyphenolic extract (50, 100, 200 mg/kg) and essential oil (10, 20, 40 mg/kg) of *C. rotundus* produced significant improvement of memory dysfunction. The fact that rivastigmine reversed the scopolamine-induced memory dysfunction confirms the validity of this memory paradigm.

Conclusion: Using the current method of the memory evaluation, none of the tested doses of the plant extract or essential oil changed the memory status of the animals, indicating either a lack of effective ingredient or unsuitable method for evaluation.

Key Words: *Cyperus rotundus*, memory dysfunction, object recognition, scopolamine

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INTRODUCTION

Memory is one of the most important and complicated behavioral process that study of its physiologic and anatomic principles can help prevents memory loss in different conditions. The cause for most memory deficient cases (such as Alzheimer, Parkinson, amnesia, and dementia) is still unknown. Accumulation of amyloid proteins that causes the degeneration of cholinergic neurons, which in turn results in cholinergic deficiency and synapse loss is the best know hypothesis that has been put forward.[1] Based on these studies, acetylcholinesterase (AChE) inhibitors have been used to treat patients with cognitive impairment.[2] Classical AChE inhibitor drugs however, provide short-term alleviation of symptoms without decelerating the progression of

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the disease.[3] Therefore, the development of new drugs that somehow counteract the progression of the disease would be highly desirable for treating such patients. Mental stimulation, exercise and balanced diet have been suggested as other useful ways to delay symptoms.[4,5]

Cyperus rotundus L. (family *Cyperaceae*), also known as purple nutsedge or nutgrass, is a common perennial weed and scaly creeping rhizome. The tubers are externally brown in color and reddish white inside with a characteristic odor.[6] The rhizome of *C. rotundus* is a well-known functional food and traditional herbal medicine in Asia.[7] In traditional herbal medicine in Iran, the rhizome of *C. rotundus* is used for memory, dermatological and psychological disorders.[8,9] The rhizome of this plant has been reported to show antioxidant and free radical scavenging activities, these effects play a major role in protection of neurodegenerative disorders.[10] It addition to these, the plant has also antifungal, anti-inflammatory, anti-diabetic, cyto protective, anti-microbial, antioxidants, and cytotoxic properties.[6,11,12] Considering the wide-spread central action of the plant and in order to verify its traditional use as a memory enhancer, the present study was undertaken to determine the effect of *C. rotundus* on memory performance after scopolamine-induced neuronal damage.[4] Memory is assessed by the novel object recognition task that originally developed by Ennaceur and Delacour in 1988 and it is based on natural tendency of rodent to explore a novel object more than familiar one.[13]

MATERIALS AND METHODS

Animals

Male mice (8-12 weeks, Pasteur Institute, Tehran, Iran) with average weight of 18-35 g were housed in cage of six in the experiment place a day before study. Tap water and standard food pellets were available *ad libitum*. All the studies were performed between 8 AM and 12 AM in a special noise-free room with controlled illumination. There were at least six mice in each group.

Drug treatments

As a control group mice was treated with saline, and for inducing memory dysfunction scopolamine was injected at doses 0.5 mg/kg and 1 mg/kg (the dose 1 mg/kg was administered only for negative control group). For positive control group rivastigmine was administered 0.6 mg/kg and the test groups were injected with hydroalcholic extracts (100, 200, 400 mg/kg), polyphenolic extracts (50, 100, 200 mg/kg) and essential oil (10, 20, 40 mg/kg) 5 min after scopolamine injection. Then the animals were kept in their home cage for 25 min and finally the mice were placed in the test box for starting T1 [Figure 1].

Sample preparation

Scopolamine sulphate (Daru pakhsh, Iran) was diluted in normal saline (0.9%). Rivastigmine (Modava, Tehran, Iran) and was prepared in normal saline 0.9% and tween 80 (1% v/v) containing 1% (w/v) methylcellulose.[14] All polyphenolic extracts were diluted with water, hydroalcholic extracts and essential oil were diluted with water and tween 80 (1% v/v). Control animals received either normal saline or vehicle. All of the drugs were injected intraperitoneally (i.p.).

Tubers of *C. rotundus* were collected on October 2010 from plants growing wild in Baharan homestead in Kelishad area in Isfahan province in Iran at altitude of ca 1650 m. The plant was authenticated by Dr. Lili Ghaem maghami (Biology Department, Isfahan University, Iran), as *C. rotundus L.* The plant herbarium was deposited in the School of Pharmacy, at Isfahan University of Medical Sciences with code number of 2262.

For preparation of hydroalcholic extract, air-dried and powdered tubers of plant (300 g) were macerated with Ethanol: H_2O (7:3) for 48 h. The extract was then shacked, filtered and evaporated in a rotating evaporator.[15] For preparation of the polyphenolic extract, air-dried and powdered tubers of the plant (300 g) were extracted in two steps: firstly with Ethanol: H_2O (9:1) and secondary with Ethanol: H_2O (1:1) For 48 h. The two extracts were combined and evaporated to about 1/3 of the original volume. The resultant aqueous extract was cleared by extraction in a separating funnel with chloroform and then evaporated to dryness under reduced pressure in a rotating evaporator.[16] The essential oil was isolated by hydrodistillation of the air-dried powdered tubers of the plant for 3-4 h based on our previous studies. Essential oil sample was homogenized and dried over anhydrous sodium sulfate and stored in fridge.[15]

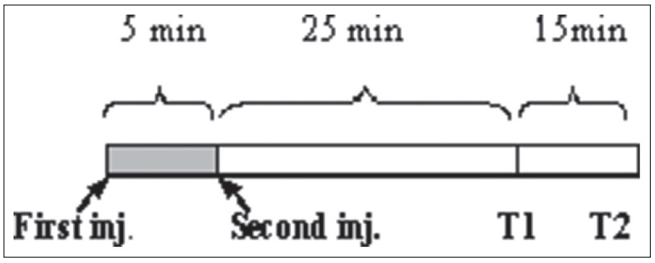

Figure 1: Effect of rivastigmine on scopolamine induced memory deficit. The first injection was made 30 min prior to test and consisted of saline for control groups and scopolamine for others. This was followed 5 min later by a second injection (rivastigmine, extracts, and essential oil). The animals were kept in the home cage for 30 min before starting the test

Object recognition test

Novel object recognition task is a relatively sensitive procedure for evaluating compounds effects on cognition activity. The apparatus was consisting of square wooden cage (35 × 35 × 40 cm) with the black walls, which located in noise free room. The day before the test, each animal was submitted to a habituation session in the open field and allowed to freely explore the arena in the presence of two objects for at least 15 min on experimental day, animals were submitted to two trials spaced by an intertribal interval (15 min). During the first trial (acquisition trial, T1), animals were placed in the arena containing two identical objects for an amount of time necessary to explore the objects for 20 s. Mice that did not explore the objects for 20 s within the 12-min period was exclude from the experiments. Exploration is defined as the animal directing the nose within 2 cm of the object while looking at, sniffing, or touching it. For the second trial (test trial. T2), one of the objects presented in the first trial was replaced by a new object, animals were placed back in the arena for 5 min and total time spent in exploration of each object was determined. Animals behavior were recorded by using an ordinary web camera mounted above the experimental apparatus, records were analyzed later.[17]

Data processing and statistical analysis

The parameters were measured, time required to achieve 20 s of object exploration on T1, time spent in active exploration of the familiar (F) or novel (N) object on T2. Recognition index (RI) was defined using the following formula: (N – F)/(N + F) ×100. All the procedures were recorded using a camera, which was placed above the experimental apparatus.[17] T1 was recorded for each mouse, RI was calculated, and the data were analyzed using two-way ANOVA and student *t*-test, $P < 0.05$ was considered as significant result. Results were expressed as mean + SEM. Sigma stat software was used for statistical analyses.

RESULTS

The essential oil of *C. rotundus* was a yellow liquid bearing the characteristic pungent and aromatic odor of *Cyperaceae* plants. The essential oil content of the plant was 0.2% (w/w) yield. Evaporation and solvent removal of hydroalcholic extract gave a semisolid mass with a yield of 19.0% (w/w) and yield of polyphenolic was 8.3% (w/w).

Effect of scopolamine on RI

In this study, mice received 0.5 mg/kg and 1 mg/kg of scopolamine were significantly different with saline group [Figure 2]. Figure 2 shows the RI in the second trial of object recognition task after scopolamine.

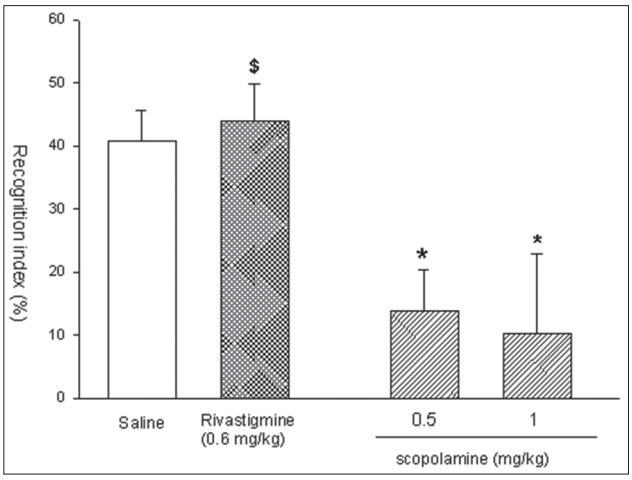

Figure 2: Effect of different doses of scopolamine on memory performance (expressed as recognition index) and effect of rivastigmine (0.6 mg/kg) on reversal of memory deficit induced by scopolamine. Results are expressed as mean + SEM ($n = 6$). $^{*}P < 0.05$ compared with saline. $^{\$}P < 0.05$ compared with scopolamine

The ANOVA revealed a significant treatment effect ($P < 0.05$). The RI score was decreased in the scopolamine condition compared to the saline condition, as shown by the *post-hoc* analysis.

Effect of rivastigmine on RI

Based on previous studies 0.6 mg/kg of rivastigmine was used to test reversal of memory impairment by scopolamine. At this dose the memory was reversed to control level. There was no significant difference between rivastigmine and saline.

Figure 2 shows the RI in the second trial of object recognition task after scopolamine and rivastigmine administration. The ANOVA revealed a significant treatment effect ($P < 0.05$). The RI score was increased in the scopolamine induced memory deficit compared to the saline condition.

Effect of extracts and essential oil in scopolamine-induced memory deficit

The RI of mice received 50, 100, and 200 mg/kg of polyphenolic extract, 100, 200, and 400 mg/kg hydroalcholic extracts and 10, 20, and 40 mg/kg essential oil of *C. rotundus* were not significantly different with saline [Figures 3-5].

DISCUSSION

Herbal drugs are proved to be of great commercial and pharmacological importance. Traditional herbal medicine forms an important part the health-care system. Plants have been used for centuries as remedies for human diseases.[18] *C. rotundus*, a member of *Cyperaceae* family is grown throughout the world from tropics to temperate regions. In Iranian traditional medicine *C. rotundus* has long been used

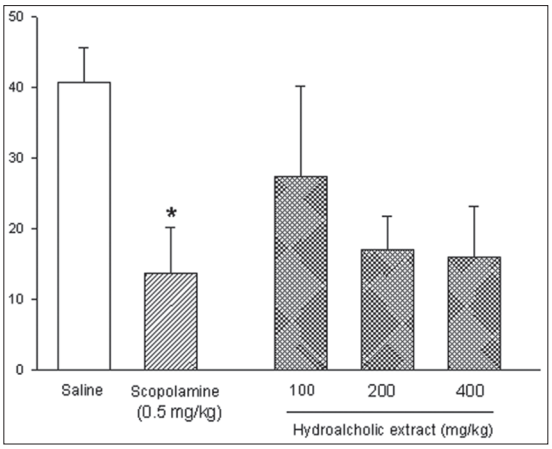

Figure 3: Effect of scopolamine on memory performance (expressed as recognition index) and effect of different doses of *Cyperus rotundus* hydroalcholic extract on reverse of memory deficit induced by scopolamine. Results are expressed as mean + SEM ($n = 6$). *$P < 0.05$ compared with saline

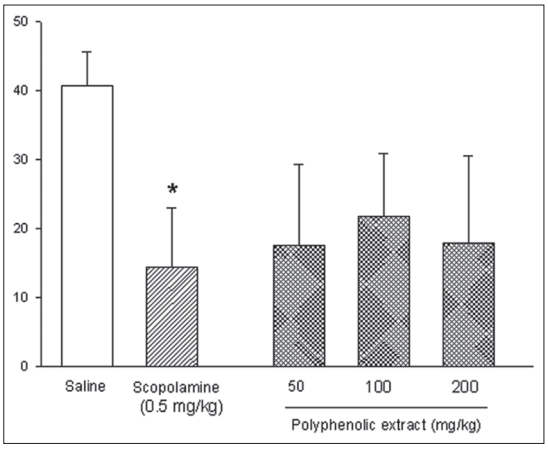

Figure 4: Effect of scopolamine on memory performance (expressed as recognition index) and effect of different doses of *Cyperus rotundus* polyphenolic extract on reverse of memory deficit induced by scopolamine. Results are expressed as mean + SEM ($n = 6$). *$P < 0.05$ compared with saline

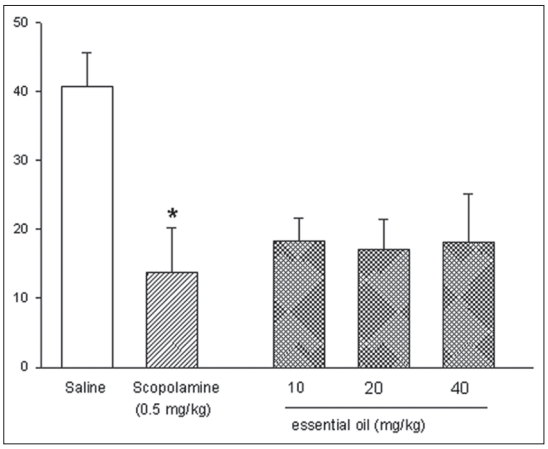

Figure 5: Effect of scopolamine on memory performance (expressed as recognition index) and effect of different doses of *Cyperus rotundus* essential oil on reverse of memory deficit induced by scopolamine. Results are expressed as mean + SEM ($n = 6$). *$P < 0.05$ compared with saline

to improve memory.[8] Despite its wide-spread use as a memory enhancer, no pharmacological data is available to support such claim. Keeping in view its suitability as food ingredient, the current investigation was designed to investigate the effect of *C. rotundus* on scopolamine-induced memory dysfunction in mice using object recognition task.

In the current work, scopolamine, a competitive muscarinic antagonist reduced the RI by 66%. Amnesic properties of scopolamine, have long been known in humans and animals, and in fact, scopolamine amnesia has been proposed as a pharmacological model for human dementia and for Alzheimer's disease.[19] The range of memory dysfunction in the similar studies that have used scopolamine was 70%.[17]

Reversal of scopolamine induced memory deficit has been used as an initial screening method for identification of therapeutic candidate for cognitive disorders.[19] Rivastigmine, an acetylcholine esterase inhibitor is routinely used as a control drug for testing the validity of the method.[20,21] In the present work, the scopolamine-induced memory deficit was reversed to control value by 0.6 mg/kg of rivastigmine. Although, no other study has used rivastigmine as a control in object recognition test, in other memory paradigm model, the result of rivastigmine is very similar to that of ours reverse scopolamine induced in some dose.[14]

The plant extract and essence used in varying doses in the present study failed to improve the impaired memory by scopolamine. This could be due to several factors, such as the animal model or the underlying mechanism that was studied here. Several animal models are available for studying such function that include, radial maze, water maze, and object recognition paradigm.[22] It is well-established that in object recognition model of memory both the short- and long-term memory is studied.[23] Since in the present experimental setting the whole procedure takes 24 h to complete, we are only looking at the short term memory.[23] It is possible that for the effective action, the plant extract requires to be present over a longer period of time. As far as the memory performance model is concerned, this model is considered as a simple and reliable method that is fast and reproducible.[17] However, there are some studies that shows some drugs are effective in one model without a significant effects on other model.[24]

Since in several Iranian traditional medicine resources and literatures the *C. rotundus* is recommended to be used in combination with other plants such as *Crocus sativus, Zingiber officianale,* and *Boswellia papyrifera*[25] it is possible that the effect that

is produced to be due to the presence of other plant ingredients like *B. papyrifera*. The memory improvement property of *B. papyrifera* has already been shown by number of studies.[26]

As mentioned above, in the object recognition model we used scopolamine to impair the animal's memory. Therefore, any drugs that could reverse the action of scopolamine such as rivastigmine could produce effective action and drugs that are not acting through this remain ineffective. One other possibility for not seeing significant memory improvement effect by the plant extract in this study could be due to lack of cholinergic action. It is well-known that *C. rotundus* has antioxidant properties.[10,27] It is therefore, possible that the memory enhancement by *C. rotundus* to be due to the presence of such components in the extract of the plants. Different experimental memory set up is required to evaluate the antioxidant properties of the drugs.[28]

CONCLUSION

In summary, the present study shows that the *C. rotundus* extracts or essence do not provide any memory impairment in mice. Lack of active ingredient in the plant seems to be the major reason for not observing any effects as this preclinical model. Further studies are underway to test the combination of this plant and other known so-called memory enhancer plants.

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