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RESEARCH ARTICLE

Beneficial effects of *Urtica dioica* on scopolamine-induced memory impairment in rats: protection against acetylcholinesterase activity and neuronal oxidative damage

Simagol Ghasemi[a], Malihe Moradzadeh[b,c], Mahmoud Hosseini[a], Farimah Beheshti[d,e] and Hamid Reza Sadeghnia[f]

[a]Division of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; [b]Department of New Sciences and Technology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [c]Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran; [d]Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; [e]Department of Basic Science and Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran; [f]Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

This study was conducted to investigate protective effects of *Urtica dioica* extract on acetylcholinesterase (AChE) activity and the oxidative damage of brain tissues in scopolamine-induced memory impairment model. The rats were treated with (1) saline (control), (2) scopolamine, and (3–5) the plant extract (20, 50, or 100 mg/kg) before scopolamine. The traveled distance and the latency to find the platform in Morris water maze (MWM) by scopolamine-treated group were longer while the time spent in target quadrant was shorter than those of the control. Scopolamine decreased the latency to enter the dark in passive avoidance test. Besides, it also increased AChE activity and malondialdehyde (MDA) concentration in the hippocampal and cortical tissues while decreased thiols content and superoxide dismutase (SOD) and catalase (CAT) activities in the brain ($p < 0.01$–$p < 0.001$). Treatment by the extract reversed all the effects of scopolamine ($p < 0.05$–$p < 0.001$). According to the results of present study, the beneficial effects of *U. dioica* on memory can be attributed to its protective effects on oxidative damage of brain tissue and AChE activity.

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Learning; memory; *Urtica dioica*; oxidative damage; scopolamine; acetylcholinesterase

1. Introduction

Alzheimer's disease (AD), as a major cause of dementia in the elderly population, is an irreversible and progressive neurodegenerative disorder with a wide range of behavioral disturbances (Querfurth and LaFerla 2010). It is estimated that the number of world's population with dementia will increase by 29.9% from 2005 to 2040 (Ferri *et al*. 2006).

Brain tissues oxidative injury has a well-established role in the pathogenesis of different central nervous system (CNS) disorders and neurobehavioral diseases including AD (Qureshi *et al*. 2004, Sharma *et al*. 2009). CNS tissues have a high content of fatty acids (Halliwell 1992), making them liable to oxidative damages (Frölich and Riederer 1995, Qureshi *et al*. 2004).

Cholinergic system has a critical role in learning and memory. Cholinergic system dysfunction has shown a role in the etiology of aging-related cognitive disorders and senile dementia (Schliebs and Arendt 2011). Scopolamine, a muscarinic antagonist drug, induces memory impairment symptoms similar to AD and dementia (Azizi-Malekabadi *et al*. 2012). Recently, it has been shown that scopolamine-induced memory impairment is associated with an oxidative stress status in the brain tissues (Fan *et al*. 2005, Lee *et al*. 2009).

Antioxidants show beneficial effects on neurological activities (Harrison *et al*. 2009a, 2009b, Harrison and May 2009). Several studies show that sufficient amounts of antioxidants can enhance cognitive execution (Harrison *et al*. 2009a, 2009b, Harrison and May 2009). It is also suggested that the antioxidants might make prophylactic effects against CNS diseases. For example, blueberry extract not only enhances memory function but also inhibits acetylcholinesterase (AChE) activity, which is a synaptic enzyme involved in the etiology of the AD. AChE inhibition is one of the mechanisms behind protective effects of AD medicine, such as tacrine, donepezil, and rivastigmine (Kamat *et al*. 2008).

Urtica dioica (stinging nettle), a medicinal plant distinguished by stinging hairs, grows in numerous countries (Kavalalı *et al*. 2003). Traditionally, all parts of the plant are used to treat nasal and menstrual hemorrhage, rheumatic pain, anemia, nephritis, hematuria, jaundice, menorrhagia, and diarrhea. Also, *U. dioica* shows some effects such as blood purification and diuretic properties and some therapeutic effects on diabetes, atherosclerosis, cardiovascular diseases, and prostate cancer (Konrad *et al*. 2000). Chemical analyzes show that *U. dioica* extract contains iron, vitamins such as A, B, and B12, and other compounds such as flavonoids, carotenoids, thymol, quercetin, carvacrol, salicylic acid,

CONTACT Mahmoud Hosseini hosseinim@mums.ac.ir Neurocognitive Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

and other compounds such as acetylcholine and serotonin (Wessler *et al*. 2001, Nahata and Dixit 2012, Otles and Yalcin 2012). Carvacrol shows a protective impact against cerebral ischemia/reperfusion damage (Yu *et al*. 2012) while modifying dopamine and serotonin concentration in the prefrontal cortex and hippocampus (Hornick *et al*. 2011). *Urtica dioica* extract has been reported to improve spatial and associative memory dysfunction associated with chronic diabetes (Patel and Udayabanu 2013). The aim of present study was, the possible protective effect of a hydroalcoholic extract of *U. dioica* on scopolamine-induced memory impairment in rats, highlighting the effects on AChE activity and the brain tissues oxidative stress as possible mechanisms of action.

2. Materials and methods

2.1. Preparing the plant extract

Aerial parts of *U. dioica* were gathered from Mashhad (Iran) area and confirmed by botanists. To provide hydro-alcoholic extract, the ground plant (50 g) was mixed with ethanol (50%) and soaked for 48 h. Finally, it was filtered to separate the solution by using a paper filter. To remove the solvent, a rotary vacuum evaporator was used (Naghibi *et al*. 2012).

2.1.1. Animals and the experimental protocol

Male Wistar rats (200–240 g, 2 months old) were used in this investigation. The rats were maintained at standard conditions including 22 ± 2 °C and a periodic light/dark (light ON at 7 AM) condition. The animals had *ad libitum* access to the food and water. Animal examinations were carried out by the procedures approved by the Committee on Animal Research of Mashhad University of Medical Sciences (Ethical approval number: IR.MUMS.REC.1395.152). They were grouped as follows ($n = 8$–10 in each): (1) Control group: the animals received saline (2 ml/kg, daily), (2) Scopolamine (Sco) group: the animals received saline during two weeks and then were injected by scopolamine (1.5 mg/kg dissolved in saline) injection (intraperitoneally, IP) (Sigma Chemical Co) 30 min before Morris water maze (MWM) test. (3–5) the treatment groups: the animals were daily treated with 20 (Urtica 20-Sco), 50 (Urtica 50-Sco), or 100 mg/kg (Urtica 100-Sco) of *U. dioica* extract IP for two weeks. Finally, scopolamine was injected before each trial in MWM. Treatments were similarly carried out for passive avoidance (PA) test, except for scopolamine that was injected 30 min before retention trials.

2.2. Morris water maze apparatus and procedures

In order to carry out the MWM test, a round dark pool (136 cm diameter and 60 cm deep) was used. Water with 23–24 °C temperature was used to fill the pool up to 30 cm. A round platform (10 cm distance across, 28 cm high) was set inside the pool and submerged around 2 cm underneath the surface of the water in the center of the southwest quadrant. Some posters and other objects such as computer were put around the pool as visual cues. All examination sessions were recorded by a camcorder put simply over the focal point of the maze. Prior to each investigation, each rat was accustomed to the maze for 30 s without the platform. Then, the rats did four trials every day for a 5-day period. The trials were started by releasing the rats into the water. The release directions including North (N), East (E), South (S), and West (W) were selected randomly. The rats were then allowed to search for the hidden platform inside the pool. If the rat was able to find the platform during 60 s, it was permitted to remain on it for 20 s; otherwise, it was helped by the experimenter to find the hidden platform. The rat was then returned to the cage after drying. Twenty-four hours later, the platform was removed, and a probe test was carried out. The time spent and the traveled path in the target (Q1) and non-target (Q2–Q4) quadrants were compared between groups (Abareshi *et al*. 2012, Jamialahmadi *et al*. 2013). Each behavioral test was conducted within a time range of 4:00 to 6:00 PM. The time latency to find the platform and the length of the swimming way was recorded.

2.3. Passive avoidance apparatus and procedures

A passive avoidance (PA) apparatus (Borjsanat, Tehran, Iran) was utilized to evaluate PA learning. The apparatus included two compartments each containing a grid floor. One of the compartments was light while the other was dark. Additionally, there was a small gate between the compartments. On days 1 and 2, the animals were accustomed to the apparatus. On day 3, the rats were located inside the light-room, facing away from the darkroom and the time elapsed to enter the dark was recorded. The rats received an electrical shock (2 mA, 2 s duration) when they were completely entered the dark room. They were then returned to their cages. One, 24, and 72 h later, the retention phases were done. The rats were located inside the light room of the apparatus, the gate was opened, and the time elapsed to enter the dark was recorded. Also, at the retention phases, the gate of the apparatus was opened and the rats were allowed to move between the light and dark rooms during 300 s and the time spent by the animals in both the dark and the light rooms were recorded.

2.4. Biochemical assessment

In this step, the animals were sacrificed. Cerebral cortex and hippocampus were removed and homogenized in phosphate-buffered saline (PBS) to provide a 10% homogenate (0.1 g tissue was added 1 ml PBS). To prepare PBS, 0.9 g NaCl (sodium chloride) + 0.071 g Na2HPO4 (disodium hydrogen phosphate) was added to 100 ml distilled water (pH = 7.4).

To measure AChE activity, Elman method described in (Hosseini *et al*. 2015) was used. Briefly, the supernatant (40 µl) was mixed with a solution containing PBS (2.55 ml) and dithiobisnitrobenzoic acid (DTNB; 0.1 ml of 10 mM) and incubated for 5 min at 37 °C. The absorbance of the solution was read at 412 nm. Next, 0.02 ml of 75 mM acetylthiocholine was added to the mixture and incubated at 37 °C for 5 min. The difference of the absorbance during 5 min was noted.

AChE activity was calculated and expressed as nmol/min/mg protein.

To measure MDA levels, 2 ml of TBA (thiobarbituric acid)/TCA (trichloroacetic acid)/HCL (hydrochloric acid) reagent was mixed with 1 ml of brain tissue homogenate and heated in a boiling water bath. After 40 min, the samples were removed from the bath and allowed to reach the room temperature. The samples were centrifuged (10 min) and the supernatants were separated. The absorbance of the samples was determined at 535 nm. The MDA concentration was calculated using a formula described in (Hosseini *et al*. 2013).

Total thiol contents were determined using DTNB, which reacts with thiol groups and yields a yellow solution. To provide Tris-EDTA (ethylenediaminetetraacetic acid) buffer 0.05 g EDTA +3 g Tris was added to 100 ml distilled water. To measure thiol content, the supernatants of the samples (50 µl) was mixed with Tris-EDTA buffer (pH = 8.6). The absorbance was read at 412 nm (A1). DTNB (20 µl) was then added to the mentioned mixture. The samples were kept at room temperature. After 15 min, the absorbance of the samples was read again (A2). The absorbance of DTNB was read and considered as a blank (B). The brain tissues total thiol content was calculated using a formula described in (Hosseini *et al*. 2013).

Superoxide dismutase (SOD) activity was measured by a procedure described in (Madesh and Balasubramanian 1998). This protocol is a colorimetric assay based on the production of superoxide by pyrogallol auto-oxidation and inhibition of superoxide-dependent reduction of the tetrazolium dye to its formazan by SOD. The colorimetric changes were measured at 570 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in the tetrazolium dye reduction rate.

CAT activity was measured based on the method described in (Aebi 1984). This essay is principally based on the determination of the rate constant k (dimension: s-1, k) of hydrogen peroxide decomposition. 50 µl of brain tissue homogenate was added to a cuvette containing 1 ml of 30 mM H_2O_2. Catalase activity was measured at 240 nm for 1 min using a spectrophotometer.

The rate constant of the enzyme was determined by measuring the drop in absorbance at 240 nm per minute (Abareshi *et al*. 2012).

2.5. Statistical analysis

All data were expressed as means ± SEM. SPSS software version 11.5 was used to evaluate the obtained data. The data from different treatment groups were compared using one-way ANOVA followed by Tukey's *post hoc* comparisons test. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. MWM test

Our results demonstrated that scopolamine administration decreased the time spent to reach the platform and also the traveled distance to reach the platform in Sco group compared to control group ($p < 0.01$–$p < 0.001$) (Figure 1(A,B)). Treatment with all doses of Urtica extract decreased the time spent to reach the platform and also traveled distance to reach the platform compared to Sco group ($p < 0.01$–$p < 0.001$) (Figure 1(A,B)).

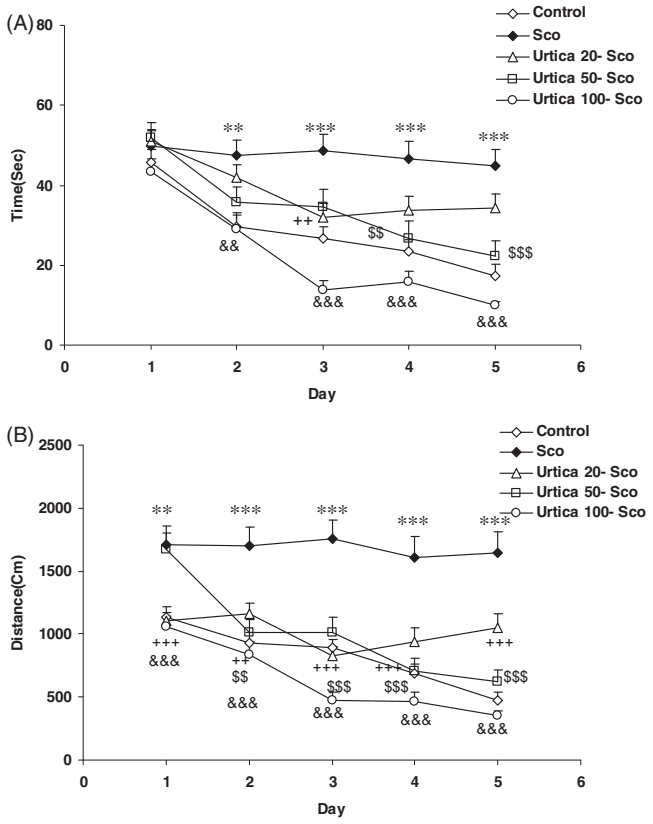


Figure 1. Comparison of time latency (A) and path length (B) to reach the platform in MWM test among Control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are presented as mean ± SEM ($n = 8$–10 per group). $^{*}p < 0.01$ and $^{***}p < 0.001$ the difference between scopolamine and control group, $^{++}p < 0.01$ and $^{+++}p < 0.001$ the difference between Urtica 20- Sco and Sco group, $^{\$\$}p < 0.01$ and $^{\$\$\$}p < 0.001$ the difference between Urtica 50- Sco and Sco group, $^{\&\&}p < 0.01$ and $^{\&\&\&}p < 0.001$ the difference between Urtica 100- Sco and Sco group.

The results of probe trial showed that the time spent in target quadrant (Q_1) in Sco group was lower than that in the control group ($p < 0.01$) (Figure 2(A)), while all doses of Urtica extract were able to increase the time spent in Q_1 compared to Sco group ($p < 0.05$–$p < 0.01$) (Figure 2(A)).

In addition, scopolamine administration increased the time spent in the non-target (Q_3) quadrant ($p < 0.05$), while different doses of the extract decreased the time spent in Q_3 ($p < 0.05$–$p < 0.01$) (Figure 2(A)). Additionally, the traveled distance in target quadrant in Sco group was lower than the control group ($p < 0.05$). Only 100 mg/kg of Urtica extract was able to attenuate this effect of scopolamine and increase the time spent in target quadrant compared to Sco group ($p < 0.05$) (Figure 2(B)). Furthermore, scopolamine injection increased the traveled distance in non-target quadrants including Q_2, Q_3, and Q_4 ($p < 0.001$), while all doses of the plant extract decreased the traveled distance in Q_2 and Q_3 ($p < 0.01$–$p < 0.001$) (Figure 2(B)). Additionally, both 50 and 100 mg/kg of the extract decreased the traveled distance in Q_4 ($p < 0.001$); however, the lowest dose of extract did not affect this parameter (Figure 2(B)).

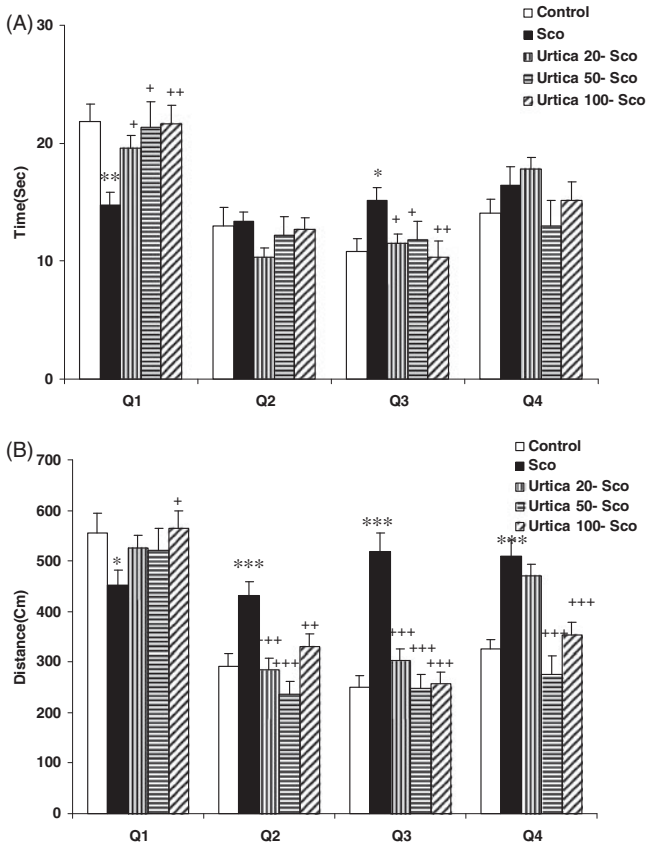


Figure 2. Comparison of the total time spent (A) and traveled distance (B) in the target quadrant in MWM test among control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are presented as mean ± SEM ($n = 8$–10 per group). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ in comparison with control group, $^{+}p < 0.05$, $^{++}p < 0.01$, and $^{+++}p < 0.001$ in comparison with Sco group.

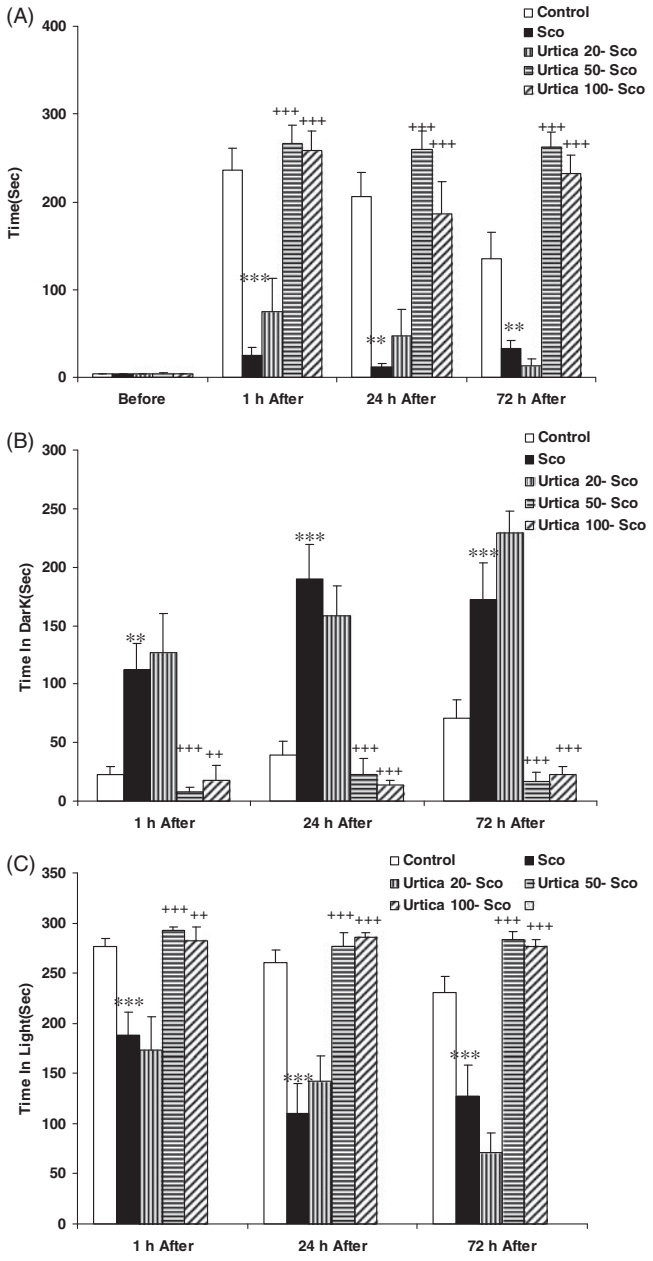


Figure 3. Comparison latency time (A), the time spent in the dark (B), and light (C) compartments in PA test among Control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are presented as mean ± SEM ($n = 8$–10 per group). $^{**}p < 0.01$ and $^{***}p < 0.001$ in comparison with control group, $^{++}p < 0.01$ and $^{+++}p < 0.001$ in comparison with Sco group.

3.2. PA test

The results of PA test showed that scopolamine administration decreased the time latency for entering the dark room at 1, 24, and 72 h after the shock compared to the control group ($p < 0.01$–$p < 0.001$) (Figure 3(A)). Pretreatment by 50 and 100 mg/kg of Urtica extract increased the time latency at 1, 24, and 72 h after the shock compared to Sco group ($p < 0.001$) (Figure 3(A)). Additionally, the time spent in a dark room in Sco group at 1, 24, and 72 h after the shock were higher than that in the control group ($p < 0.01$–$p < 0.001$) (Figure 3(B)). Treatment by 50 and 100 mg/kg of Urtica extract decreased the time spent in the darkroom compared to Sco group ($p < 0.01$–$p < 0.001$) (Figure 3(B)). Injection of scopolamine also decreased the time spent in the lightroom after the shock compared to the control group ($p < 0.001$) (Figure 3(C)). Treatment by 50 and 100 mg/kg of extract increased the time spent in the lightroom compared to Sco group ($p < 0.01$–$p < 0.001$) (Figure 3(C)).

3.3. Biochemical results

3.3.1. Hippocampal tissues

Biochemical analyses of brain tissues showed that AChE activity in the hippocampal tissues of Sco group was higher than the control group ($p < 0.001$), while treatment by all doses of Urtica extracts decreased AChE activity in the hippocampus compared to Sco group ($p < 0.05$–$p < 0.001$) (Figure 4).

Scopolamine injection also increased MDA concentration in the hippocampal tissues of Sco group compared to control group ($p < 0.001$) (Figure 5(A)). All doses of Urtica extract were effective to reduce this effect of scopolamine by decreasing MDA concentrations in the hippocampus compared to Sco group ($p < 0.05$–$p < 0.001$) (Figure 5(A)). Scopolamine reduced total thiol concentrations in the hippocampus of Sco group ($p < 0.001$) (Figure 5(B)), whereas different doses of Urtica extract improved total thiol concentrations in hippocampal tissues compared to Sco group ($p < 0.05$–$p < 0.001$) (Figure 5(B)).

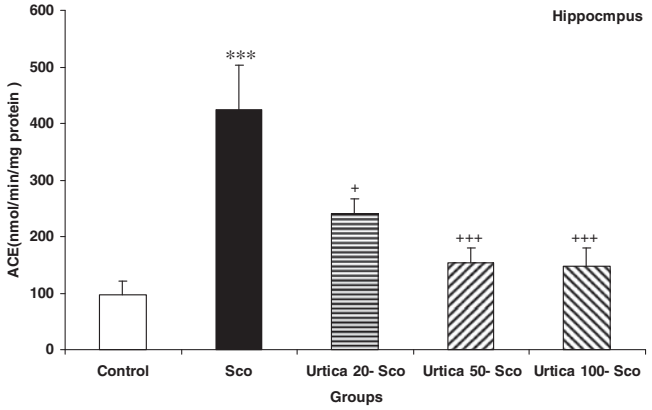


Figure 4. The ACE activity in hippocampal tissues of control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are shown as mean ± SEM of 8–10 animals per group). $^{***}p < 0.001$ in comparison with control group, $^{+}p < 0.05$, $^{+++}p < 0.001$ in comparison with Sco group.

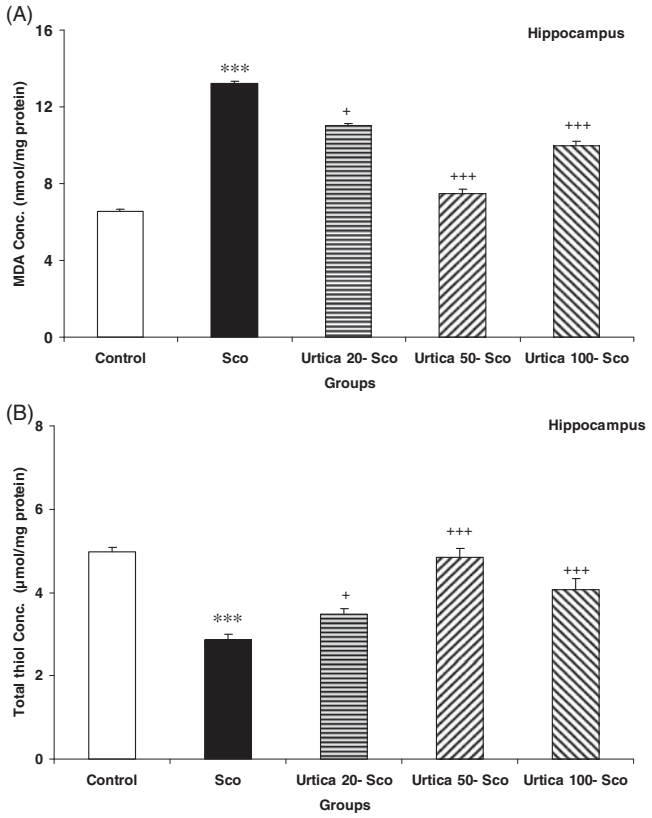
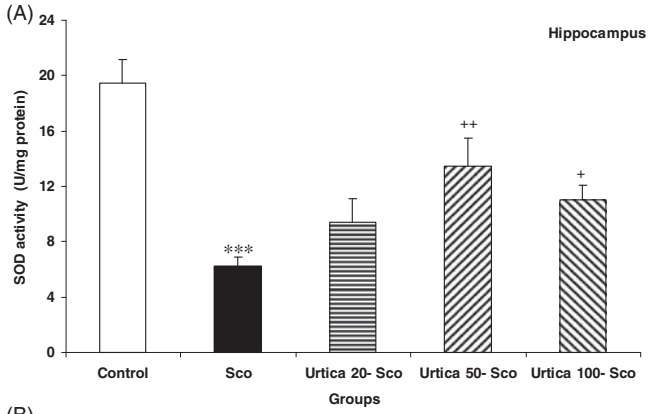


Figure 5. The MDA concentrations (A) and total thiol concentrations (B) in hippocampal tissues of control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are shown as mean ± SEM ($n = 8$–10 per group). $^{***}p < 0.001$ in comparison with control group, $^{+}p < 0.05$ and $^{+++}p < 0.001$ in comparison with Sco group.



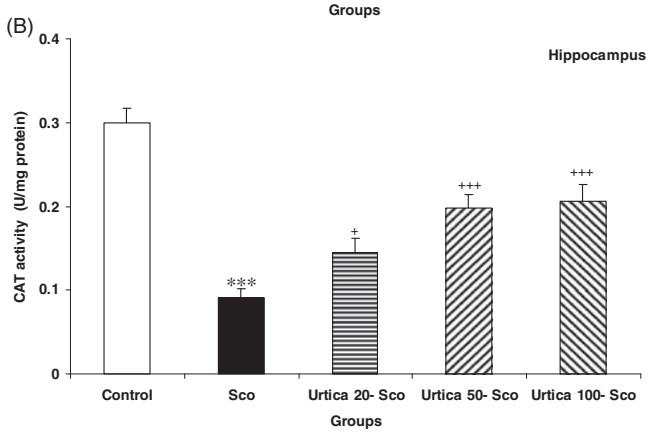


Figure 6. The SOD (A) and CAT (B) activities in hippocampal tissues of Control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are shown as mean ± SEM ($n = 8$–10 per group). $^{***}p < 0.001$ in comparison with Control group, $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ in comparison with Sco group.

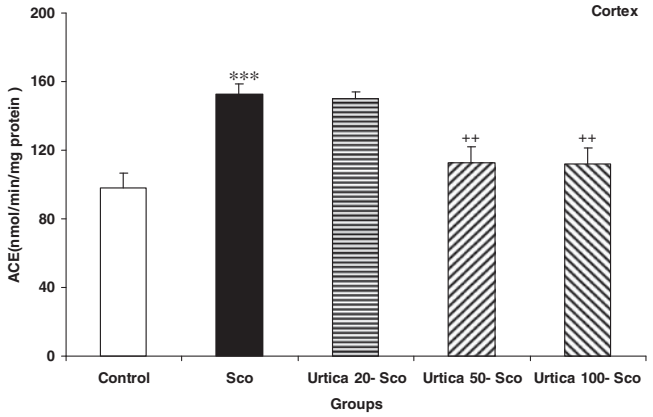


Figure 7. The ACE activity in cortical tissues of control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are shown as mean ± SEM ($n = 8$–10 per group). $^{***}p < 0.001$ in comparison with control group, $^{++}p < 0.01$ in comparison with Sco group.

Results of this study also showed that scopolamine lowered SOD activity in the hippocampus of Sco group in comparison to the control group ($p < 0.001$) (Figure 6(A)). Both 50 and 100 mg/kg of Urtica extract increased SOD activity in the hippocampus compared to the Sco group ($p < 0.05$–$p < 0.01$); however, 20 mg/kg of the extract was not effective (Figure 6(A)). Additionally, CAT activity in the hippocampal tissues of Sco group was lower than that in the control group ($p < 0.001$) (Figure 6(B)). Pre-treatment by all doses of Urtica extract increased CAT activity compared to Sco group ($p < 0.05$–$p < 0.001$) (Figure 6(B)).

3.3.2. Cortical tissues

Biochemical analyses of the cortical tissue showed that scopolamine administration was associated with an increased level of AChE activity in Sco group compared to control group ($p < 0.001$) (Figure 7). Treatment by 50 and 100 mg/kg of Urtica extract decreased AChE activity in cortical tissues compared to Sco group ($p < 0.01$), however, 20 mg/kg of the

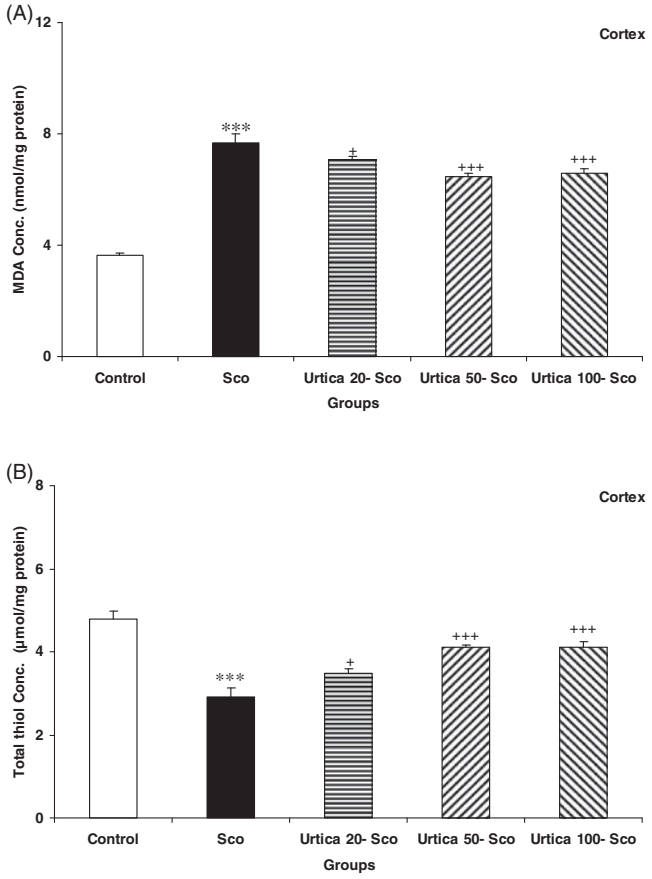


Figure 8. The MDA concentrations (A) and total thiol concentrations (B) in cortical tissues of Control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are shown as mean ± SEM ($n = 8$–10 per group). $^{***}p < 0.001$ in comparison with control group, $^{+}p < 0.05$ and $^{+++}p < 0.001$ in comparison with Sco group.

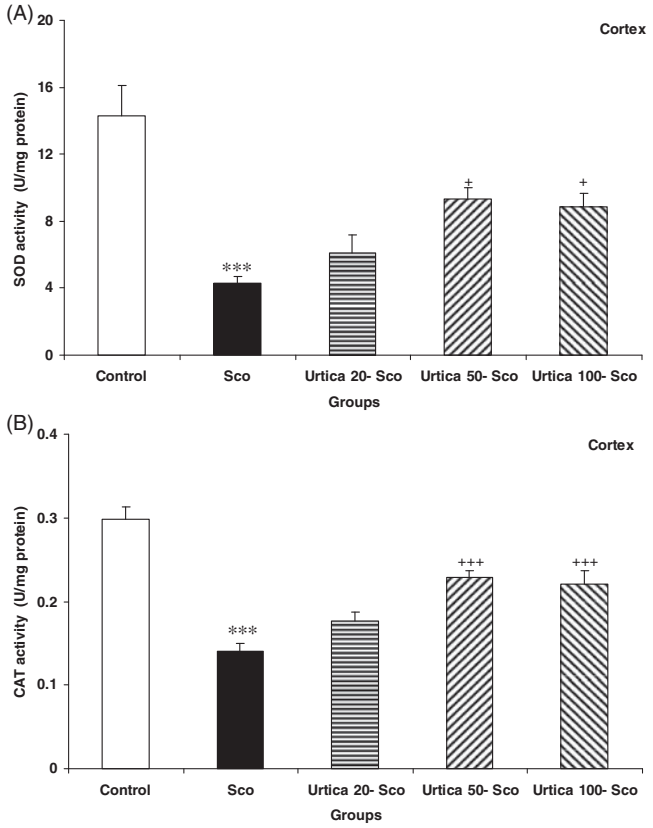


Figure 9. The SOD (A) and CAT (B) activities in hippocampal tissues of Control, Sco, Urtica 20- Sco, Urtica 50- Sco, and Urtica 100- Sco groups. Data are shown as mean ± SEM ($n = 8$–10 per group). $^{***}p < 0.001$ in comparison with control group, $^{+}p < 0.05$ and $^{+++}p < 0.001$ in comparison with Sco group.

plant extract was not significantly effective on cortical AChE activity (Figure 7).

MDA concentration in the cortex of Sco group was higher than the control group ($p < 0.001$) (Figure 8(A)). All doses of Urtica extract decreased MDA concentration in the cortex compared to the Sco group ($p < 0.05$–$p < 0.001$) (Figure 8(A)). Additionally, scopolamine injection reduced total thiol concentration in the cortex of Sco group compared to control group ($p < 0.001$) (Figure 8(B)). All doses of Urtica extract were able to attenuate this effect of scopolamine by increasing total thiol concentrations in the cortical tissues compared to Sco group ($p < 0.05$–$p < 0.001$) (Figure 8(B)).

In addition, SOD activity in cortical tissues of Sco group was lower than that of the control group ($p < 0.001$) (Figure 9(A)). Both 50 and 100 mg/kg of Urtica extract improved SOD activity in the cortex compared to Sco group ($p < 0.05$); however, the lowest dose was not significantly effective (Figure 9(A)). Furthermore, CAT activity in cortical tissues of Sco group was lower than the control group ($p < 0.001$) (Figure 9(B)). Treatment by 50 and 100 mg/kg of Urtica extract increased CAT activity compared to Sco group ($p < 0.001$); however, 20 mg/kg was not effective (Figure 9(B)).

4. Discussion

The present study confirmed that scopolamine administration adversely affects learning and memory capabilities of the rats as demonstrated in MWM and PA test results. MWM results showed that scopolamine administration prolonged the time spent and the traveled distance to reach the platform during 5 days learning period. It also reduced the time spent in the target quadrant in probe day when the animals were examined to recall the area of the platform. Moreover, the latency to enter the dark compartment of PA apparatus decreased in scopolamine-treated animals. Scopolamine administration was also associated with an increase in the time spent in the darkroom and a reduction in the time spent in the lightroom after the shock. These results confirmed previous findings of learning and memory-impairing effects of scopolamine (Jamialahmadi *et al*. 2013, Karimi *et al*. 2015, Hejazian *et al*. 2016). An increased level of AChE activity is accompanied with scopolamine-induced memory impairment (Lee *et al*. 2009, Kwon *et al*. 2010, Shi *et al*. 2010, Azizi-Malekabadi *et al*. 2012). Similarly, this study showed that scopolamine administration was associated with an elevation of AChE activity in both hippocampal and cortical tissues of scopolamine-treated rats. Additionally, previous studies demonstrated that scopolamine-impaired memory was accompanied with an elevated level of MDA concentrations and a decreased level of GSH in the brain tissue of scopolamine-treated rats, confirming an oxidative stress status in the (El-Sherbiny *et al*. 2003). Confirming previous findings, the results of the current study showed that scopolamine treatments result in an increase in MDA concentrations and a decrease in total thiol concentrations and SOD and CAT activities in both hippocampus and

cortex tissues. We have also previously confirmed that scopolamine-induced learning and memory impairment was accompanied with the brain tissues oxidative damages (Hosseini *et al*. 2015, Karimi *et al*. 2015, Mohammadpour *et al*. 2015, Hejazian *et al*. 2016). In line with our results, it has been previously shown that scopolamine increases lipid peroxidation and AChE activity in the brain (Goverdhan *et al*. 2012). Elsewhere, it was reported that the brain cholinergic system is involved in hippocampal neurogenesis and cognitive function by modulating neurogenic mechanisms, such as mechanisms related to brain-derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB) (Bruel-Jungerman *et al*. 2011). Learning and memory impairments, which are associated with AD and aging, are also primarily related to cholinergic dysfunction, including impaired acetylcholine release and increased AChE activity in CNS (Ul Islam *et al*. 2017).

Moreover, the anti-oxidant agents such as ascorbic acid are reported to improve scopolamine-induced learning and memory deficits in mice via attenuating AChE activity and protecting the brain tissues against oxidative damages (Harrison *et al*. 2009a). We previously showed that some plant extracts, such as *Nigella sativa* and *Rosa damascena*, improve learning and memory and protect brain tissues against oxidative damages (Hosseini *et al*. 2015, Mohammadpour *et al*. 2015). Previous studies show the antioxidant effects of *U. dioica* (Namazi *et al*. 2012, Oguz *et al*. 2015). In the current work, we investigated the protective effects of *U. dioica* against scopolamine-induced learning and memory impairments. The results showed that all doses of the plant extract improved the time spent and traveled distance to reach the platform during 5 days of learning in MWM test. Plant extract administration also helped the animals to better recall the location of the platform, which was reflected in a longer time spent in the target quadrant. Also, treatment with *U. dioica* prolonged the delay time to enter the dark room and also the time spent in the light compartment, while decreasing the time spent in a dark room in PA test. Consistent with these results, another study showed that *U. dioica* improved depressive-like behavior and cognitive dysfunction induced by dexamethasone (Patel and Udayabanu 2014). In this study, we found that *U. dioica* extract decreased AChE activity in both hippocampus and cortex tissues. Similarly, another study demonstrated that *U. dioica* leaves extract had a potential to reverse spatial memory deficiency associated with streptozotocin-induced diabetes in mice. In the same study, it was observed that diabetes-mediated alterations in the muscarinic cholinergic system in the hippocampus and *U. dioica* extract improved memory functions (Patel *et al*. 2015). Moreover, it was reported that *U. dioica* extract was able to improve granule cell loss in the dentate gyrus of diabetic rats and ameliorated cognitive impairment in diabetes (Fazeli *et al*. 2008). In another work, a chemical compound of *U. dioica*, scopoletin, was able to increase acetylcholine secretion, leading to an improvement in hippocampal long-term potentiation (LTP), learning, and memory (Hornick *et al*. 2011). To better understand the possible mechanisms involved in *U. dioica* effects, MDA, total thiol concentrations, CAT, SOD, and AChE activities were evaluated in the hippocampus and cortex. The results of the current study showed that *U. dioica* extract decreased AChE activity in both hippocampal and cortical tissues. Similarly, another study revealed that *U. dioica* leaves extract attenuated hippocampal tissues oxidative damage and improved cholinergic system function in the brain of diabetic mice (Patel *et al*. 2015). Furthermore, the antioxidant activity of *U. dioica* was reported in (Alpinar *et al*. 2009). As shown in previous studies, *U. dioica* has inhibitory effects on fatty acid peroxidation and reducing free radicals. These properties make the *U. dioica* extract a powerful natural antioxidant material (Gülçin *et al*. 2004). In addition, it was shown that *U. dioica* leaf supplementation significantly reduced the level of free electron accumulation in several brain areas, such as the right frontal lobe, in rats (Pieroni *et al*. 2002). In conjunction with its antioxidant activity, the *U. dioica* extract was able to upregulate AP-1 and had anti-apoptotic and cell-survival supporting effects (Toldy *et al*. 2005). There is a strong link between learning and memory impairment, neuronal injury, and oxidative stress (Khodabandehloo *et al*. 2013). In addition, antioxidants have been frequently demonstrated to have some beneficial effects in prevention of memory impairment (Silva *et al*. 2004). We found that *U. dioica* extract administration reduced oxidative stress in both hippocampus and cortex tissues, which were reflected in a decreased level of MDA concentration and an increase in total thiol concentration and CAT and SOD activities. In another study, *U. dioica* treatment significantly attenuated oxidative stress in the hippocampus of streptozotocin-induced diabetic mice (Patel *et al*. 2015). The results of the present study may also confirm previous findings of protective effects of *U. dioica*-rich diet on N-methyl-d-aspartate (NMDA)-induced brain injury in Wistar rats, probably via reducing reactive oxygen species levels that were measured by electron paramagnetic resonance (Toldy *et al*. 2009). The compounds responsible for the learning and memory-improving effects of the *U. dioica* extract were not evaluated in this study. The major compounds of *U. dioica* are known to be scopoletin, quercetin, carvacrol, 5-hydroxytryptamine, acetylcholine, and choline acetyltransferase (Collier and Chesher 1956), which may be involved in the beneficiary effects of *U. dioica* on learning and memory; however, it needs to be more investigated.

5. Conclusion

Urtica dioica extract was found effective in dealing with learning and memory impairment induced by scopolamine. Protection against AChE activity and the oxidative damage of brain tissues was suggested as a possible mechanism involved in the beneficial effects of *U. dioica* on scopolamine-induced memory impairment.

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ORCID

Malihe Moradzadeh http://orcid.org/0000-0003-3216-1098

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