Original Article

Protection against Brain Tissues Oxidative Damage as a Possible Mechanism for Improving Effects of Soy on Scopolamine-Induced Learning and Memory Impairments in Ovariectomized Rats

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Abstract

Background: Modulatory and antioxidant effects of soy extract on the central nervous system have been reported. **Aim:** The effects of soy on learning and memory impairments induced by scopolamine and the brain tissues oxidative damage was investigated. **Materials and Methods:** The ovariectomized rats were divided: (1) Ovariectomized (OVX), (2) Ovariectomized – Scopolamine (OVX-Sco), (3) Ovariectomized -Scopolamine - Soy 20 (OVX-Sco-Soy 20) and (4) Ovariectomized -Scopolamine-Soy 60 (OVX-Sco-Soy 60). Soy extract was administered (20 or 60 mg/kg; i.p.) daily for 6 weeks before training in the passive avoidance (PA) test. Sco (2mg/kg) was injected 30 min after training in the PA test. **Results**: In PA test, the time latency to enter the dark compartment in OVX- Sco group was higher than OVX group (P<0.01). Pre-treatment by both doses of soy prolonged the latency to enter the dark compartment compared to OVX-Sco group (P<0.05- P<0.01). The brain tissues mallondialdehyde concentration as an index of lipid peroxidation was increased while, thiol content was decreased in OVX- Sco group compared to OVX group (P<0.05). Pre-treatment by soy lowered the concentration of MDA while, increased thiol concentration compared to OVX- Sco group (P<0.05- P<0.01). **Conclusion:** It was observed that soy prevented learning and memory impairments induced by scopolamine in OVX rats. The mechanism(s) might at least in part be due to protection against the brain tissues oxidative damage.

Keywords: Scopolamine, Soy extract, Memory, Oxidative damage

Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly [1]. Middle-aged women repeatedly report memory problems during the menopausal transition [2]. It has been well documented that, deprivation from ovarian hormones impairs learning and memory which is improvable by hormone replacement therapy [3,4]. Treatment by estrogen, has been reported that improve several kinds of memory in postmenopausal women [3,4]. An improving effects of 17β-estradiol on learning and memory of the rodents has also been documented which was accompanied with an enhanced level in neurogenesis in the hippocampus [5]. Learning and memory impairments due to depletion of ovarian hormones have been reported to be connected with cholinergic system dysfunctions [6]. Beneficial effects of estrogen replacement on cognitive deficits due to aging and AD has been suggested to be mediated by improving of cholinergic system functions [7]. Ovariectomy and estrogen therapy are suggested to interact with basal forebrain cholinergic pathways [7].

Phytoestrogens are bioactive plant chemicals found in soy, which have a similarity in structure to natural estradiol (the most abundant circulating estrogen). This structural likeness enables phytoestrogens to interact with estrogen receptors in the brain, potentially affecting cognition [8]. Previously, treatment with soy isoflavones has been associated with an improved performance across several cognitive domains such as nonverbal memory, visual memory, fluency and speed of verbal skills and appears to have a beneficial outcome on enhancing cognitive capacity in postmenopausal women [9]. Dietary soy phytoestrogens has been reported that influence learning and memory and alter the expression of proteins involved in neural protection and inflammation in rats [10]. Duffy et al. reported that cognitive function in postmenopausal women was improved after 12 weeks of consumption of a soya extract containing isoflavones [11]. Hogervorst et al. reported that soy consumption was associated with better recall in younger, but not in aged people [12]. However, no effect [13,14]. or even possible deleterious effects [15]. of the plant has also been reported. For example, White et al.

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[15] proposed that consumption of tofu, a whole soybean protein rich in isoflavones was associated with brain atrophy in older Japanese Americans. A large trial enrolling postmenopausal women age 45 to 92 found a benefit however, just for a visual memory [16].

Altogether, there is little known about soy isoflavone's effect on cognition in older adults, especially older adults with AD. It has also been reported that soy isoflavones enhance learning and memory impairment induced by Aβ1–42 in rats [17]. It has been suggested that soy isoflavones may protect Aβ-impaired learning and memory in rats and the mechanism of activity may be connected with the regulation of vascular Aβ transportation and vascular inflammatory reaction [18]. Among the animal models, a neuroprotective effect of soy isoflavones on spatial learning and memory in a rat model of AD has been reported [19]. An increase level of inflammatory mediators, such as cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) in astrocytes exposed to Aβ induces were preventable by estradiol or genistein as an important components of soy [20]. Soy has been reported to be able to decrease the serum levels of IL-1β and IL-1β positive neurons in several brain regions including hippocampus [21].

A moderate positive effect of soy on cognition of the persons diagnosed with AD has also been reported [9]. It is also suggested that the soy isoflavones influence the cholinergic system of the brain to reduce neuronal loss and cognition decrease in male rats [22]. Soy has also been able to prevent from brain tissue oxidative damage in an ischemia reperfusion model in ovariectomized rats [23]. To the best of our knowledge, there is no report to evaluate possible mechanism(s) for learning and memory improving effects of soy. Therefore, the aim of present study was to protective effects against brain tissues oxidative damage as a possible mechanism for improving effects of soy on scopolamine-induced learning and memory impairments in ovariectomized rats.

Materials and Methods

Animals and drugs

Female Wistar rats, 12 weeks 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 ± 2°C) and 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 ovariectomized and were then randomly divided into four groups (n=9-10 in each group) and treated: (1) Ovariectomized (OVX), (2) Ovariectomized –Scopolamine (OVX-Sco), (3) Ovariectomized -Scopolamine - Soy 20 (OVX-Sco-Soy 20) and (4) Ovariectomized -Scopolamine - Soy 60 (OVX-Sco-Soy 60). The animals in the OVX- Sco-Soy groups (the groups 3and 4) were treated by daily injections of Soy extract (20 or 60 mg/kg; intraperitoneally; IP) for 6 weeks before the behavioral tests. The animals of OVX and OVX-Sco groups received 1 ml/kg of saline instead of Soy. Scopolamine (2 mg/kg) was injected after training in passive avoidance test. The sample size was based on the previous studies [24,25].

Ketamine and xylazin were purchased from Alfasan Company Woerden-Holand. Scopolamine was purchased from Sigma Aldrich Company, USA. Other chemicals such as those which were used for biochemical assessments were purchased from Merck Company.

Plant extracts

Soy was procured from Gorgan City, Golestan Province, in the north of Iran, and was scientifically identified by the Department of Botany of Ferdowsi University of Mashhad, Iran, and the voucher specimen of the soybean was deposited. To prepare the hydroalcoholic extract, 50 g of the crumbled, dried plant was extracted with 300 mL ethanol-water (70/30, v/v), using a rotary vacuum evaporator in order to reduce to the dryness of the extracts [23,26,27].

Ovarietomy surgery

The animals were ovariectomized 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. 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 [28].

Passive avoidance test

Passive avoidance learning test is based on negative reinforcement and is used for evaluating of non-saptial learning and memory [29,30]. The apparatus has 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 third day, the animals were placed in the 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, 2s duration). Then, the rats were injected by scopolamine and were then returned to their home cage. 3 and 24 hours 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 [29].

Biochemical measurements

Malondialehye (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 (TCA))/hydrochloric acid (HCL) and it was then boiled in a water bath for 40 minutes.

After reaching to the room temperature, the solution was centrifuged at 1000 g for 10 minutes. The absorbance was read at 535 nm. The MDA concentration was calculated according to follow equation [29,30]. C (M) = Absorbance / 1.56 × 10^5

Total thiol groups were measured using DTNB (2, 2'-dinitro-5, 5'-dithiodibenzoic acid), a reagent that reacts with the SH groups and produce a yellow colored complex which has a peak absorbance at 412 nm. Briefly, 1 ml Tris-EDTA buffer (pH = 8.6) was added to 50 µl brain homogenate in 1 ml cuvettes and the absorbance was read at 412 nm against Tris-EDTA buffer (A1). Then 20µl DTNB reagents (10 mM in methanol) were added to the mixture and after 15 min incubation in room temperature, the absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated based on an equation previously described by Hosseini et al [28].

Statistical analysis

The data were expressed as mean (standard error of the mean). One way ANOVA was run followed by tukey post hoc comparisons test. The criterion for the statistical significance was P<0.05. Data analysis was carried out using the statistical software package SPSS for windows version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

The results of passive avoidance test

In the present study, administration of scopolamine impaired learning and memory of OVX- Sco group was reflected by increasing of the latency to enter the dark compartment at both 3 and 24 hours after the shock(P<0.01). Treatment of the animals by both doses including 20 and 60 mg/ kg of the extract for 6 weeks after removal of ovaries attenuated impairing effects of scopolamine which was presented by prolonging of latency to enter the dark compartment after the shock at both 3(P=0.049 for lower dose and P=0.010 for higher dose) and 24 (P=0.048 for lower dose and P<0.01 for higher dose) hour post shock time [Figure 1]. The results of PA test also showed that the animals of OVX- Sco group had a higher number of entries to the dark compartment compared to the OVX group when they were examined 3(P<0.01) and 24(P=0.011) hours after the shock. Administration of both doses of the extract decreased the number of entries to the dark in both OVX-Sco-Soy 20 and OVX-Sco-Soy 60 groups compared to OVX-Sco groups at both 3(P=0.027 for lower dose and P<0.01 for higher dose) and 24 (P=0.023 for lower dose and P<0.01 for higher dose) hours after the shock [Figure 2]. Additionally, at both 3(P<0.01) and 24(P=0.021) hours after the shock, the animals of OVX- Sco group had increased levels of the time spent in the dark compared to OVX group. At both 3(P=0.048 for lower dose and P<0.01 for higher dose) and 24 (P=0.037 for lower dose and P<0.01 for higher dose) hours post shock times, the animals of both OVX- Sco -Soy 20 and OVX- Sco -Soy 60 groups spent shorter times in the dark compartment where they had previously received a shock compared to OVX-Sco group [Figure 3]. Treatment of the OVX rats by scopolamine shortened the time spent in the light compartment of PA test at both 3(P<0.01) and 24(P=0.021)

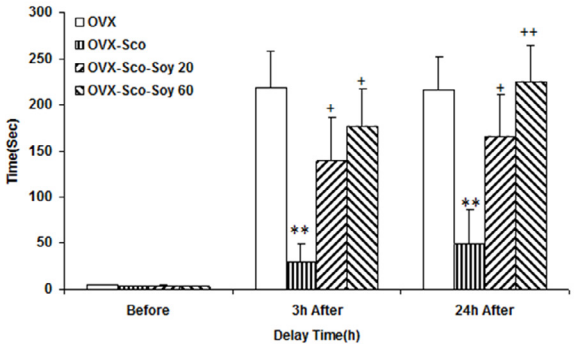

Figure 1: Comparison of time latency for entering the dark compartment at 3 and 24 h after receiving the shock in the experimental groups. Data are presented as mean ± SEM (n= 9-10 in each group). **P<0.01 compared to OVX group, +P<0.05, ++P<0.01 compared to OVX- Sco group.

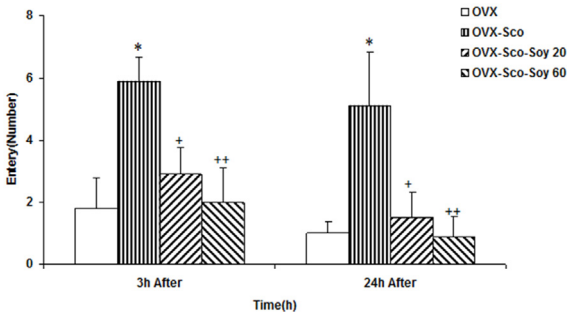

Figure 2: Comparison of the number of entries to the dark compartment at 3 and 24 h after receiving the shock in the experimental groups. Data are presented as mean ± SEM (n= 9-10 in each group). *P<0.05 compared to OVX group, +P<0.05, ++P<0.01 compared to OVX- Sco group.

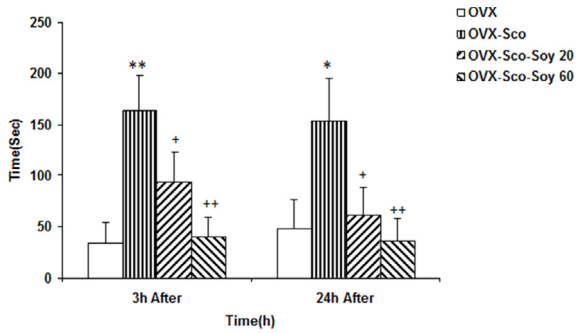

Figure 3: Comparison of the total time spent in the dark compartment at 3 and 24 h after receiving the shock in the experimental groups. Data are presented as mean ± SEM (n= 9-10 in each group). *P<0.05, **P<0.01 compared to OVX group, +P<0.05, ++P<0.01 compared to OVX- Sco group.

hours after the shock compared to the OVX group. Pretreatment of OVX rats by both doses of the extract attenuated the effects of scopolamine which was reflected in a higher time spent in the light by the animals of both OVX-Sco-Est 20 and OVX-Sco-Est 60 groups compared to OVX-Sco group at both 3 (P=0.030 for lower dose and P<0.01 for higher dose) and 24(P=0.037 for lower dose and P<0.01 for higher dose) hours after the shock [Figure 4].

The biochemical results

To evaluate the brain tissues oxidative damage status, MDA concentration and thiol contents of both the hippocampal and cortical tissues were compared among the groups. Administration of scopolamine increased lipid peroxidation in

the hippocampal tissues of OVX rats which was reflected in an increased level of MDA concentration in the hippocampus of OVX-Sco group compared to OVX group (P=0.015) [Figure 5A]. Daily injection of 20 mg /kg of soy which was started at the day after ovariectomy and was continued for 6 weeks was able to prevent from the effects of scopolamine. It was observed the animals of OVX-Sco-Soy 20 had a lower level of hippocampal MDA concentration compared to OVX-Sco group (P<0.01, [Figure 5]. There was also a significant difference between the OVX-Sco-Soy 60 and OVX- Sco groups (P<0.01, [Figure 5A].

In contrast to MDA, total thiol contents was decreased in the hippocampal tissues of OVX- Sco group after administration of scopolamine compared to OVX group(P=0.049). Chronic treatment by higher dose of the extract prevented from lowering effects of scopolamine on hippocampal tissues thiol concentrations which was revealed by a low level of thiol groups in the hippocampal tissues of OVX-Sco-Soy 60 group compared to OVX-Sco group (P=0.026, [Figure 5B]. There was no significant difference between OVX-Sco-Soy 20 group and OVX-Sco groups in thiol concentrations [Figure 5B].

Additionally, the cortical tissues of OVX- Sco showed a higher concentration of MDA compared to OVX group (P=0.013, [Figure 6A]. No significant differences were observed when the MDA concentrations in the cortical tissues of OVX- Sco-Soy 20 and OVX- Sco-Soy 60 groups were compared to that of the OVX-Sco group [Figure 6A]. The total thiol concentrations in the cortical tissues of OVX- Sco group was lower than that of OVX group [P=0.048), [Figure 6B]. The animals of OVX-Sco-

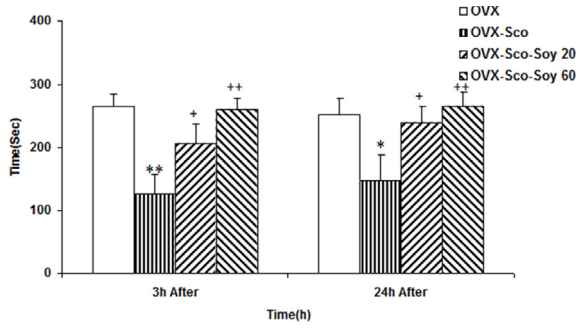

Figure 4: Comparison of the total time spent in the light compartment. Data are presented as mean ± SEM (n= 9-10 in each group). **P<0.01 compared to OVX group, +P<0.05 compared to OVX- Sco group. *P<0.05, **P<0.01 compared to OVX group, +P<0.05, ++P<0.01 compared to OVX- Sco group.

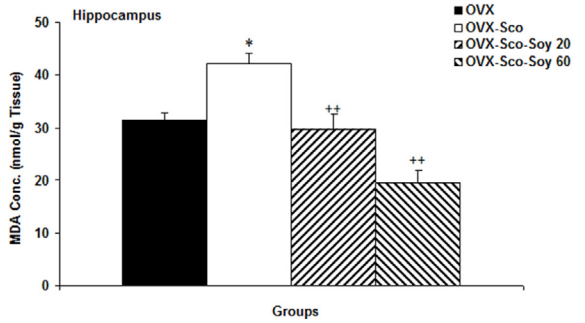

Figure 5: Values are mean ± standard error of hippocampal malondialdehyde (A) and total thiol (B)concentrations in the experimental groups. Data are presented as mean ± SEM (n= 9-10 in each group). *P<0.05 compared to OVX group, +P<0.05, ++P<0.01 compared to OVX- Sco group.

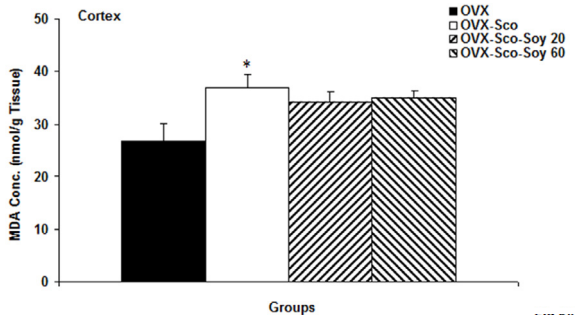

Figure 6: Values are mean ± standard error of cortex malondialdehyde (A) and total thiol (B) concentrations in the experimental groups. Data are presented as mean ± SEM (n= 9-10 in each group). *P<0.05 compared to OVX group, +P<0.05 compared to OVX- Sco group.

Soy 60 group had a higher total thiol contents in thetheir cortical tissues compared to OVX- Sco group (P=0.043), [Figure 6B]. There were no significant differences between OVX-Sco-Soy 20 and OVX-Sco groups in the thiol cortical tissues [Figure 6B].

Discussion

The results of present study indicated that soy extract prevented from scopolamine induced learning and memory impairments in OVX rats which were accompanied by improving effects on the brain tissues oxidative damage criteria. Scopolamine, a nonselective muscarinic antagonist, has been surely documented to block cholinergic signaling and produce memory and cognitive impairment [31]. Consequently, IP administration of scopolamine has been widely and successfully used as a pharmacological model for AD in rats [32-35]. In the present study, IP injection of scopolamine impaired memory which was reflected by decreasing of the latency to enter the dark while, the time spent and the number of entering to the dark where the animals had previously received a shock enhanced. Besides of dis-regulation of the cholinergic neuronal pathway, scopolamine is proposed to be accompanying with the brain tissues oxidative injury [36].

This fact can be confirmed with our past studies in which learning and memory impairments induced by scopolamine in rats was accompanied by growing of lipid peroxidation and a low level of thiol contents in the brain [32-34]. Regarding the results of present study, inducing of an oxidative stress status in the brain by scopolamine to induce learning and memory impairments in rats might be postulated. The results showed that administration of scopolamine increased MDA concentrations in both hippocampal and cortical tissues while, decreased thiol concentration in the brain. Interestingly, it seems that lipid peroxidation was more pronounced in hippocampal tissues rather than in cortical tissues. Confirmedly, it has also been previously reported that hippocampal tissues are more susceptible than the cortical tissues to lipid peroxidation [37] considering a more important role of hippocampus in learning and memory, these results might be conceivable. In this respect, a recovery of cholinergic function together with the suppression of oxidative damage has been reported to be a rational strategy for the beneficial treatment of AD [38].

The molecular structure of soy phytoestrogens is similar to that of estrogen therefore, they bind to estrogen receptors and show some functions similar to the effects of estrogen [39]. It has also been shown that these herbal compounds have beneficial

effects on memory and the cognition of both menopausal women and OVX rats [8,40]. In addition, it has been shown that they have behavioral benefices in AD and Parkinson [41-42]. The results of present study certified beneficial effects of soy extract on scopolamine - induced learning and memory as an animal model of AD. The animals pre-treated by both 20 and 60 mg/kg of the extract had a longer latency to enter the dark compartment at both 3 and 24 hours after receiving the shock. Interestingly, the animals of OVX-Sco-Soy 20 and OVX-Sco-Soy 60 groups has a lower number of entries to and spent a lower time in the dark compartment where they had previously received a shock. Conversely, the animals of the groups pre-treated by two doses of the extract spent a longer time in the light compartment compared to the OVX-Sco group.

In supporting of the results of present study, it has been previously reported that soy isoflavones can effect on the brain cholinergic system and decrease age-related neuron loss and cognition decline in male rats [22]. The study of Lee demonstrated that soy isoflavones affected on the cell density of cholinergic neurons in medial septum and CA1 area of hippocampus, as well as the cholinergic enzyme activity in the basal forebrain, hippocampus, and cortex [22]. As well as, it was reported that soy isoflavones down-regulated acetylcholinesterase activity in all regions of the cortex, hippocampus, and basal forebrain [22]. Another study showed that soy phytoestrogens effects on the neuronal cholinergic system of OVX rats to augment reference memory [43]. It is also suggested that the improving effect on cognitive ability in phytoestrogen-treated females may in part be due to an increased level of choline acetyltransferase mRNA in the frontal cortex [43]. The result of present study might be consider as an evidence for modulatory effects of soy or its components on cholinergic system. However more precise studies are need to be done to better understand this issue.

Protective effects of soy and its derivations against the brain tissues oxidative damage has been previously suggested [23]. Additionally, it is suggested that the natural compounds with a protective effect against brain tissues oxidative injury, has also an improving effect on learning and memory impairment induced by scopolamine [33,34]. Thus, a protective effect against brain tissues oxidative damage as a possible mechanism for positive effect on scopolamine – induced learning and memory impairment was examined. The results showed that both doses of the extract attenuated the MDA concentration as an index of lipid peroxidation in hippocampal tissues however; none of two doses were effective to change cortical MDA. The mechanism(s) responsible for such an effect was not understood in the present study and needs to be investigated in the future. The results also showed that 60 mg / kg of the extract enhanced the thiol contents in the cortical tissues. Surprisingly, isoflavones are proposed to be very effective antioxidants having greater in vitro antioxidant activity than vitamin C or E [44]. It has also been reported that genistein as a main compound of soy protected the neuronal cells from H2O2-induced toxicity [45]. It has been indicated that Aβ is related to oxidative stress and mitochondrial dysfunction in the Alzheimer's brain [46]. On the other hand, soy isoflavones has been suggested to counteract this process by increasing of serum and brain tissue antioxidant levels [46]. In addition, soy isoflavones has been reported to be able to decrease reactive oxygen species and ameliorate Aβ induced cellular apoptosis [47]. Regarding these facts and the results of present study, it seems that the effects of soy on learning and memory impairments induced by scopolamine are at least in part due to the protective effects against the brain tissues oxidative damage however, a more precise biochemical tests are needed to be done in the future.

Besides of having an antioxidant activity, genistein has been recommended to protect neuronal cells by activating of estrogen receptors (ERs) and improving of the brain-derived neurotrophic factors [48]. It has also been reported that the Isoflavones including genistein and daidzein are selective estrogen receptor modulators, in having greater affinity for ERβ, a receptor that is widely distributed in brain tissue, than for ERα [49]. Additionally, soy isoflavones work to show a neuroprotective effect on brain tissue via estrogenic receptor pathways [50] and thereby protect against cell apoptosis and neurotoxicity [51]. In the present study, the beneficial effects of the plant extract might be related to both anti- oxidative and modulatory effects on estrogen receptors. However, each of these mechanisms needs to be more investigated.

Conclusion

Finally, the results of present study showed that the soy extract improved learning and memory impairments induced by scopolamine in passive avoidance test. The plant extract also improved the brain tissues oxidative damage criteria and therefore, a protective effect against brain tissues oxidative damage as a possible mechanism for improving effects of soy on scopolamine-induced learning and memory impairments might also be suggested.

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