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Original Article

The Antioxidant Effect of Sidr (*Zizyphusspina-Christi*) Leaf Extract Helping to Improve the Scopolamine Induced Memory Impairment in Male Rats

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Abstract

Medicinal plants have attracted global attention due to their high levels of antioxidant components for prohibiting or improving various diseases including brain and memory disorders. This study was designed to investigate if the antioxidant effect of Sidr (*Zizyphusspina-christi*) extract can help to improve the scopolamine induced impairment of memory in Wistar rats. Animals were randomly divided into 6 groups: Control, Scopolamine at 1 mg/kg, Scopolamine+50 mg/kg Sidr extract (Zsc50+sco), Scopolamine+100 mg/kg Sidr extract (Zsc100+sco), Scopolamine+200 mg/kg Sidr extract (Zsc200+sco) and Scopolamine+diazepam at 1 mg/kgbw (D+sco). Antioxidant activity and total phenolic content of *Z. spina-christi* as well as the Passive avoidance test, antioxidant capacity and MDA level of plasma and brain were evaluated.Scopolamine increased the recorded initial latencies but decrease the step-through latency in comparison with those of control group. The use of Sidr at 50, 100 and 200 mg/kg attenuate these abnormalities and the impaired passive avoidance memory was improved. Although scopolamine didn't cause any change in the antioxidant capacity plasma and brain, it increased the MDA level of plasma and brain tissues. Also use of Sidr increased the antioxidant capacity and decreased the MDA level of plasma and brain tissues.The results suggest that *Z. SC* might act to attenuate scopolamine induced brain and memory impairments due to its high antioxidant contents. The separation of the specific compound responsible for observed antioxidant activity with protective effect against brain and memory disorders still need further researches.

Keywords: *Zizyphusspina-christi*, Antioxidant, Memory, MDA, Scopolamine

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1. Introduction

The occurrence of brain disorders such as dementia and Alzheimer's disease is dramatically increasing with the increasing of people age [1]. Amyloid plaques, neurofibrillarytangles, inflammatory processes, and disturbance of neurotransmitters as well as brain cells wither and death are of the most

pathological features of brain disorders [1]. The increased rete in free radicals production during brain cellular respiration as well as lipid peroxidation has also been reported to cause damage to cell membranes, enzymes, DNA, lipids, and proteins and subsequently impairing their functionality [2]. Considering the very high sensitivity of brain tissue due to the high content of polyunsaturated lipid-rich neural parenchyma, high oxidative metabolic demands and low antioxidative enzymes as well as the low levels of reserved oxygen as compared to other organs, it can be expected that the above mentioned pathological features can cause much severe problems in the brain functionality [3,4].Cholinergic play a significant role in the cognitive deficits associated with aging and neurodegenerative disorders [5] Impairment of memory, cognitive, learning and motor activity could be induced by administration of scopolamine in experimental animals. Scopolamine is a cholinergic antagonist known to interfere with acetylcholine transmission in the central nervous system [2]Endogenous antioxidants including the enzymes superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase as well as other low molecular weight antioxidants can be effective in elimination the adverse effects of free radicals and other oxidative stress agents [2] therefore they can be play significant role in prevention or improving the generated impairment in brain functions. ROS can interact with both endogenous and exogenous antioxidants agents influencing either the activity or availability of antioxidant systems [6].

Healthy habits of nutrition and life style along with the consumption of natural and synthetic supplement sare common issues in nutritional science. Recently, medicinal plants and their derivatives compounds have attracted global attention due to their high levels of antioxidant components including flavonoids and phenols [7]. Successful treatments for dementia have been developed from herbal drugs [1]. *Zizyphusspecies* belonging to Rhamnaceae family have been used in traditional medicine for the treatment of various diseases such as digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anaemia, diarrhea, and insomnia[8].The main bioactive components of Zizyphus species are including cyclopeptide alkaloids, flavonoids, sterols, tannins, and triterpenoidsaponins[8].

Zizyphusspina-christi, also known as Sidr in Iran, is a wild tree, with bark light-grey, very cracked, trunk twisted, crown thick, shoots whitish, flexible, spiny branches and small, orange-yellow fruits which can grow to a height of ten meters. Leaves are glabrous on upper surface, finely pubescent below, ovate-lanceolate or ellipsoid, apex acute or obtuse, margins almost entire, lateral veins conspicuous. Its leaves contain tannin, phytosterols such as Beta-*sitosterol*, Beta-*sitosterolglucoside* and saponinslactones[8,9].*Z. spina-christi* is proved as potential source of natural antioxidants to present hepatoprotective[10],

antidiabetic[11], antioxidant [12], antibacterial [13] activity. The fruit extract of *Z. spina-christi* showed beneficial effect on central nervous system in Male Albino Rats [14]. It has also been reported that different extracts of Zizyphus species could show positive effect on reversing the scopolamine-induced memory and cognitive impairment [14, 15]. It is also reported that Sidr leaves poses antibacterial, anti-cancer, anti-inflammatory, anti-hypertensive, anti-diarrhea properties and it has also positive effects on the CNS[16,17].Considering the important role of reactive oxygen species and other free radicals in brain disorders, and also because of the antioxidant effect of Sidr leaf, this study was designed to investigate the effects of Sidr (*Z. spina-christi*) leaf extract on the scopolamine induced impairment of memory, passive avoidance learning and motor activity in male rats.

2. Materials and Methods

2.1. Preparation of Hydroalcoholic Extract of Z. Spina-Christi

The fresh leaves of Sidr (*Z. spina-christi*) were dried under shade, finely powdered, and macerated with 70% Alcohol for 7 days. The resulting extract was then filtered, and the filtrate was concentrated to dryness using vacuum distillation at 40℃. Different concentrations of the extract were prepared using distilled water [18].

2.2. Animals and Treatments

All experiments were conducted on adult male Wistar rats, weighing 250–300g. Rats were kept under standard laboratory conditions (12h light/12h dark cycle at 22°C±2°C) with free access to water and standard laboratory food. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by Research and Ethics Committee at Shahrekord University of Medical Sciences.

Male Wistar rats were divided randomly into 6 groups, each group consist of eight animals:(Control)did not received scopolamine and only received distillated water intraperitoneally; (scolplamine) administration of scolplamine at 1 mg/kgbw;(Zsc50+sco) administration both Sidr leaf extract at 50 mg/kgbw and scolplamine; (Zsc100+sco)administration both Sidr leaf extract at 100 mg/kgbw and scolplamine; (Zsc200+sco)administration both Sidr leaf extract at 200 mg/kgbw and scolplamine; (D+sco) administration both of diazepam at 1 mg/kgbw and scolplamine.

The rats were received extract by gavage and the scopolamine was injected intraperitoneally, half an hour before gavage administration of extract. Injections were performed one week before behavioral testing as well as during behavioral testing period. After performing behavioral testing, the animals were subsequently put under deep anesthesia, cardiac blood samples were collected, and brain was removed. After removal of the brain, hippocampus, cortex and sub-cortex were separated on ice and used for

biochemical analysis. Blood was centrifuged and plasma was separated and used for biochemical analysis.

2.3. Determination of Antioxidant Activity of Z. spina-Christi Leaf Extract

Final concentrations of 10, 20, 40, 60, 80 and 100 µg/ml were prepared from stock solutions of Sidr extract and BHT (1 mg/ml in ethanol). 2 ml of these solutions were transferred to the tube and to which 2 mL of DPPH solution (0.1 mM, in ethanol) was added. After 15 min at room temperature, the absorbance values were measured at 517 nm. The mixture of ethanol (2mL) and DPPH solution (2 mL) served as control. The scavenging activity percentage was determined by the following equation:

$(A\ A\ \%) = 100 \times (A_{control}-A_{sample})/A_{control}$

The antioxidant activity was expressed as IC50 (the concentration of extract required to inhibit the formation of DPPH radical by 50%). The IC50 values were determined by plotting the graph with concentrations in x axis and percentage of inhibition in y axis [19].

2.4. Determination of Total Phenolic Content of Z. Spina-Christi Leaf Extract

Briefly, 0.1 ml of diluted extract (0.01 g in 10 ml of 60 °C methanol) was mixed with 0.5 ml of Folin-Ciocalteu reagent. After 3-5 minute, 0.4 mL of 7.5% sodium carbonate solution was added to the mixture and allowed to stand at room temperature for 30 min. The absorbance of reaction mixture was measured at 750 nm against distillated water blank. A standard calibration curve was plotted using different concentrations of Gallic acid. The phenolic content was expressed as "mg of Gallic acid equivalents per g of sample [19].

2.5. Passive Avoidance Test

Passive avoidance test was performed using a shuttle box apparatus. The apparatus consisted of a lighted and dark compartment with a grid floor. This test was performed for each rat during the 4 days. Initial latency (t1) was recorded on third day and step-through latency (t2) for animals was recorded on forth day[19].

2.6. Measurement of Plasma Antioxidant Capacity

Three solutions were used for this purpose: Solution 1) 1.5 ml of sodium acetate and 8 ml of concentrated acetic acid, diluted to 500 mL with distilled water; Solution 2) 270 mg of Iron (III) chloride, dissolved in 50 mL of distilled water; Solution 3) 47 mg of treeazin, dissolved in 40 mL of HCl. Working solution was prepared by mixing 10 mL of solution 1, 1 ml of solution 2 and 1 ml of solution 3. Thereafter, 25 microliters of plasma sample was added to 5.1ml of working solution. The resulting mixture was incubated at 37°C for 15 minutes and then the absorbance was measured at 593 nm [19].

2.7. Measurement of Brain Antioxidant Capacity

The antioxidant capacity of brain was determined by ferric reducing antioxidant power (FRAP) assays. FRAP working solution was prepared by mixing 25 ml of acetate

buffer, 2.5 ml of TPTZ (2, 4, 6-tripyridyl-s-triazine) and 2.5 ml of $FeCl_3$. Brain tissue was homogenized and the homogenate was centrifuged at 1000 g for 10 min. 50 ml of the resulting supernatant was mixed with 5.1 mL of FRAP working solution. After 10 minutes of incubation, Fe^{3+} TPTZ complex was reduced to the ferrous (Fe^{2+}) form which produced an intense blue color. The absorbance of mixture was measured at 590 nm [19].

2.8. Measurement of Plasma MDA Level

Briefly, 50 microliters of plasma were mixed with 50 µL of 0.05% BHT, 400 µL of 0.44 M H_3PO_4 and 100 µL of 42 Mm TBA. The mixture was vortexed and then heated in a boiling water bath for 1 h. After cooling at 0℃ for 5 min, 250 µL of n-butanol was added to the mixture, vortexed, and then centrifuged at 14000 rpm for 5 min. the absorbance of the supernatant was measured at 532 nm [19].

2.9. Measurement of Brain MDA Level

Brain tissue was homogenized in (1:10 wv^{-1}) pre-chilled KCL solution and transferred into a 20ml tube. After incubation for 60 min at 37℃, the suspension was mixed with 1 ml of 5% tetrachloroacetic acids and 1 ml of 67% TBA and centrifuged for 15 minutes at 2000 rpm. The resulting supernatant was transferred into a new tube and placed in a boiling water bath for 10 min. After cooling, its absorbance was measured at 535nm [18].

2. 10. Statistical Analysis

All data were expressed as the mean ± SD. The Kolmogorov–Smirnov test was used for the normality test. All data had p-values greater than 0.05, which indicates the normal distribution of data. The homogeneity of variances was determined using Levene's Test. In the case of homogenous variances, one-way ANOVA was used to compare the means between experimental groups, while in the case of non-homogenous variances, Dunnett's T3 was used. $P<0.05$ was considered statically significant.

3. Results and Discussion

Z. spina-christi is proved as potential source of natural antioxidants to present [12] hepatoprotective [10], antidiabetic [11] antibacterial [13] activity. It has also been reported that different extracts of Zizyphus species could show positive effect on central nervous system and reversing the scopolamine-induced memory and cognitive impairment [14, 15, 16]. It is also reported thatSidrleavesposes antibacterial, anti-cancer, anti-inflammatory, anti-hypertensive, anti-diarrhea propertiesand it has also positive effectson the CNS [17]. This study was designed to investigate if the antioxidant effect ofSidr (*Z. spina-christi*) leaf extract can help to improve thescopolamine induced impairment of memory in male Wistar rats.

3.1. Antioxidant Capacity of Z. Spina-Christi Leaf Extract

Table 1 shows the antioxidant capacity of *Z. spina-christi* leaf extract in DPPH method.

Table 1. Antioxidant capacity of *Zizyphusspina-christi* leaf extract.

DPPH radical scavenging activity Inhibition (%)	***Z. spina-christi* extract (µg/ml)**
IC_{50} (µg/ml)	
70	96.1
60	83.4
50	75.8(IC_{50})
40	63.5
30	47.5
20	35.2
10	14.3

The IC50 of *Z. spina-christi* extract was 75.8µg/ml. Al-Jassabi and Abdullah showed that themethanolic extract of *Z. spina-christi* could neutralize 52% of free radicals at the concentration of 198.72 µg/ml [12]. The radical scavenging properties of Sidr leaf extract might be due to the presence of bioactive antioxidants such as poly phenols and by this mechanism this plant is effective as e traditional medicine [20].

3.2. Total Phenol Content of Ziziphusspina-Christi Leaf Extract

Total phenolic compounds in *Ziziphusspina-christi* leaf extract was 290 mg gallic acid equivalent per one gram dried extract. This result was higher than the previously reported TPC of the prepared extracts from *Ziziphusspina-christi*. Al-Jassabi and Abdullah reported the total phenolic contents in the *Z. spina-christi* extract prepared using five different solution ranged from 11.04 to 56.44 mg QE/g [12]. The difference in the total phenolic contents in zizyphus extract maybe due to the type solvent used in extraction, the method used for extraction and the part of plant as well as the season in which the samples were collected. Because of the scavenging ability on free radicals due to their hydroxyl groups, phenols such as tannins and favonoids may contribute directly to the antioxidant activity [21]. Thus, the high total phenolic compounds in Sidr leaf extract could be considered for its use in various acute and chronic ailments.

3.3. Effects of Zizyphusspina-Christi Leaf Extract on Passive Avoidance Test

The passive avoidance learning was investigated using shuttle box apparatus. Results for the initial latency (t1) and the step-through latency (T2) in the passive avoidance test are shown in figure1; Aand B, respectively. Control group had the shortest initial latencies (T1) in comparison with different studied groups. The administration of scopolamine increased the recorded initial latencies or the second delay in the shuttle box test in entrance to a dark room ($p<0.05$). The use of Sidr extract at 50 and 100 mg/kg reduced second delay compared with scopolamine group; however, the reduction was not statistically significant. The administration of scopolamine significantly reduced ($p<0.05$) the step-through latency (T2) or the initial delay in shuttle box test in the

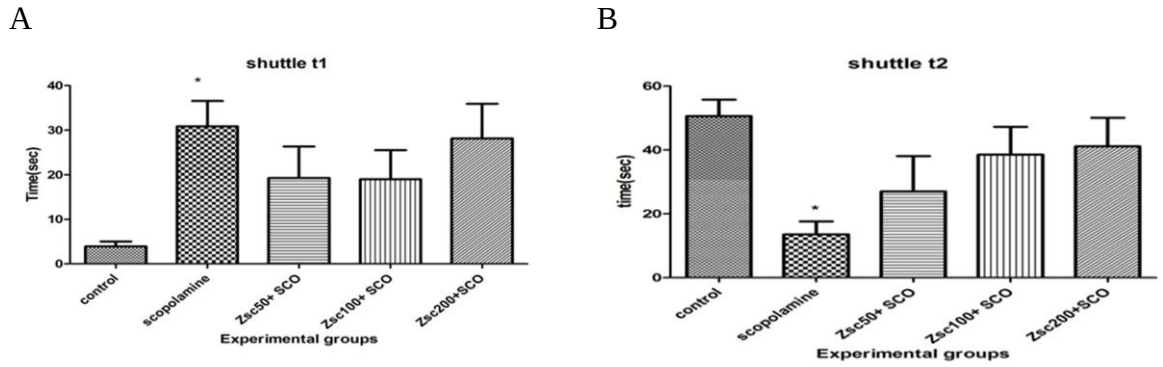

Figure 1. A) the initial latency (t1) and B) step-through latency (t2) in the passive avoidance response. The data are expressed as mean ± SD; n = 8 in each group. Zsc*Zizyphusspina-christi*, sco scopolamine.

entrance to a dark room compared with that of control group. The gavage administration of Sidr leaf extract at doses of 50, 100 and 200 mg/kg in rats treated with scopolamine could prolong the shortened step-through latency, however, any significant difference was not shown among these three concentrations ($p>0.05$)(fig 1). The results of the present study suggested that the administration of Sidr leaf extract can improve passive avoidance memory impairment induced by scopolamine injection.

Scopolamine has extensively been used to induce experimental models of Alzheimer's disease and brain and memory impairments [3]. Scopolamine is a muscarinic receptor antagonist and act as a blocker of cholinergicneurotransmission. Cholinergic interneurons in the striatum contain even richer source of acetylcholinesterase and would also be affected strongly by enzyme inhibitors [22] It significantly can result in increasing of AChE, brain oxidative stress and malondialdehyde (MDA) levels in the cortex and hippocampus [24] There are many studies which used scopolamine for inducing the brain and memory impairment in animal model [2, 22, 23, 24, 25].

Our results were in harmony with those studies reported the longer initial latency but shorter step-through latency for the scopolamine treated mice compared with the normal control [3, 23]. Also, there are many studies indicating the reversing effect of natural antioxidant and medical herbs on the generated abnormalities in initial latency and step-through latency due to scopolamine administration [2, 19, 3].

It's reported that the oxidative stress is one of the earliest events in pathogenesis of memory impairment [3, 26]. The scopolamine induced impairment in brain and memory functionality are probably caused by the oxidative stress through the interference with acetylcholine [2,3]. Sidr leaf extract due to its high antioxidant capacity can prevent or improve the adverse effects of oxidative stress and free radicals. Our results also suggest that effect of Sidr leaf extract on enhancing learning and memory in scopolamine treated rat models may be relatedto mediation of the cholinergic

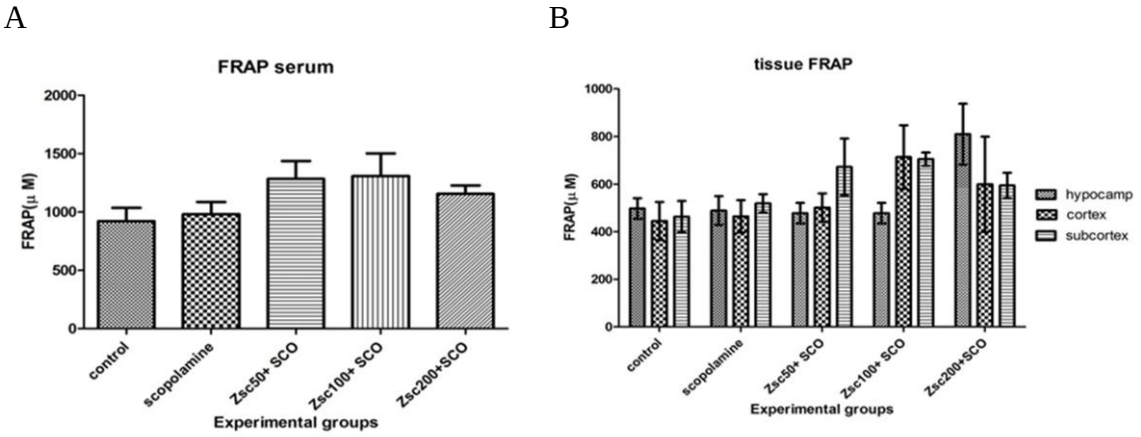

Figure 2. A) Plasma antioxidant capacity and B) brain antioxidant capacity (FRAP) in different groups receiving *Zizyphusspina-christi* leaf extract and scopolamine. The data are expressed as mean ± SD; n = 8 in each group. Zsc*Zizyphusspina-christi*, sco scopolamine.

neurotransmitter system which is in harmony with similar studies [19].

3.4. Effects of Zizyphusspina-christi Leaf Extract on Plasma and Brain Antioxidant Capacity

Figure 2-A shows the effect of *Z. spina-christi* leaf extract on the plasma antioxidant capacity. Scopolamine administration didn't cause any significant difference in the plasma antioxidant capacity compared to the control group; however, the use of *Z. spina-christi*leaf extract increased the antioxidant capacity of plasma. The effect of *Z. spina-christi* leaf extract on the brain antioxidant capacity are shown in figure 2-B. The administration of *Z. spina-christi* leaf extract at concentrations of 50, 100 and 200 mg/kg increased the brain antioxidant capacity of scopolamine treated rats. Although this increase was not statistically significant, the greater increase in antioxidant capacity of hippocampus, cortex and subcortex was achieved respectively by 200, 100 and 100 mg/kg of Sidr leaf extract.

3.5. Effects of Zizyphusspina-Christi Leaf Extract on Plasma and Brain MDA Level

Because of the complications of measuring free radicals directly *in vivo*, the quantification of cellular components which can react with these free radicals is of significant importance. MDA is one of the most known secondary products of lipid peroxidation, and it can be used as a marker of oxidative damage to cells and tissues [27]. The results of the effect of *Z. spina-christi* leaf extract on the plasma and brain MDA level are presented in figures 3-A and 3-B, respectively. Scopolamine treatment increased the MDA level of plasma in comparison with control group. The use of 50, 100 and 200 mg/kg of *Z. spina-christi* leaf extract significantly decreased the MDA level of plasma in comparison with that of scopolamine group. As shown in figure 3-B, the administration of scopolamine resulted in

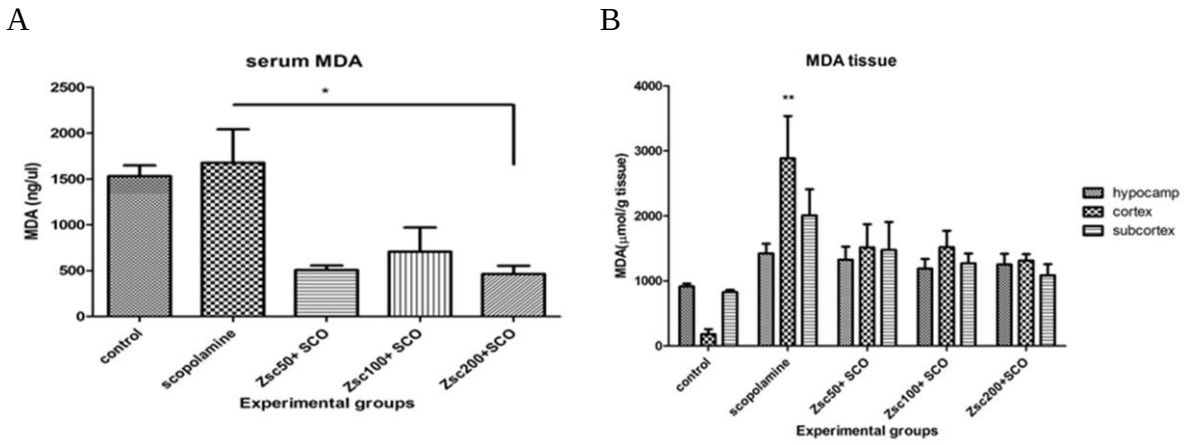

Figure 3. A) Plasma MDA level and B) brain MDA level in different groups receiving *Zizyphusspina-christi*leaf extract and scopolamine. The data are expressed as mean ± SD; n = 8 in each group. Zsc*Zizyphusspina-christi*,sco scopolamine

increasing of MDA level of brain tissues including hippocampus, cortex and subcortex. This increase was significant in the case of cortex compared with that of control group. *Z. spina-christi* leaf extract at 50, 100, and 200 mg/kg decreased the brain MDA level compared with scopolamine group. Our results are in line with the results of … who reported that the administration of hydroalcoholic extract of ZJ significantly increased the brain glutathione levels and significantly decreased brain MDA levels in rat [16].

The administration of *Z. spina-christi* extract increased the antioxidant capacity and decreased MDA level of plasma and brain samples in comparison with scopolamine group. Our results are in harmony with the results of … who reported that Lavender extract reduced serum and brain MDA levels that proved lavender extract may be increased antioxidant capacity in brain and serum [19]. This can be associated with the increased activity of antioxidant defense system and inhibition of oxidative stress in the brains of rats. Different studies also reported the antioxidant activity of *Z. spina-christi extract* [28, 29].The main phytochemical constituents of this plant are including flavonoids, alkaloids and saponins [28].It has been reported that plants with high antioxidant activity have ability to attenuate oxidative stress [29]. Alhakmani et al (2014) showed that *Z. spina-christi* extract has significant antioxidant activity and can be used to prevent oxidative stress and related diseases [21].Considering the oxidative stress and free radicals as the main risk factors in memory impairment and due to the high antioxidant activity of Sidr leaf extract, Therefore the positive effect on scopolamine induced memory impairment observed in this study may be related to the antioxidant capacity of Sidr extract.

4. Conclusion

The results of our study suggest that *Z. spina-christi* might act to attenuate scopolamine induced brain and memory impairments. This activity may be related to the antioxidant effects of *Z. spina-christi* leaf

extract. However the plant extracts contain various types of bioactive compounds or phytochemicals with different polarities and the separation of the specific compound responsible for observed antioxidant activity with protective effect against brain and memory disorders still need further researches.

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