Original Article

The effects of tamoxifen on spatial and nonspatial learning and memory impairments induced by scopolamine and the brain tissues oxidative damage in ovariectomized rats

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Abstract

Background: Modulatory effects of tamoxifen (TAM) on the central nervous system have been reported. The effects of TAM on spatial and nonspatial learning and memory impairments induced by scopolamine and the brain tissues oxidative damage was investigated.

Materials and Methods: The ovariectomized (OVX) rats were divided and treated: (1) Control (saline), (2) scopolamine (Sco; 2 mg/kg, 30 min before behavioral tests), (3–5) Sco-TAM 1, Sco-TAM 3 and Sco-TAM 10. TAM (1, 3 or 10 mg/kg; i.p.) was daily administered for 6 weeks.

Results: In Morris water maze (MWM), both the latency and traveled distance in the Sco-group were higher than control ($P < 0.001$) while, in the Sco-TAM 10 group it was lower than Sco-group ($P < 0.05$). In passive avoidance test, the latency to enter the dark compartment was higher than control ($P < 0.05 – P < 0.01$). Pretreatment by all three doses of TAM prolonged the latency to enter the dark compartment compared to Sco-group ($P < 0.05 – P < 0.001$). The brain tissues malondialdehyde (MDA) concentration was increased while, superoxide dismutase activity (SOD) decreased in the Sco-group compared to control ($P < 0.05 – P < 0.01$). Pretreatment by TAM lowered the concentration of MDA while, increased SOD compared to Sco-group ($P < 0.05 – P < 0.001$).

Conclusions: It is suggested that TAM prevents spatial and nonspatial learning and memory impairments induced by scopolamine in OVX rats. The possible mechanism(s) might at least in part be due to protection against the brain tissues oxidative damage.

Key Words: Memory, morris water maze, superoxide dismutase, lipid peroxidation, scopolamine, tamoxifen

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INTRODUCTION

Alzheimer's disease (AD) has been accepted as the most common cause of sporadic dementia, afflicting almost 13 million people worldwide.[1] Studies have found a remarkably higher incidence of AD in postmenopausal women. The neuro-protective effects of estrogen led to an increased interest to investigate its role in cognitive functioning or dementia.[2] Estrogen has known to

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be able to inhibit the neurotoxic effect of glutamate or aggregation of amyloid-beta peptide in the brain. It also has an antioxidant property so, its protection against cognitive dysfunction is conceivable.[3,4]

The possibility of using selective estrogen receptor modulators (SERMs) to perform estrogen-like neuro-protective actions in the brain has emerged their use as an alternative to estradiol.[5] SERMs bind to estrogen receptors (ER) and bring on specific changes in their three-dimensional conformation allowing a tissue-selective mobilization of transcriptional co-factors.[6] SERMs may also exert neuro-protective actions by the control of local brain inflammation, which is mainly regulated by microglia and astroglia.[5] SERMs interact with both ERs, ER alpha (ERα), and ER beta (ER$_\beta$) and may behave as agonists or antagonists. Their effects in different tissues hinge upon which ER is expressed and what response components are incorporated in the estrogen-responsive genes. Moreover, SERMs may act in a different way depending on the presence or absence of Estrogen.[7] Triphenylethylene SERMs, such as tamoxifen (TAM), are known as the first-generation of SERMs.[6] TAM, a synthetic, non-steroidal ER modulator is administered widely in the breast cancer treatment. In the central nervous system, TAM counteracts ERs and causes apoptosis in a wide range of cells.[8] Although, some human studies also propose that TAM may minify the risk of AD.[9] The neuro-protective actions of TAM in various forms of neural injury have also been reported.[10,11] In contrast, raloxifene, another drug of SERMs, does not mimic the effects of estrogen on cognitive performance as assessed by the acquisition of a simple spatial memory task in ovariectomized (OVX) rats.[12] It has been reported that TAM acts as an estrogen-like agonist to increase cholinergic system activity and hippocampal mediated learning in human.[13]

Selective estrogen receptor modulators have also been proposed to have pro-oxidative effects. TAM was shown that induced lipid peroxidation, protein carbonyl content and inhibited the enzymes of the antioxidant defense system.[14] Some studies suggest that reduction of oxidative damages to biological macromolecules *in vitro* and *in vivo* may at least in part explain the anti-carcinogenic and chemopreventive actions of TAM.[15] It seems that the SERMs sometimes protect tissues from oxidative injuries.[16,17] With keeping this in mind, it has been suggested that estradiol, TAM, and raloxifene improve prefrontal cortex-related cognitive performance and modulate prefrontal cortex morphology in OVX rats.[18] TAM was able to enhance acetylcholine transferase expression in a manner similar to that of estrogen in several basal forebrain regions.[19]

The aim of present study was to evaluate the effects of TAM on spatial and nonspatial learning and memory impairments induced by scopolamine in OVX rats. Malondialdehyde as an index of lipid peroxidation and superoxide dismutase was also evaluated in the brain tissues.

MATERIALS AND METHODS

Animals and drugs

Female Wistar rats, 12-week-old (200 ± 10 g), were obtained from animal house of School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran and used in the present study. The animals were housed in 4–5 per standard cages, at room temperature (22°C ± 2°C) on a 12 h light/dark cycle. Food and water were available *ad libitum* properly. All efforts were made to maintain the animals in good general health, in accordance with the European Communities Council Directive (2010/63/UE). Animal handling and all related procedures were approved by the Mashhad Medical University Committee on Animal Research. All of the animals were OVX and were then divided into five groups and treated: (1) Control, (2) scopolamine (Sco), (3) scopolamine-TAM 1 mg/kg (Sco-TAM1), (4) scopolamine-TAM 3 mg/kg (Sco-TAM 3) and (5) scopolamine-TAM 10 mg/kg (Sco-TAM 10).

The animals in the Sco-TAM groups (the groups 3–5) were treated by daily injections of TAM (1, 3 or 10 mg/kg; Intraperitoneally; i.p.) for 6 weeks before the behavioral tests. The animals of Control group received 1 ml/kg of saline instead of TAM. Scopolamine (2 mg/kg) was injected 30 min before the behavioral tests.

Ketamine and xylazin were purchased from Alfasan Company Woerden-Holand. TAM (was kindly provided by Iran Hormone Company, Tehran, Iran). Scopolamine was purchased from Sigma Aldrich Company, USA. TAM was kindly provided by Iran Hormone Company (Tehran, Iran). Other chemicals such as those which were used for biochemical assessments were purchased from Merck Company.

Surgery

Before the surgery, the rats were permitted 15 days for acclimatization to the animal house. The animals were OVX under ketamine anesthesia (100 mg/kg, i.p.) and xylazin 15 mg/kg, i.p. anesthesia was confirmed by reduced respiratory rate and no response to gentle pinching of foot pad. The abdominal incision was made through the skin of the flank of the rats and ovaries, and ovarian fats were removed. Ovaries were isolated by ligation of the most proximal portion of the oviduct before removal.[20]

Passive avoidance test

Passive avoidance (PA) learning test is based on negative reinforcement and is used for evaluating of nonsaptial learning and memory.[21-25] The apparatus consisted of a light and a dark compartment with a grid floor adjoining each other through a small gate. The rats were accustomed to the behavioral apparatus for 5 min during 2 consecutive days before the training session. On the 3rd day, the animals were placed in light compartment and the time latency to enter the dark compartment was recorded. On a training trial, the rats were placed in the light compartment facing away from the dark compartment. When the rats were entered completely into the dark compartment, they received an electric shock (1 mA, 2 s duration). Then, the rats were returned to their home cage. 3 and 24 h later, the rats were placed in the light compartment and the latency time to enter the dark compartment as well as, the times spent by the animals in dark and light compartments was recorded and defined as retention trial.[21,25]

Morris water maze apparatus and procedures

Morris water maze (MWM) test is sued for evaluation of spatial learning and memory. A circular black pool (136 cm diameter, 60 cm high, and 30 cm deep) was filled with water (24–26°C). A circular platform (10 cm diameter, 28 cm high) was placed within the pool and was submerged ~2 cm below the surface of the water in the center of the South-West quadrant. Outside the maze, fixed visual cues (i.e., a computer, hardware, and posters) were present at various locations around the room. Before the experiment, each rat was handled daily for 3 days and habituated to the water maze for 30 s without a platform. The animals performed four trials daily, for 5 days. Each trial began with the rat being placed in the pool and released facing the side wall at one of the four positions (the boundaries of the four quadrants, labeled north [N], east [E], south [S], and west [W]). Release positions were randomly predetermined. For each trial, the rat was allowed to swim until it found and remained on the platform for 15 s. If an animal was not able to find the platform in 60 s, it has being guided to the platform by the experimenter and allowed to stay on the platform for 15 s. The rat was then removed from the pool, dried, and placed in its holding bin for 5 min. The time latency to reach the platform and the length of the swimming path were recorded by a video tracking system.[21,23,26]

Biochemical measurements

Malondialdehyde (MDA) level is as an index of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) and produces a red complex. Briefly, 1 mL of the brain homogenates was added to 2 mL of a complex solution containing TBA/trichloroacetic acid/hydrochloric acid, and it was then boiled in a water bath for 40 min. After reaching to the room temperature, the solution was centrifuged at 1000 g for 10 min. The absorbance was read at 535 nm. The MDA concentration was calculated according to follow equation.[21,23]

$$C\ (M) = \text{Absorbance}/1.56 \times 10^5$$

Superoxide dismutase (SOD) activity was measured by the procedure described by Madesh and Balasubramanian. A colorimetric assay involving generation of superoxide by pyrogallol auto-oxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) to its formazan by SOD was measured at 570 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in the MTT reduction rate.

Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). All data were expressed as means ± SEM. The data related to time, distance speed, during 5 days of MWM were compared using repeated measures analysis of variance (ANOVA) followed by Tukey's *post-hoc* comparisons test. The data due to probe trail in MWM, the data of PA test and biochemical data were compared by one-way ANOVA followed by Tukey's *post-hoc* comparisons test. The criterion for the statistical significance was $P < 0.05$.

RESULTS

Behavioral results

In scopolamine group, the escape latency and traveled path to find the platform was significantly higher than that of control group [$P < 0.05 - P < 0.001$; Figures 1 and 2]. Pretreatment by 10 mg/kg but not

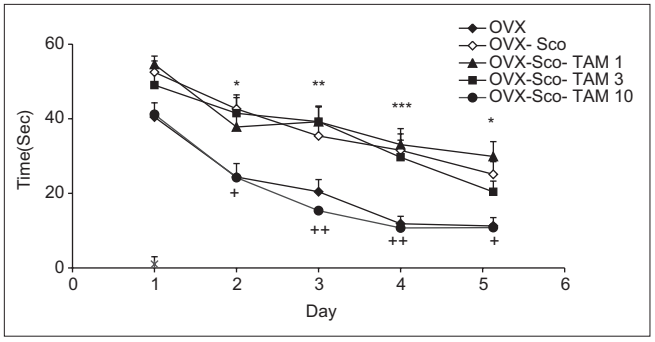

Figure 1: Comparison of time latency to reach the platform in Morris water maze between five groups. Data are presented as mean ± standard error of the mean ($n = 10$ in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to control group. $^{+}P < 0.05$, $^{++}P < 0.01$ compared to Sco-group

1 and 3 mg/kg of TAM prevented spatial learning and memory impairments induced by scopolamine presented as shorter time and traveled distance to find the platform in Sco-TAM 10 group compared to Sco-group [$P < 0.05 - P < 0.001$; Figures 1 and 2]. As shown in Figure 3, there were no significant differences between the groups in swimming speed.

The ability of animals to remember the location of the platform was evaluated in probe trial. The results showed that the scopolamine administration reduced the spent time in target quadrant in OVX-Sco-group compared to the OVX group [Figure 4; $P < 0.05$]. Treatment of animals with 10 mg/kg of TAM improved the spent time in target quadrant [Figure 4; $P < 0.05$].

The results of PA test showed that there were no significant differences between the groups before receiving the shock. Administration of scopolamine shortened the latency to enter the dark compartment at both 3 ($P < 0.01$) and 24 ($P < 0.05$) h after the shock. Treatment of the OVX rats by 1, 3, and 10 mg/kg TAM prevented the impairing effects of scopolamine on memory presented in higher latencies of the animals treated by TAM to enter the dark compared to scopolamine group ($P < 0.05 - P < 0.001$) [Figure 5].

The results of biochemical assessments showed that the MDA concentration in the hippocampal tissues of Sco-group was significantly higher than that of control group ($P < 0.05$), the MDA concentrations in the hippocampal tissues of in Sco-TAM1, Sco-TAM3, and Sco-TAM10 groups were lower than Sco-group ($P < 0.01 - P < 0.001$) [Figure 6].

The results of the homogenates of cortical tissues also showed that the MDA concentration in the cortical tissues of Sco-group was significantly higher than that of control group ($P < 0.05$). The MDA concentrations in the cortical tissues of Sco-TAM10 group was lower than Sco-group ($P < 0.05$) [Figure 7].

The results also showed that the activity of SOD enzyme in the brain tissues of Sco-group was lower than control group ($P < 0.01$). Administration of all three doses of TAM enhanced the activity of the antioxidant enzyme SOD in comparison with the Sco-group ($P < 0.05 - P < 0.01$) [Figure 8].

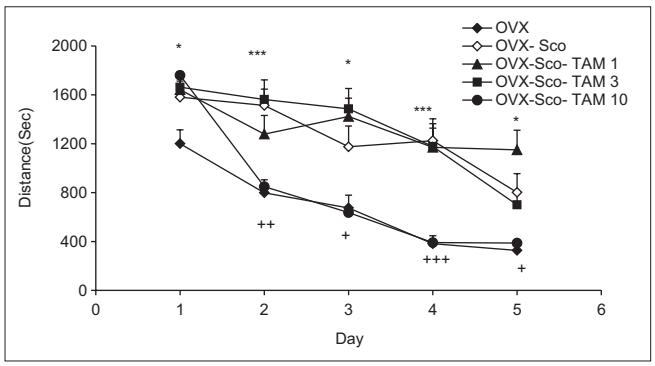

Figure 2: Comparison of traveled distance to reach the platform in Morris water maze between five groups. Data are presented as mean ± standard error of the mean ($n = 10$ in each group). $^{*}P < 0.05$, $^{***}P < 0.001$ compared to control group. $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared to Sco-group

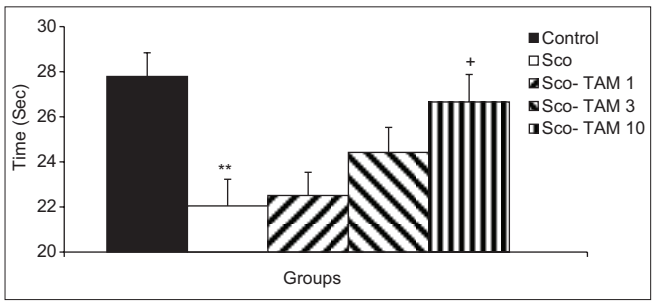

Figure 4: Comparison of the time spent in target quadrant during the probe trial on day 6 (24 h after the last secession of learning). Data are presented as mean ± standard error of the mean ($n = 10$ in each group). $^{**}P < 0.01$ compared to control group, $^{+}P < 0.05$ compared to Sco-group

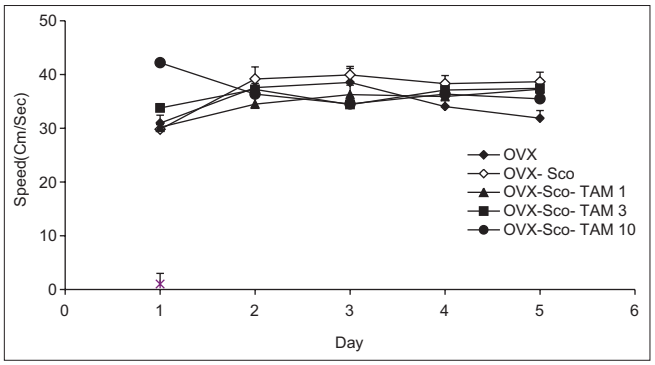

Figure 3: Comparison of swimming speed in Morris water maze between five groups. Data are presented as mean ± standard error of the mean ($n = 10$ in each group)

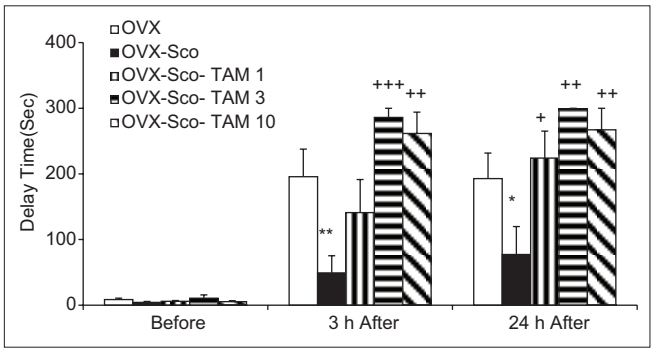

Figure 5: Comparison of time latency for entering the dark compartment at the 3 and 24 h after receiving shock in the experimental groups. Data are presented as mean ± standard error of the mean ($n = 10$ in each group). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control group. $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared to Sco-group

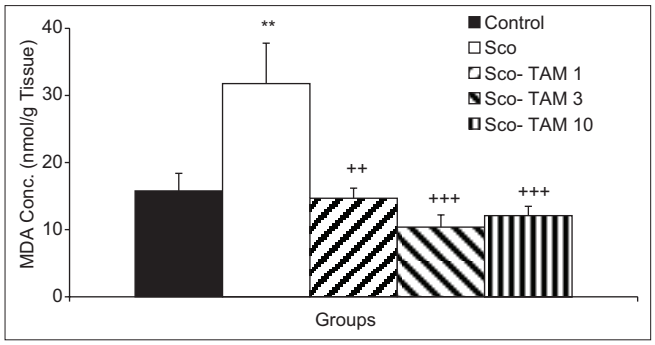

Figure 6: Comparison of the malondialdehyde concentrations in hippocampal tissues of five groups. Data are presented as mean ± standard error of the mean (n = 10 in each group). $^{**}P < 0.01$ compared to control group. $^{++}P < 0.01$, $^{+++}P < 0.001$ compared to Sco-group

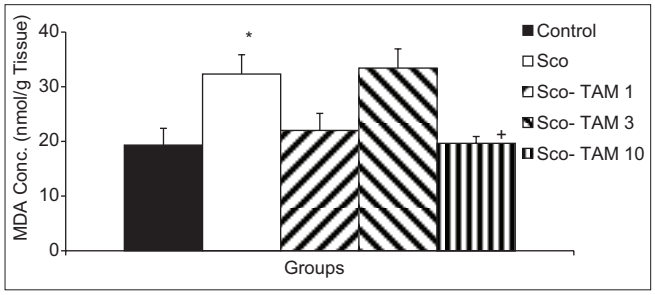

Figure 7: Comparison of the malondialdehyde concentrations in cortical tissues of five groups. Data are presented as mean ± standard error of the mean (n = 10 in each group). $^{*}P < 0.05$ compared to control group. $^{+}P < 0.05$ compared to Sco-group

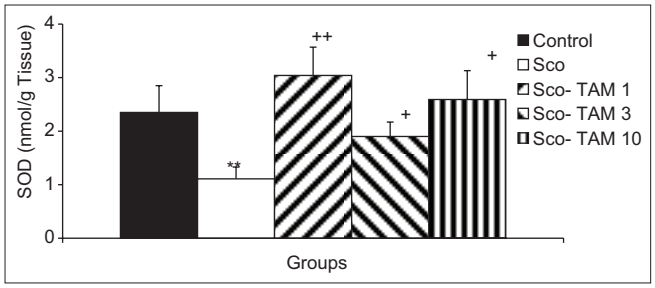

Figure 8: Comparison of the activity of superoxide dismutase in the brain tissues between five groups. Data are presented as mean ± standard error of the mean (n = 10 in each group). $^{**}P < 0.01$ compared to control group. $^{+}P < 0.05$, $^{++}P < 0.01$ compared to Sco-group

DISCUSSION

The results of present study showed that treatment of OVX rats by scopolamine impaired both spatial and non-spatial memory that was accompanied with increased levels of brain tissues oxidative damage criteria. The results also showed that TAM prevented memory impairment related to blockade of acetylcholine receptors that accompanied with lowering of oxidative damage criteria. Modulatory effects of female and male sex hormones on nervous system functions as well as their effects on neurological disorders such as Parkinson, epilepsy, and AD have been reported.[20,27,28] Learning and memory impairments that have been reported in OVX rats confirm the beneficial effects of endogenous estradiol on learning and memory.[29,30] Other researchers also showed that estrogen administration can reverse the effects of ovariectomy on avoidance and spatial memory.[18,31] In contrast to these findings, it was shown that chronic treatment of female rats with high doses of estradiol had deleterious effects on learning and memory.[32] It has been suggested the SERMs may have an estrogen-like neuroprotective effect and may be useful to improve memory tasks.[21] The results of our previous study showed that TAM improved learning and memory impairments due to deletion of ovarian hormones in rats.[21] The modulatory effects of ovarian hormones on learning and memory have been attributed to the interaction with the cholinergic system. The interaction of estrogen with the central cholinergic system, the most important neurotransmitter system involved in cognitive functions has also been suggested. Estrogen has been reported to stimulate choline acetyltransferase expression and activity and potassium-stimulated acetylcholine release in rat hippocampus while it inhibits the activity of acetylcholine esterase.[33,34] Considering the mentioned evidence, an interaction between TAM and the cholinergic system to modulate learning and memory was postulated. The results showed that TAM prevented scopolamine-induced learning and memory impairment that was presented by shorter time and distance to reach the platform in MWM. The animals pre-treated by 10 mg/kg of TAM also had a longer latency to enter the dark compartment in PA test. The results confirmed an interaction between the cholinergic system and TAM. In consistent with the results of present study, it has been reported that TAM acts as an estrogen-like agonist to enhance cholinergic system activity and hippocampally mediated learning in human.[13] TAM was also able to enhance acetylcholine transferase expression in a manner similar to that of estrogen in several basal forebrain regions.[19] In another study, administration of 20 mg of tamoxifen significantly attenuated the verbal episodic memory and spatial navigation impairment due to cholinergic blockade in postmenopausal women compared with placebo.[13]

The role of cholinergic system in cognition and memory has been well-documented.[35-37] The levels of acetylcholine in the hippocampus seem to be related to learning and memory.[38] It has also been shown that the release of acetylcholine increases during learning.[39] The scopolamine-induced memory deficit model has been widely used for identifying

the potential of drug candidates to reverse the effects of cholinergic blockade.[40] Scopolamine causes memory impairment through inhibition of cholinergic system.[41] It was shown that muscarinic acetylcholine receptors, causing the consolidation of spatial memory.[39] Using PA and MWM tests, it has been well-documented that cholinergic system of several areas of the brain including amygdala and hippocampus play an important role in learning and memory.[42] The PA model has been used to study learning and memory for a stressful stimulus. The practice is based on the innate preference of rodents for the dark section of the apparatus and the suppression of this innate preference following exposure to an inescapable shock that is considered as a measure of learning and memory.[43] This test is mainly used to evaluate the contextual and nonspatial learning and memory that is sometimes considered a nonhippocampal mediated memory.[44] The MWM task, a well-established test of spatial navigation, is mainly hippocampal dependent.[45]

It has been suggested that besides acetylcholine antagonizing effects of scopolamine, it may have deleterious effects on memory by other mechanisms. It has been reported that scopolamine-induced memory deficit is accompanied with oxidative damage.[46,47] The results of the present study also showed that the impairing effects of scopolamine on learning and memory were accompanied with an elevation of MDA levels in the brain while, the SOD activity was diminished. It has also been previously reported that scopolamine causes oxidative stress in the brain, and possibly in this way can also be involved in the creation of memory disorder.[47,48] In a study conducted in 2009 by Ciobica and colleagues, it was observed that the administration of scopolamine reduced the levels of SOD and GPX while increased MDA levels compared to saline groups.[49]

Estrogen has been shown that exerts its memory-related effects via multiple pathways including antioxidant properties, which may also have a role in its neuroprotective effects.[50] The antioxidant property of the estradiol has been attributed to its free phenolic hydroxyl group on the A-ring of the steroid. Removal or blocking of the phenolic hydroxyl group eliminates the antioxidant effect, as well the neuroprotection properties.[50] In the current study, the estrogen-like compound TAM mimicked the antioxidant effects of estrogen and prevented of increasing in lipid peroxidation induced by scopolamine in the brain tissues of rats. The SOD activity in the brain tissues of TAM pretreated rats was also lower than that of scopolamine group. In agreement with this, TAM was shown to be able to inhibit the increased TBARS concentrations in hypoxic–ischemic rat model.[51] The results of our previous study also confirmed the protective effects of TAM against the brain tissue oxidative damage of OVX rats.[21] Regarding these results, a protective effect against brain tissues oxidative damage as a possible mechanism for improving effects of TAM in animal model of scopolamine memory impairment might be postulated; however, it needs to be more investigated in the future. In supporting of our results, it was previously shown that raloxifene also increased the level of GSH in the brain cortex of OVX rats treated by kainic acid.[16] A protective role for raloxifene against oxidative stress associated endothelial dysfunction has also been suggested.[17]

Finally, it is concluded that TAM has an interaction with the cholinergic system to modulate learning and memory. Regarding the brain tissues oxidative damage associated with scopolamine memory impairment which has been previously reported and was also confirmed in the present study, it seems that beneficial effects of TAM on memory impairment induced by scopolamine that was seen in the present study may at least in part be due to the protection against the brain tissues oxidative damage.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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