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Original Article

The effects of captopril on learning and memory impairment induced by scopolamine in rats: anti-oxidative effects

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Abstract

Introduction: Angiotensin converting enzyme (ACE) inhibitors are suggested to have some beneficial effects on the brain. In the present study the protective effects against brain tissues oxidative damage as possible mechanism for learning and memory improving effects of captopril was investigated in scopolamine treated rats.

Methods: Fifty male Wistar rats were divided into seven groups and treated: saline as a control group, Sco (scopolamine) and Sco-Capto10, 50 and 100 (captopril 10, 50 and 100mg/kg before scopolamine). Treatment was passive avoidance test and then the cortical tissues were collected to measure malondialdehyde (MDA), nitric oxide (NO) metabolites, thiol, super oxide dismutase (SOD) and catalase (CAT).

Results: Scopolamine decreased the latency to enter the dark in passive avoidance test compared to control group. It also increased MDA and NO metabolites while decreased thiol, SOD and CAT in comparison with control group. Captopril increased the latency to enter the dark. It also decreased MDA and NO metabolites while, increased thiol, SOD and CAT.

Conclusion: Captopril protected brain tissues oxidative damage and improved learning and memory impairment induced by scopolamine.

Keywords:
Scopolamine;
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Introduction

Alzheimer's disease (AD), the most common type of human dementia, is accompanied by a decline in cognitive functions and changes in behavior and social adaptability. Cholinergic hypofunction seems to have a main role in cognitive dysfunction and memory loss in AD. Acetylcholine plays an important role in learning and memory processes (Drachman,

1977). Scopolamine, a muscarinic acetylcholine receptor antagonist (Huang et al., 2001), has been frequently used to produce learning and memory impairments (Glick and Zimmerberg, 1972). Scopolamine-induced dementia in human showed a model dementia ever since researchers reported similarities in cognitive dysfunctions between scopolamine treated young subjects and untreated demented individuals (Fuld, 1984). Also, it was induced certain aspects of cognitive impairment due to aging and dementia in animal models (Drachman and Leavitt, 1974; Ebert and Kirch, 1998). Recently, it has been suggested that scopolamine-induced memory deficits are accompanied with brain tissues oxidative damage (Fan et al., 2005).

Brain tissue in particular is more susceptible to the deleterious effects of reactive oxygen species (ROS) because of its high rate of oxygen consumption and reduced antioxidant defense systems. ROS initiate lipid peroxidation, which triggers degeneration of several neuronal population especially central cholinergic pathways (Tabet et al., 2000). Post-mortem studies have confirmed elevated levels of malondialdehyde (MDA), an index of lipid peroxidation in AD brains, which further supports the role of oxidative stress in the pathogenesis of the disease (Sultana et al., 2013). There are increasing evidence to support the role of antioxidant supplementation in the prevention and treatment of age-related diseases (Uttara et al., 2009). Indeed, several compounds with antioxidant property have been shown to improve cognitive dysfunctions and to slow down the progression of AD (Kelsey et al., 2010).

We were looking for a drug that could improve memory impairment through antioxidant properties. The renin-angiotensin system (RAS) is one of the neuropeptide systems in the brain. The substrate of this system, angiotensinogen, is suggested to be synthetized in several regions of the brain and is cleaved by the enzyme renin to form the decapeptide angiotensin (Ang I). Ang I is then converted to an octapeptide Ang II by angiotensin converting enzyme (ACE) (Wright and Harding, 2004) which is extensively located within the central nervous system (CNS) areas (McKinley et al., 2003).

Ang II as the main effector of RAS binds to specific receptors to perform multiple actions in the brain (Bodiga and Bodiga, 2013). The brain RAS has also been shown to have a role in AD and the other diseases associated with memory impairments including stroke, depression and emotional stress (Lenkei et al., 1997). These impairing effects of Ang II are suggested to be preventable by ACE inhibitors including captopril (Gard, 2002).

Some studies showed that captopril have antioxidative effects (de Cavanagh et al., 2000; Abareshi et al., 2017). In our previous study we showed that captopril improved learning and memory impairment induced by lipopolysaccharide by decreasing the brain tissue oxidative damage (Abareshi et al., 2016b). We, therefore, decided to test the protective effects against brain tissues oxidative damage as a possible mechanism for learning and memory improving effects of captopril in scopolamine treated rats.

Materials and methods

Animals and experimental protocol

Fifty male Wistar rats (230±20g, 10 weeks old) were kept at 22±2^{o}C and 12h light/dark cycle starting at 7am. All behavioral experiments were carried out between 10am and 2pm. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the study was approved by Mashhad University of Medical Sciences Ethical Committee. Permission NO was: IR.MUMS.fm.REC.1396.201. The animals were randomly divided into five groups of 10 rats in each and treated according to a designed protocol for two weeks. In the first week the rats were treated by saline or captopril. In the second week the rats were treated in a manner similar to the first week and additionally they were injected by scopolamine.

Rats in group 1 (control received saline intraperitoneally (ip) instead of captopril during two weeks. In group 2 (Sco group) saline was injected instead of captopril during two weeks, but treated by scopolamine (2mg/kg, Sigma Chemical Co) 30min before each behavioral test during the second week. Groups 3-5 were treated daily with 10, 50 and 150mg/kg captopril (Li et al.,2010) dissolved in saline (Sco-Capto 10, Sco-Capto 50 and Sco-Capto 100 groups) for two weeks, and they were also injected by scopolamine (2mg/kg, ip) 30min before behavioral tests.

Behavioral procedures

The animals were handled for 1 week before starting the experiments. Passive avoidance (PA) learning test based on negative reinforcement was used to examine learning and memory. The apparatus was consisted of a light and a dark chamber with a grid floor adjoining each other through a small gate. The rats were accustomed to the behavioral apparatus for 5min during 2 consecutive days before the training session. On the third day, the animals were placed in light chamber and the time latency to enter the dark chamber was recorded. On a training trial, the rats were placed in the light chamber facing away from the dark chamber. When the rats entered completely into the dark chamber, they received an electric shock (2mA, 2s duration). Then, the rats were returned to their home cage. One, 24 and 48 hours later, the rats were placed in the light chamber and the latency time to enter the dark chamber as well as, the times spent by the animals in dark chamber were recorded and defined as retention trial (Nassiri-Asl et al., 2010).

Biochemical assessments

After the behavioral tests, the animals were euthanized, the brains were dissected and the cortical tissues were detached on an ice-cold surface. One tenth of each sample was homogenized using phosphate buffer solution. The samples were centrifuged at 1500rpm for 10min to use for measuring the MDA, total thiol and nitric oxide (NO) metabolites concentration and also superoxide dismutase (SOD) and catalase (CAT) activity. The chemical material used for biochemical experiments were obtained from Merck Company, Darmstadt, Germany.

MDA was measured as a biomarker of lipid peroxidation. The measurement method was described previously (Beheshti et al., 2017). Briefly, one ml of the sample solution was mixed with 2ml of thiobarbituric acid + trichloroacetic acid (TCA + hydrochloric acid) solution and was boiled for 45min. The solutions were then centrifuged within 1000g for 10min and its absorbance was measured at 535nm. MDA concentration was assayed based on the formula which was previously reported (Beheshti et al., 2017).

A Griess reagent kit was used to measure NO metabolites (Bargi et al., 2017; Baghcheghi et al., 2018). The total thiol contents were measured in the tissue homogenates applying a method described by Ellman (Habeeb, 1972) and as it was previously reported (Beheshti et al., 2017). In summary, 50µl of the supernatant of each sample and one ml of tris-ethylenediaminetetraacetic acid (EDTA) buffer was mixed and the absorbance was read at 412nm against tris-EDTA buffer alone labeled A1. After that, 20µl of 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB) solution was added to A1 and the sample absorbance was read for the second time after 15min labeled A2. The absorbance of DTNB was used as blank (B). A previously described equation was used to calculated total thiol concentration.

SOD activity was measured based on Madesh and Balasurbamanian (1997). The method is based on the generation of SOD through auto-oxidation of pyrogallol and dependent inhibition of 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) to formazan. The reaction stopped by dimethyl sulfoxide (DMSO). In summary, the supernatant of the sample was poured into the wells of the plate (96 wells). After 5min, the DMSO was added and the plate was observed with a micro plate reader at a wavelength of 570nm. One unit of SOD was described as the amount of protein needed to inhibit 50% reduction of MTT.

For CAT activity measurement, 100µl H_2O_2 was mixed with phosphate buffer (pH=7) and used for preparation of the solution that was used for measurement (C buffer). The 650µl phosphate buffer (pH=7) used as solution blank. The cuvette for measurements was filled by the C buffer and sample homogenates. The reduction of absorption was determined by spectrophotometer at the wave length of 240nm for 5min (Aebi, 1984).

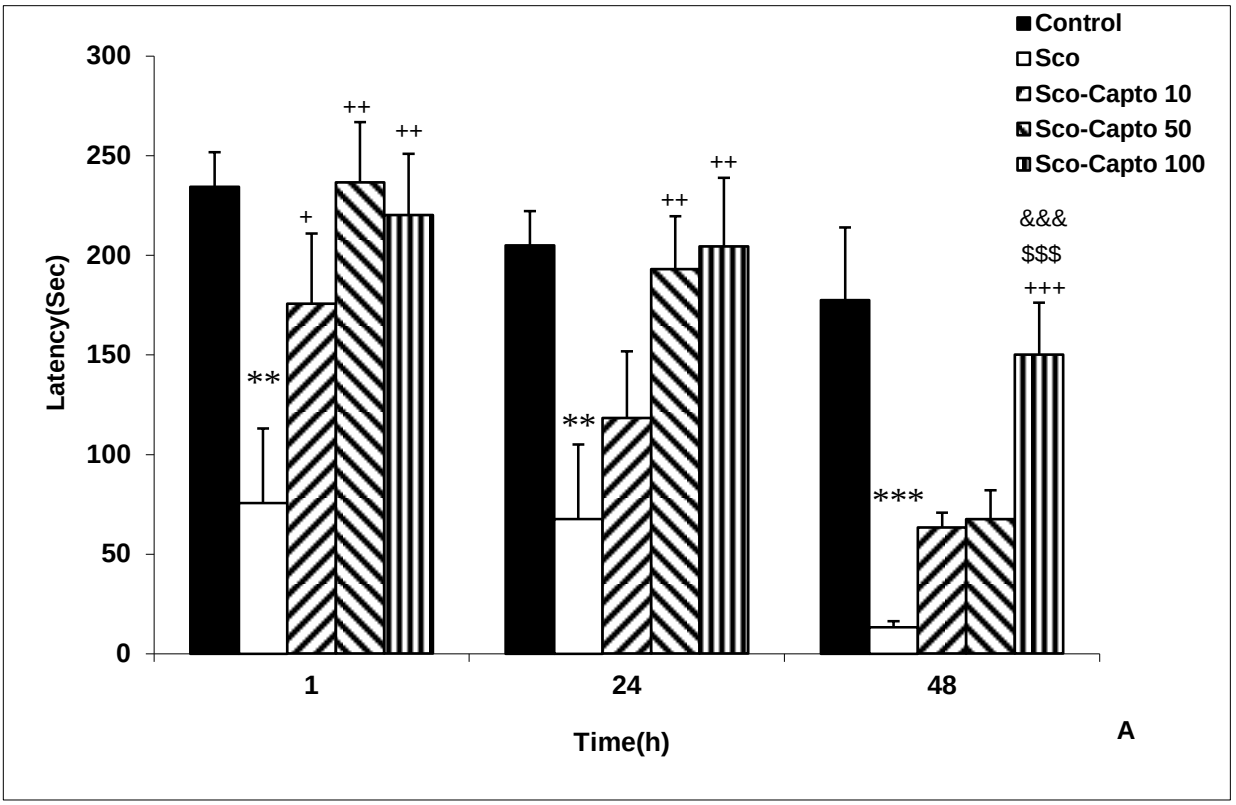
Statistical analysis

Data were expressed as mean±SEM. For all parameters one-way ANOVA test was done, followed by post hoc comparisons test. $P<0.05$ was considered statistically significant.

Results

Captopril improved learning and memory

The results showed that scopolamine impaired learning and memory of the rats which was presented by a shorter latency to enter the dark in scopolamine



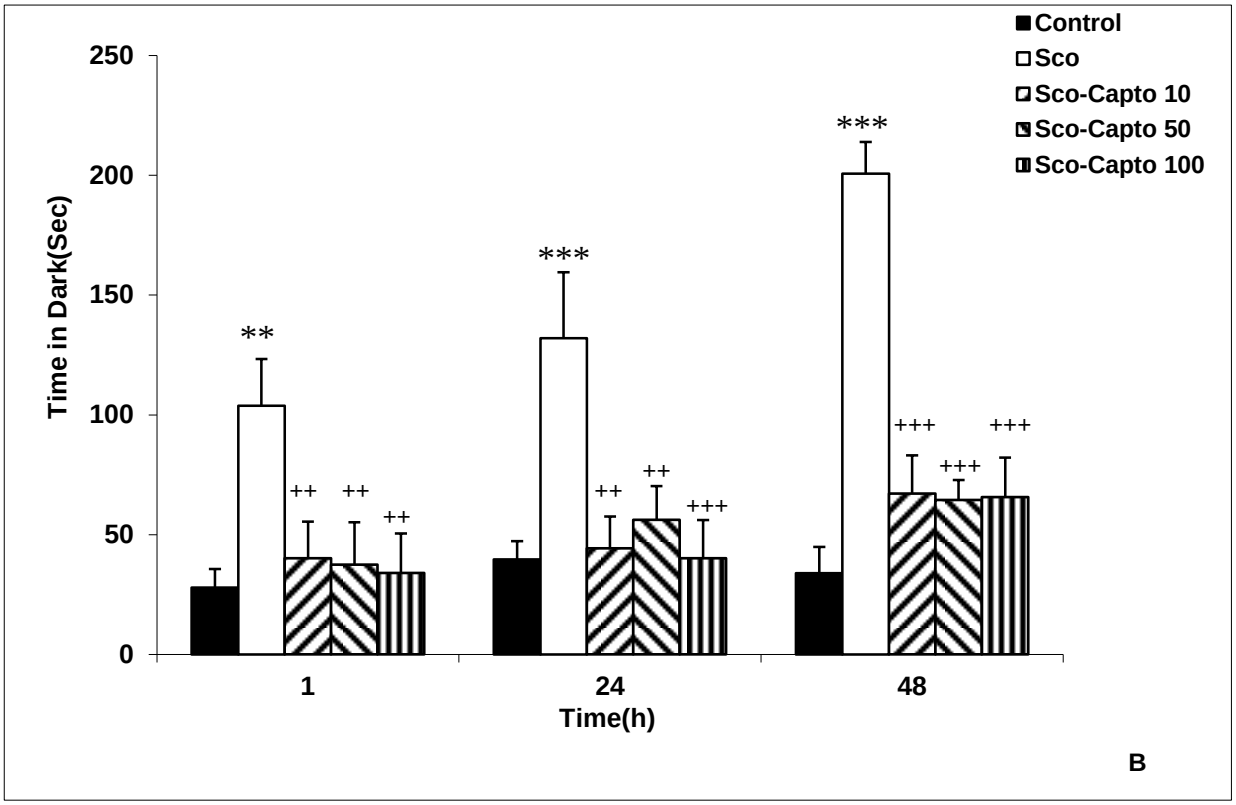


Fig.1. The passive avoidance results. Panel (A) shows the latency for the first entering to the dark after receiving a shock. Panel (B) shows the total time that the animals spent in the dark segment of the apparatus. Data were presneted as mean±SEM (n=10 rats in each group). $^{**}P<0.01$ and $^{***}P<0.001$ Sco vs control groups, $^{+}P<0.05$, $^{++}P<0.01$ and $^{+++}P<0.001$ Sco–Capto treated groups vs Sco group, $^{\$\$\$}P<0.001$ vs Sco-Capto 10 group, $^{\&\&\&}P<0.001$ vs Sco-Capto 50 group. Sco: Scopolamin; Capto: Captopril.

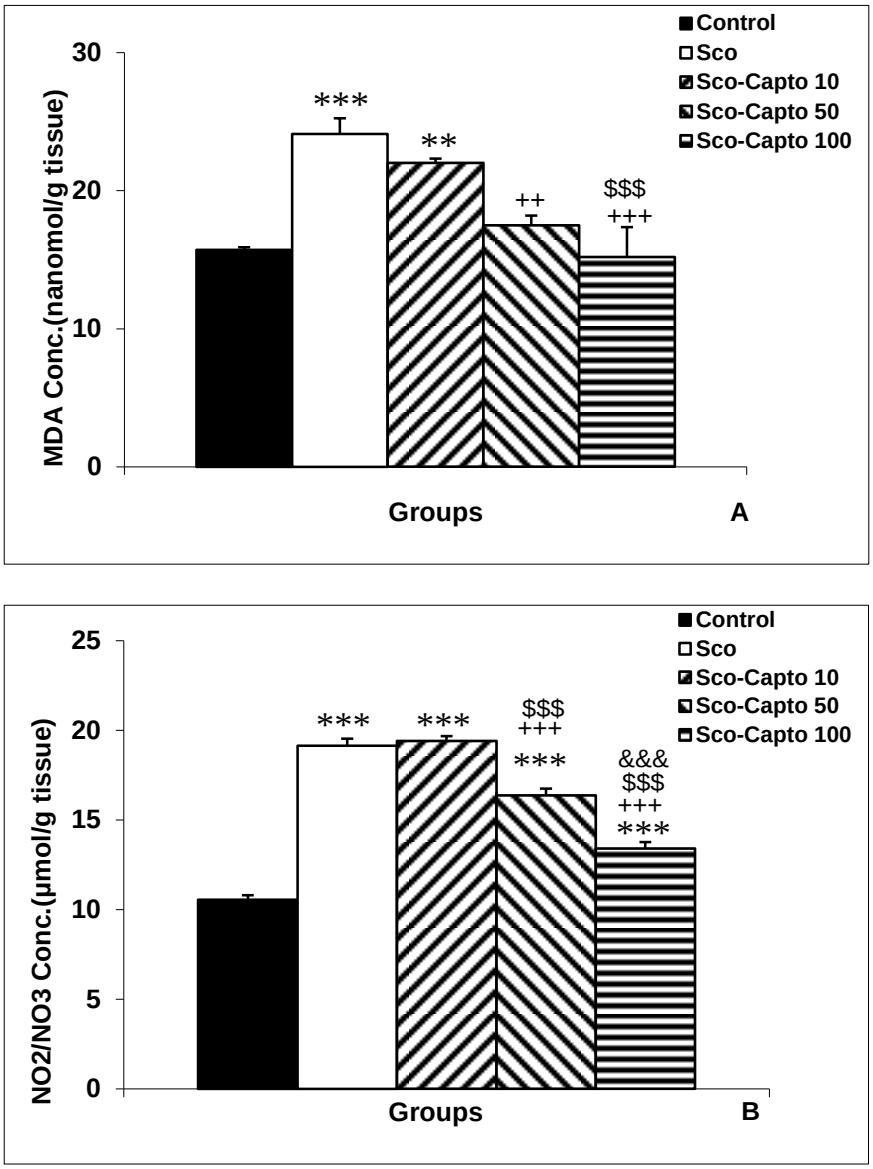
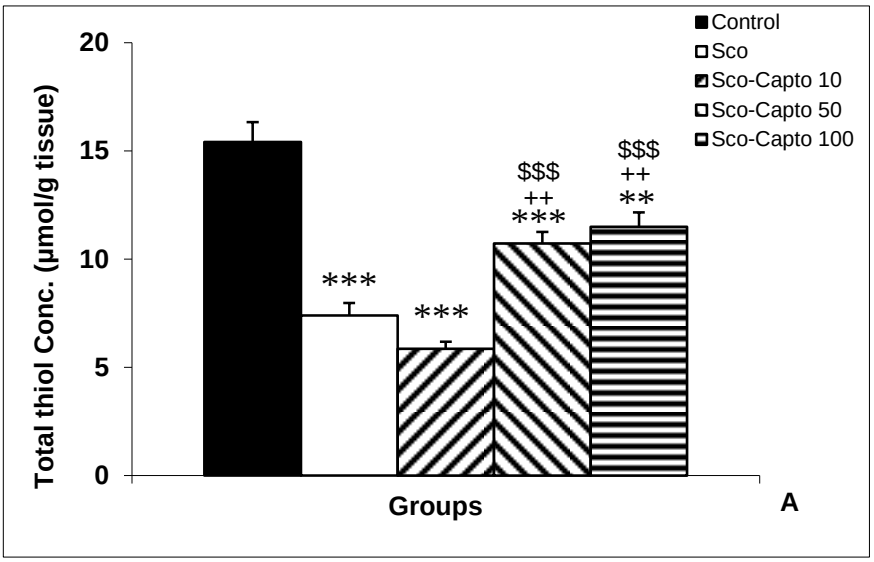
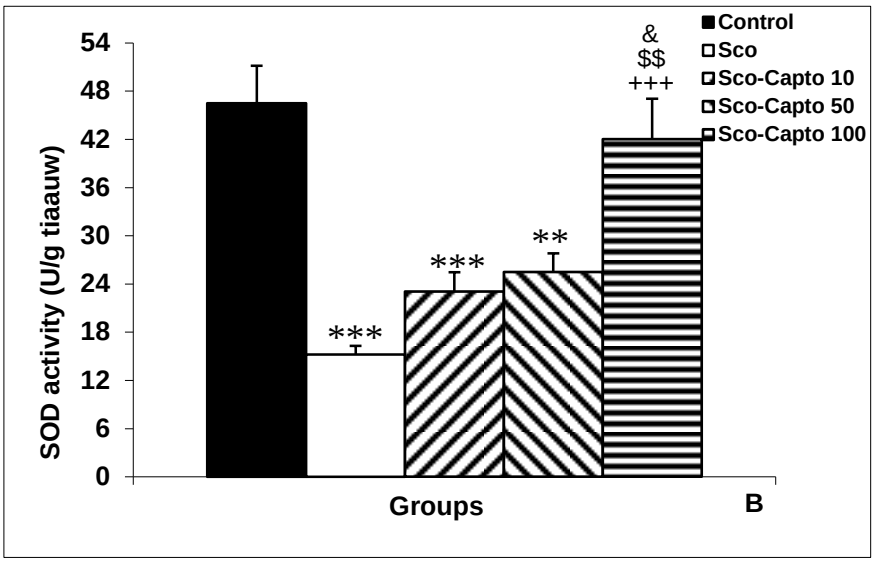


Fig.1. Panel (A) MDA and panel (B) NO metabolites in the cortical tissues. Data were presneted as mean±SEM (n=10 rats in each group). $^{**}P$<0.01 and $^{***}P$<0.001 Sco vs control group, ^{++}P<0.01 and ^{+++}P<0.001 and scopolamine - captopril treated groups vs scopolamine group, $^{\$\$\$}P$<0.001 vs Sco-Capto 10 group, $^{\&\&\&}P$<0.001 vs Sco-Capto 50 group. Sco: Scopolamin; Capto: Captopril.

treated rats compared to the control ones at all 1, 24 and 48h (*P*<0.01-*P*<0.001) post- delivery time shock (Fig. 1A). The results of passive avoidance test also showed that all doses of captopril including 10 (*P*<0.05), 50 (*P*<0.01) and 100mg/kg (*P*<0.01) increased the latency time to enter the dark at 1h after receiving a shock compared to the scopolamine (Fig. 1A). Additionally the two higher doses including 50 (*P*<0.01) and 100mg/kg (*P*<0.01) of captopril were able to increase the latency for entering the dark at 24h post-delivery shock time; however 10mg/kg was not effective (Fig.1A). When the animals were examined at 48h after the shock, the animals of the group treated by the highest dose of captopril had a longer latency to enter the dark compared to the scopolamine group (*P*<0.001); however, the two lower doses were not effective. Additionally the 100mg/kg captopril was more effective that both 10 (*P*<0.01) and 50mg/kg (*P*<0.01) to increase the latency time (Fig. 1A).

Compared to the control rats, scopolamine increased the total time spent in the dark when the animals were allowed to move between the two chambers of passive avoidance apparatus at 1 (*P*<0.01), 24 (*P*<0.001) and 48h (*P*<0.001) after the shock (Fig. 1B). The results also showed that all doses of captopril were able to reverse the effects of scopolamine and decreased the time spent in the





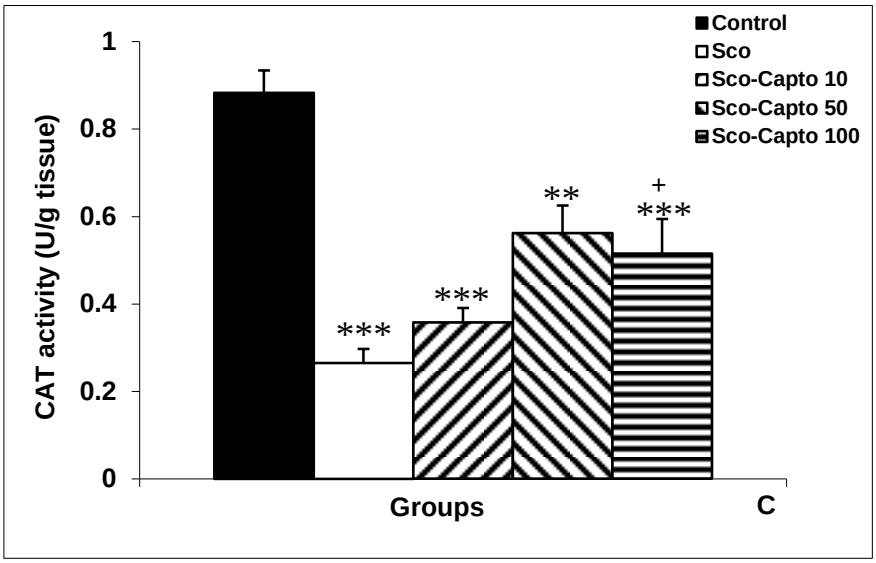


Fig.3. Panel (A) thiol, panel (B) SOD and panel (C) CAT in the cortical tissues. Data were presneted as mean±SEM (n=10 rats in each group). $^{**}P$<0.01 and $^{***}P$<0.001 Sco vs control group, ^{+}P<0.05, ^{++}P<0.01 and ^{+++}P<0.001 and scopolamine - captopril treated groups vs scopolamine group, $^{\$\$}P$<0.01 and $^{\$\$\$}P$<0.001 vs Sco-Capto 10 group, $^{\&}P$<0.05 vs Sco-Capto 50 group. Sco: Scopolamin; Capto: Captopril.

dark at all 1 (P<0.01 for all doses of captopril compared to the scopolamine), 24 (P<0.01, P<0.01 and P<0.001 for 10, 50 and 100mg/kg of captopril respectively compared to scopolamine) and 48h (P<0.001 for all doses of captopril compared to the scopolamine) post deliver shock time. There was no significant difference between the three doses of captopril in the total time spent in the dark (Fig. 1B).

Biochemical results

Captopril decreased both MDA and NO metabolites

Injection of scopolamine increased cortical tissues MDA (P<0.001 compared to the control group). Treatment by both 50 (P<0.01) and 100mg/kg (P<0.001) captopril decreased MDA in the cortex. Additionally 100mg/kg of captopril were more effective than 10mg/kg to decrease MDA (P<0.01 compared to 10mg/kg captopril). There was no significant difference between the effects of the highest and the medium doses of captopril on cortical MDA. Compared to scopolamine, the lowest dose of captopril were not able to change MDA concentration in the cortex (Fig. 2A).

The results also showed that scopolamine administration increased NO metabolites in the cortex (P<0.001 compared to the control group). The lowest dose of captopril was not able to attenuate NO metabolites in the cortical tissue; however, both 50 and 100mg/kg doses decreased NO metabolites in the cortex (P<0.001 for both doses compared to scopolamine). The medium dose of captopril was more effective than the lowest dose to attenuate NO metabolites (P<0.001 compared to the lowest dose). Additionally, NO metabolites in the cortex of the group treated by the highest dose of captopril was lower than in those treated by the medium and the lowest doses (P<0.001 compared to the lowest and the medium doses, Fig. 2B).

Captopril improved thiol, SOD and CAT

Compared to the control rats, scopolamine decreased thiol content in the cortex (P<0.001 compared to the control group). Both the medium and highest doses (P<0.01 for both doses compared to the scopolamine) but not the lowest dose of captopril were able to increase thiol content in the cortex. Both the medium and the highest doses of captopril were more effective than the lowest dose (P<0.001 for both doses compared to the lowest dose); however, there was no significant difference between the medium and the highest dose (Fig. 3A).

Scopolamine also decreased SOD activity in the cortical tissues (P<0.001 compared to the control group). Compared to scopolamine, 100mg/kg (P<0.001) dose but not 10 and 50mg/kg of captopril were able to increase SOD activity in the cortical tissue. Additionally, 100mg/kg was more effective than both 10 (P<0.01) and 50mg/kg (P<0.05) to increase cortical SOD. Additionally, there was no significant difference between the medium and the lowest doses (Fig. 3B).

The results also showed that scopolamine was able to decrease CAT activity in the cortex (P<0.001 compared to the control group). Compared to scopolamine, pretreatment by both 50 and 100mg/kg captopril increased CAT activity in the cortex (P<0.05 - P<0.01); however, 10mg/kg captopril was not effective (Fig. 3C). There was no significant difference between the effects three doses of captopril on cortical CAT (Fig. 3C).

Discussion

Using PA test, our results indicated that captopril had a protective effect of on learning and memory impairment induced by scopolamine in rats and it was at least in part due to its antioxidant effect. Scopolamine, a non-selective muscarinic antagonist, blocks cholinergic signaling and produce cognitive dysfunctions including long-term and short term memory impairment (Ishola et al., 2017). It interferes with memory in animals and humans (Hefco et al., 2003). Also, it was many evidenced that showed scopolamine has been used to induce experimental models of AD (Beatty et al., 1986; Collerton, 1986). In the present study, PA as a well-established memory task was used to evaluate a scopolamine model of AD in rats. In the present study, result of PA test showed that scopolamine induced memory impairment and decreased the latency for entering the dark room while, the time spent in dark room was increased compared to the control group.

Cholinergic deficit is a major neuropathological feature that is associated with memory loss and closely correlated with cognitive dysfunction in AD (Hasselmo, 2006). It is generally accepted that oxidative stress plays a prominent role in the

pathogenesis of AD. Both preclinical and clinical studies have confirmed an increased level of oxidative stress during early period of the disease, which often leads to sudden onset of symptoms of AD including cognitive decline (Sultana et al., 2013). Moreover, scopolamine induced memory impairment has been linked to increased oxidative stress in the whole brain, as well as specific regions associated with learning and memory (Budzynska et al., 2015). The results of the present study confirmed that the memory impairment induced by scopolamine was accompanied by an increased level oxidative stress, as shown by elevated brain levels of MDA and NO metabolites in prefrontal cortex and a decrease in antioxidant defense systems. Consistently, we previously showed scopolamine- induced learning and memory impairment was accompanied with a brain tissues oxidative damage which was improved by the antioxidant agents (Hosseini et al., 2015; Mohammadpour et al, 2015; Hejazian et al., 2016).

Considering the antioxidant effects of ACE inhibitors including captopril and some beneficial effects of the mentioned drugs on cognitive functions including learning and memory (Bild et al., 2013; Bodiga and Bodiga, 2013; Abareshi et al., 2016a; Abareshi et al., 2016b; Abareshi et al., 2017) it was assumed that captopril may improve learning and memory impairments. Interestingly, pretreatment of the rats with captopril increased the latency to enter the dark while, decreased the total time spent in the dark chamber indicating improvement in learning and memory. The results were in agreeing our previous works in which captopril improved learning and memory impairments induced by lipopolysaccharide (Abareshi et al., 2016a; Abareshi et al., 2016b). In our previous studies the beneficial effects of captopril was attributed to anti-inflammatory effects (Abareshi et al., 2016a; Abareshi et al., 2016b).

Additionally, ROS production and brain tissue oxidative damage have been well known to play an important role in learning and memory impairments (Beheshti et al., 2017). Considering the results of present study an interaction between RAS and cholinergic system to improve learning and memory might be suggested. RAS has been reported to have a critical role in ROS production (Husain et al., 2015). We also previously showed that ACE inhibitors including captopril had some antioxidant effects (Shahveisi et al., 2014; Abareshi et al., 2016b; Abareshi et al., 2017). On the other hand, It has also been documented that Ang II is able to enhance ROS production after activating of AT_1 receptors (Pacurari et al., 2014). Activation of the brain RAS has been reported to be accompanying with oxidative stress in the brain (Bodiga and Bodiga, 2013). AT_1 receptor has been reported to have a role in age-related cognitive impairments associated with oxidative stress in the brain (Li et al., 2010). The results of current study also showed that captopril decreased MDA and NO metabolites while increased thiol, SOD and CAT. Thus, the ability of captopril to reverse scopolamine-induced learning and memory impairments which was seen in the present study may at least in part be due to enhancement of antioxidant defense system and attenuation of oxidative stress in the brain (Bild et al., 2013). This action might be resulted from its ability to overcome the pro-oxidant effects of scopolamine in the brain, through increase in antioxidant defense systems including GSH, SOD and CAT and a decrease in MDA and nitrite levels in the brain (Ciobica et al., 2011). Previously, studies introduced that excessive production of NO is go along with several biochemical events for examples, lipid peroxidation, protein oxidation and oxidation of thiols to induce an oxidative stress condition (Luperchio et al., 1996). NO over-production can induce learning and memory impairment (Hosseini et al., 2010; Abdel-Zaher et al., 2017) which has been ascribed to inducible nitric oxide synthase (iNOS) activity amplification (Tabrizian et al., 2016; Abdel-Zaher et al., 2017). Additionally, There is a lot of evidences showing that inhibition of iNOS rarefy inflammatory responses and oxidative damage but improve learning and memory (Anaeigoudari et al., 2016). Based on the results obtained from the behavioral and biochemical studies, it may be suggested that captopril may act directly as a free radical scavenger or regulator to ameliorate oxidative stress in nervous system (Tota et al., 2012).

Conclusion

Captopril protected from the brain tissues oxidative damage to improve learning and memory impairment induced by scopolamine. However, further studies are required to better understanding the responsible mechanism(s).

Acknowledgments

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Conflict of interest

We declare that we have no conflict of interest.

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