Avicenna Journal of Phytomedicine (AJP)

Original Research Article

Cedrol improved brain-derived neurotrophic factor and attenuated oxidative stress in the brain and improved learning and memory in scopolamine-injected rats

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Article history:
Received: Jun 19, 2025
Received in revised form: Nov 25, 2025
Accepted: Nov 26, 2025
Epub ahead of print

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Keywords:
Cedrol
Brain-derived neurotrophic factor (BDNF)
Oxidative stress
Learning ability
Memory
Cholinergic system

Abstract

Objective: Natural antioxidant products including cedrol are suggested to improve cognitive abilities. In the current research, the possible effects of cedrol on oxidative stress, and brain-derived neurotrophic factor (BDNF) within the brain, using scopolamine-treated rats as a model of memory impairment, were investigated.

Materials and Methods: The rats received 15 or 30 mg/kg cedrol or its vehicle and then were injected with scopolamine. The animals were examined in Morris water maze test (MWMT) and passive avoidance test (PAT). The brain tissues were also collected and were used for biochemical examinations.

Results: Cedrol at both doses shortened the latency and distance required to locate the hidden platform across 5 days of MWMT ($p<0.05$ to $p<0.001$) and prolonged the time and distance in the target area in probe test of MWMT ($p<0.05$ to $p<0.001$). In the PAT, cedrol decreased the time spent in the dark part ($p<0.001$) and the entry number in to the dark ($p<0.05$ to $p<0.001$) but increased delay time to enter the dark part ($p<0.001$) and the time spent in the light part ($p<0.001$). Cedrol especially at the higher dose increased BDNF, thiols, catalase and superoxide dismutase levels, but reduced malondialdehyde in the tissues of the brain.

Conclusion: The findings revealed that cedrol could mitigate cognitive dysfunction induced by injection of scopolamine through attenuating oxidative stress and improving BDNF levels.

Please cite this paper as: Shakeri F, Rajabian A, Mansouritorghabeh F, Beheshti F, Forouzanfar F, Bibak B, Eshaghi Ghalibaf M.H, Hosseini M. Cedrol improved brain-derived neurotrophic factor and attenuated oxidative stress in the brain and improved learning and memory in scopolamine-injected rats. Avicenna J Phytomed, 2025. Epub ahead of print.

Introduction

As the leading cause of dementia, Alzheimer's disease (AD) is recognized as a progressive neurodegenerative condition that gradually diminishes cognitive capacities, particularly those related to memory consolidation and learning processes (Nie et al. 2009). Amyloid plaque (Aβ) formation and accumulation of tau proteins are suggested to have an important role in AD (Giacobini et al. 2022). Damage to cholinergic neurons which is accompanied with cholinergic system dysfunction is also suggested to be the main contributor to AD (Giacobini et al. 2022; Giacobini and Pepeu 2018; Hampel et al. 2019). Therefore, drugs such as donepezil and rivastigmine which are able to inhibit acetylcholinesterase (AChE) activity are advised to improve the conditions of AD patients because the AChE inhibitors reinforce the cholinergic system (Terry and Buccafusco 2003). Evidence also suggests a direct link between neuronal degeneration and oxidative stress (Niedzielska et al. 2016). Oxidative stress is a disruption in the equilibrium among endogenous antioxidant defenses and pro-oxidant factors, particularly reactive oxygen species (ROS), (Sayre et al. 2008; Shakeri et al. 2020). Multiple determinants underlie the susceptibility of certain brain regions to oxidative stress. Among these are the unique metabolic demands of the brain and the high concentration of polyunsaturated fatty acids, particularly within areas such as the hippocampus and prefrontal cortex, which render these regions especially vulnerable. Also, studies suggest that oxidative stress has a main role in Aβ and tau protein accumulation in the brain. Brain-derived neurotrophic factor (BDNF) is a key neurotrophic involved in the regulation of cognitive processes. It plays an essential role in neuronal development, differentiation, and proliferation, particularly within brain regions critical for learning and memory (Callaghan and Kelly 2012; Cunha et al. 2010; Hartmann et al. 2012; Keshavarzi et al. 2021). Both experimental and clinical studies have implicated BDNF dysregulation in the underlying mechanisms of AD (Saura and Valero 2011).

Scopolamine as an antagonist of the cholinergic system is well known for producing an impaired learning and memory animal model (Azizi-Malekabadi et al. 2012; Jamialahmadi et al. 2013; Tang 2019). This experimental model provides a valuable framework for investigating the underlying mechanisms contributing to learning and memory impairments associated with AD (Akbarian et al. 2022; Tang 2019). Scopolamine injection is also shown to be accompanied by an AChE over activity (Akbarian et al. 2022). Moreover, Scopolamine treatment has been shown to provoke oxidative stress–induced neuronal damage in the brain, as evidenced by experimental findings (Akbarian et al. 2022; Amirahmadi et al. 2022; Hosseini et al. 2022; Marefati et al. 2019). Conversely, antioxidant compounds have been proposed to possess preventive potential against AD and are under investigation for their capacity to ameliorate clinical symptoms in affected individuals (Behl and Moosmann 2002; Manoharan et al. 2016).

Cedrol is known to be a natural compound which is obtained from *Juniperus Virginia* oil that has some biological properties including sedative (Kagawa et al. 2003), anti-inflammatory (Rastegar-Moghaddam et al. 2024), and antioxidant functions (Emami et al. 2011). In previous studies, the anxiolytic effect of cedrol and its effects on neurotransmitters has been reported (Zhang and Yao 2018; Zhang and Yao 2019). It was also shown that cedrol attenuated brain inflammation and protected the brain against harmful effects of lipopolysaccharide (Dabouri Farimani et al. 2024). This study was designed to evaluate the impact of cedrol on cognitive impairment caused by scopolamine injection in rats, its improving effect on the levels of BDNF, and the protection against oxidative stress-related brain injury.

Materials and Methods

Animals, grouping, drugs, and treatments

Adult rats (Wistar, male, N=40, age = 9-10 weeks) were used in the current research. The standard conditions were provided for the rats, the temperature was 21 - 25˚C, with a periodic 12 hr dark / light, the lights were ON at 06 00 am and they were OFF at 06 00 PM. The study was carried out on 4 groups of the animals in the current research. Sample size determination was performed using G*Power software. Each rat was treated as an independent experimental unit. Random allocation into control and treatment groups was conducted using a computer-generated randomization approach, specifically the RAND function in Microsoft Excel.

Animals in Group I were the control group, receiving the vehicle solution in place of both cedrol and scopolamine. Group II was the Scopolamine group; in this group the animals were given vehicle instead of cedrol, the animals were also injected (intraperitoneal) with scopolamine (2 mg/ kg) (Eshaghi Ghalibaf et al 2023; Akbarian et al. 2022). Groups III and IV received intraperitoneal injections of cedrol (15 and 30 mg/kg), respectively (Dabouri Farimani et al. 2024; Asgharzade et al. 2025), followed by scopolamine administration.

The experimental procedures were conducted over a three-week period. During the initial two weeks, animals in groups III and IV received daily oral doses of cedrol at respectively 15 and 30 mg/kg, while groups I and II were administered the vehicle. During the third week, cedrol treatment continued for groups III and IV at the same respective doses, followed by scopolamine injection 30 min post-cedrol administration. Group II animals received the vehicle in place of cedrol, with scopolamine administered 30 min thereafter. In the group I, the rats were given the vehicle of cedrol and they were also injected with saline instead of scopolamine.

The rats were examined in the behavioral tests 30 min after scopolamine (in groups II-IV) and saline (in group I) (Figure 1). Cedrol and scopolamine were obtained from Tinab Shimi Khavarmianeh Company located in Mashhad, Khorasan Province, Iran, and Sigma-Aldrich (USA), respectively. A solution containing dimethyl sulfoxide (DMSO) and tween was used to dissolve cedrol. To do the behavioral and biochemical tests, the samples were selected by a person who was unaware of treatments. The research protocol (No. 990226) received ethical approval from the Ethics Committee of North Khorasan University of Medical Sciences, under the approval code IR.NKUMS.REC.1400.066.

Behavioral assessments included the Morris water maze test (MWMT) and the Passive Avoidance Test (PAT). Escape latency and path length in the MWMT were assessed over five days and compared among groups. During the probe trial, spatial memory was evaluated by measuring both the duration and distance covered within the designated target quadrant. In the PAT, cognitive performance was assessed by comparing latency to enter the dark compartment, duration spent in both dark and light areas, and the number of entries into the dark chamber. Data obtained from the MWMT probe trial were utilized to determine the required cohort size. The dosing regimen for cedrol was determined based on previously published protocols (Dabouri Farimani et al. 2024).

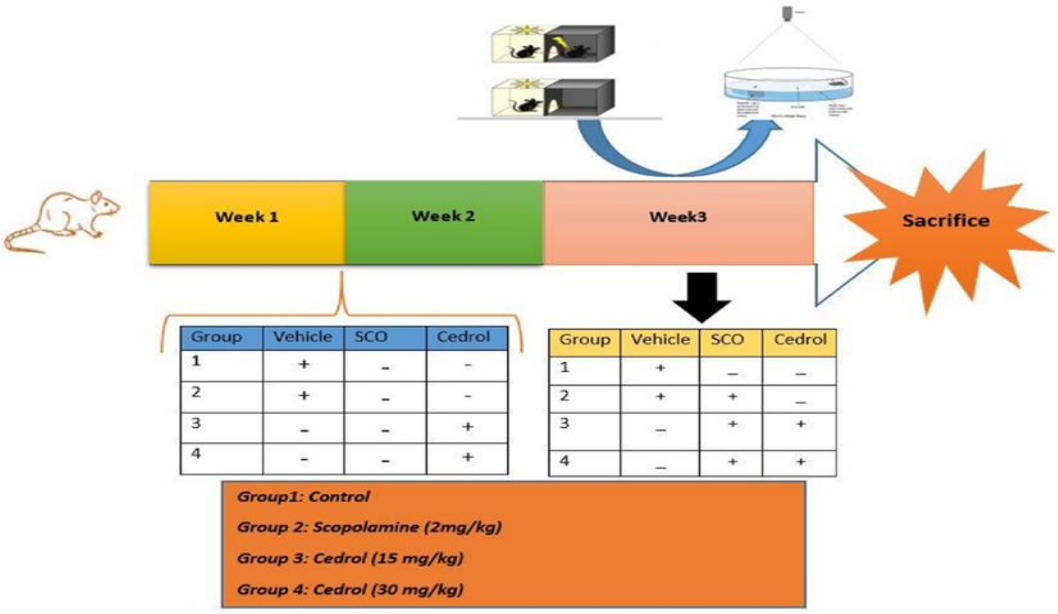

Figure 1. A diagram to show the time line of the study and the treatments.

Memory and learning assessments in PAT and MWMT

A water-filled round pool was used to examine the MWMT. The temperature of the water was adjusted to be about 23-26˚C. The round pool was hypothetically included of four areas (quadrants) and a round and clear platform (diameter = 10 cm) was located in one of the four quadrants (target area). The test was including two phases. During the initial phase of training, the rats acquired the ability to navigate the water maze and locate the submerged platform. Training was done on 5 days and in each day, 4 trials were done. To do a trial, the experimenter released each rat into the pool from one of four positions which. In each trial, each rat had a time (60 sec) to search for the platform and locate it. If the animal was not able to locate the platform after elapsing 60 secs, the experimenter helped it to find the platform. After locating the platform, each rat had 20 sec time to see the cues in the walls around the pool. These spatial cues facilitate the rats' ability to locate the submerged platform during subsequent trials. To record the traveled path of the rat to reach the platform, a camera was used which was located above the pool. The camera recorded the activity of the rat. The recorded films were transferred to the computer and data including traveling distance and time were extracted using a software. Following the acquisition phase, the platform was withdrawn, and rats swam freely for 60 secs in the probe test. Behavioral activity was recorded, and subsequent analysis of the video footage provided measurements of both the path length traversed and the duration spent within the target quadrant.

An apparatus which was made from Plexiglas was used for PAT. The apparatus was including two parts that were adjoining each other by a gate. One of the two parts was clear and lighted while the other was black and dark. The gate was closeable using a small door. The floor of the apparatus was containing still bars with a distance of 100 mm. A stimulator was connected to the bars of the dark part. At first, the rat was allowed to search inside both dark and light parts of the apparatus for 5 min. Each rat was initially placed in the lighted compartment and allowed to transition into the dark chamber upon the opening of the connecting door. When the animal had fully entered and the door was closed, a mild foot shock (2 mA, 2 sec) was administered through the grid floor to reinforce avoidance learning. After opening the adjoining door, the rat was

entered to the light part and then it was transferred into the cage. To assess memory retention, rats were evaluated at 3, 24, 48, and 72 hr following the aversive stimulus. During each assessment, the animal was positioned in the illuminated compartment, and upon opening the connecting door, the latency to enter the dark chamber was recorded. In addition, each rat was allowed to freely explore the apparatus for a duration of five minutes, during which the time spent in the dark, the number of entries into the dark, and the time spent in the light were recorded to further evaluate memory performance and exploratory behavior.

Oxidative stress indicators and BDNF measurements

A deep anesthesia was applied in the rats using urethane at a dose of 1.6 g/kg, the chest was opened, and the heart was ruptured to euthanize the rat. The brains of the rat were collected and the hippocampus and cortex were separated. After The homogenization and centrifugation, the biochemical measurements were done on the supernatants.

The concentration of hippocampal BDNF was performed with an ELISA kit (MyBioSource, USA) following the supplier's instructions. Sample absorbance values were measured and compared against a standard calibration curve to determine BDNF levels. This procedure was consistent with methodologies reported in earlier studies (Mansouri et al. 2021).

The assessment of lipid peroxidation was conducted via the thiobarbituric acid reactive substances (TBARS) assay, wherein malondialdehyde (MDA) formed a chromogenic complex with thiobarbituric acid (TBA). Following incubation, cooling, and centrifugation, absorbance was determined at 535 nm to quantify oxidative stress levels (Boskabady et al. 2021). The determination of thiol content employed 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB) reactivity with absorbance readings at 412 nm, whereas superoxide dismutase (SOD) and catalase activities were analyzed using previously validated methodologies (Boskabady et al. 2021; Mansouri et al. 2021).

Data analysis

Quantitative data are presented as mean ± standard error of the mean (SEM). Statistical evaluations were conducted using IBM SPSS Statistics software (version 26.0), and graphical outputs were generated via GraphPad Prism (version 8.4.3). The Kolmogorov–Smirnov test was employed to examine the normality of data distribution. To assess escape latency and path length across training sessions in the MWMT, repeated measures ANOVA was utilized. For other experimental variables, one-way ANOVA followed by Tukey's post hoc analysis was performed.

Results

Effects of cedrol on behavioral performance in the MWMT

Scopolamine administration significantly impaired spatial learning in rats, as evidenced by prolonged escape latency and increased path length to locate the submerged platform compared to the control group ($p<0.01$ to $p<0.001$). Cedrol treatment (15 and 30 mg/kg) effectively mitigated these deficits, resulting in significantly reduced latency and distance traveled relative to the scopolamine group ($p<0.05$ to $p<0.001$; Figures 2A, 2B). No meaningful statistical distinction was observed between the two cedrol groups.

On the sixth day of probe trial, animals treated with scopolamine exhibited impaired memory retention, demonstrated by reduced exploration time and movement range within the goal quadrant ($p<0.001$ and $p<0.05$, respectively). In contrast, cedrol-treated rats at both dosage levels showed enhanced memory performance, spending significantly prolonged search behavior in the target area (both $p<0.001$) and covering greater distances ($p<0.05$ and $p<0.001$) versus the scopolamine group (Figures 3A and 3B).

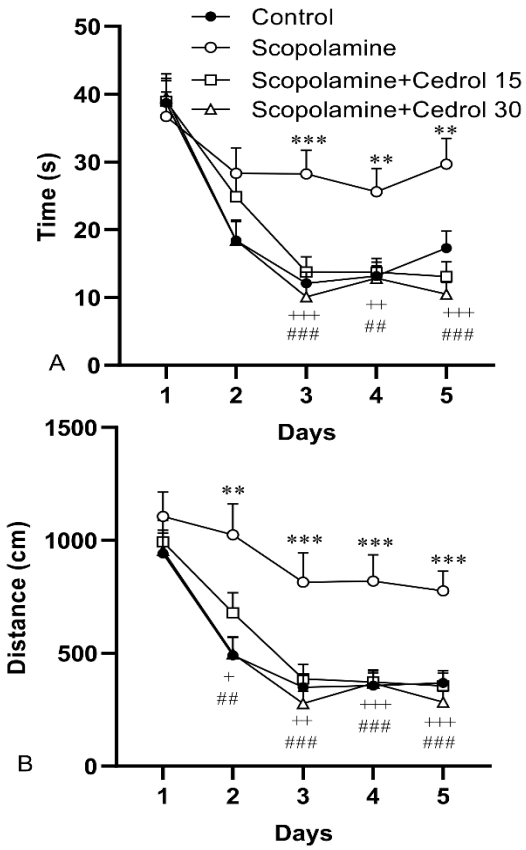

Figure 2. The results of 5 days of learning of the MWMT including time (A) and distance (B). **p<0.01, and ***p<0.001 present the difference between the Scopolamine group and Control group, $^{+}$p<0.05, $^{++}$p<0.01, and $^{+++}$p<0.001 present the difference between Scopolamine group and Scopolamine + Cedrol 15 group, $^{\#\#}$p<0.01, and $^{\#\#\#}$p<0.001 present the difference between Scopolamine and Scopolamine + Cedrol 30 groups. The data are presented as mean ± SEM (n=10).

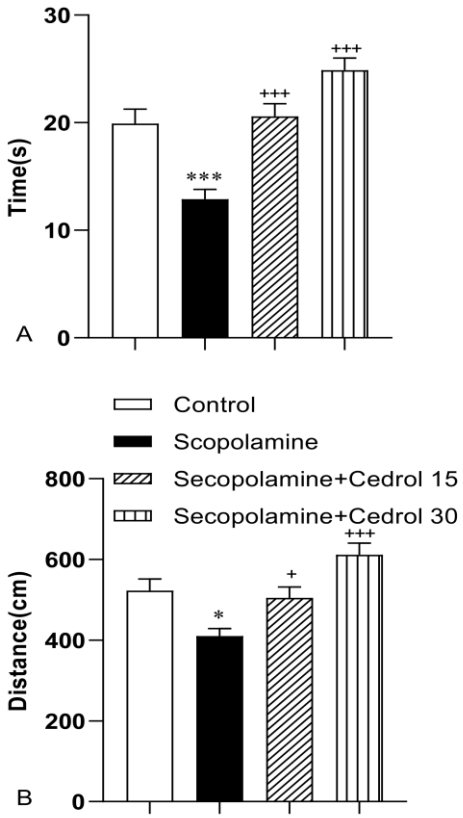

Figure 3. The results of the time (A) and distance (B) in the target area on the sixth day of MWMT in which the platform was removed and the probe trial was carried out. *p<0.05 and ***p<0.001 present the difference between the Scopolamine group and Control group, +p<0.05 and +++p<0.001 present the difference between Scopolamine and Scopolamine + Cedrol 15 groups or Scopolamine and Scopolamine + Cedrol 30 groups. The data are presented as mean ± SEM (n=10).

Effects of cedrol on behavioral performance in the PAT

Scopolamine administration significantly impaired memory retention, as shown by a lower latency to enter the aversive compartment at all post-shock time points compared to the control group (p<0.001). Cedrol treatment at both doses effectively counteracted this impairment, resulting in significantly longer delay times relative to the scopolamine group (p<0.001 at 24, 48, and 72 hr; Figure 4A–D). Additionally, animals in the scopolamine group spent more time in the dark compartment relative to the controls across all time intervals (p<0.001). Cedrol administration at both doses significantly reduced this dark time (p<0.001 at 24, 48, and 72 hr; Figure 4E–H).

Scopolamine also diminished the period spent in the illuminated compartment at all measured intervals (p < 0.001; Figure 5A–D). Cedrol treatment restored this parameter, with both doses significantly increasing light area duration at 3 all-time points (p<0.001; Figure 5A–D). Moreover, the entry count into the darkened section of the apparatus was markedly higher in the scopolamine group versus the control (p<0.001). Cedrol co-administration notably diminished this frequency relative to the scopolamine group (p<0.05 to p<0.001 at 3, 24, 48, and 72 hr; Figure 5E–H).

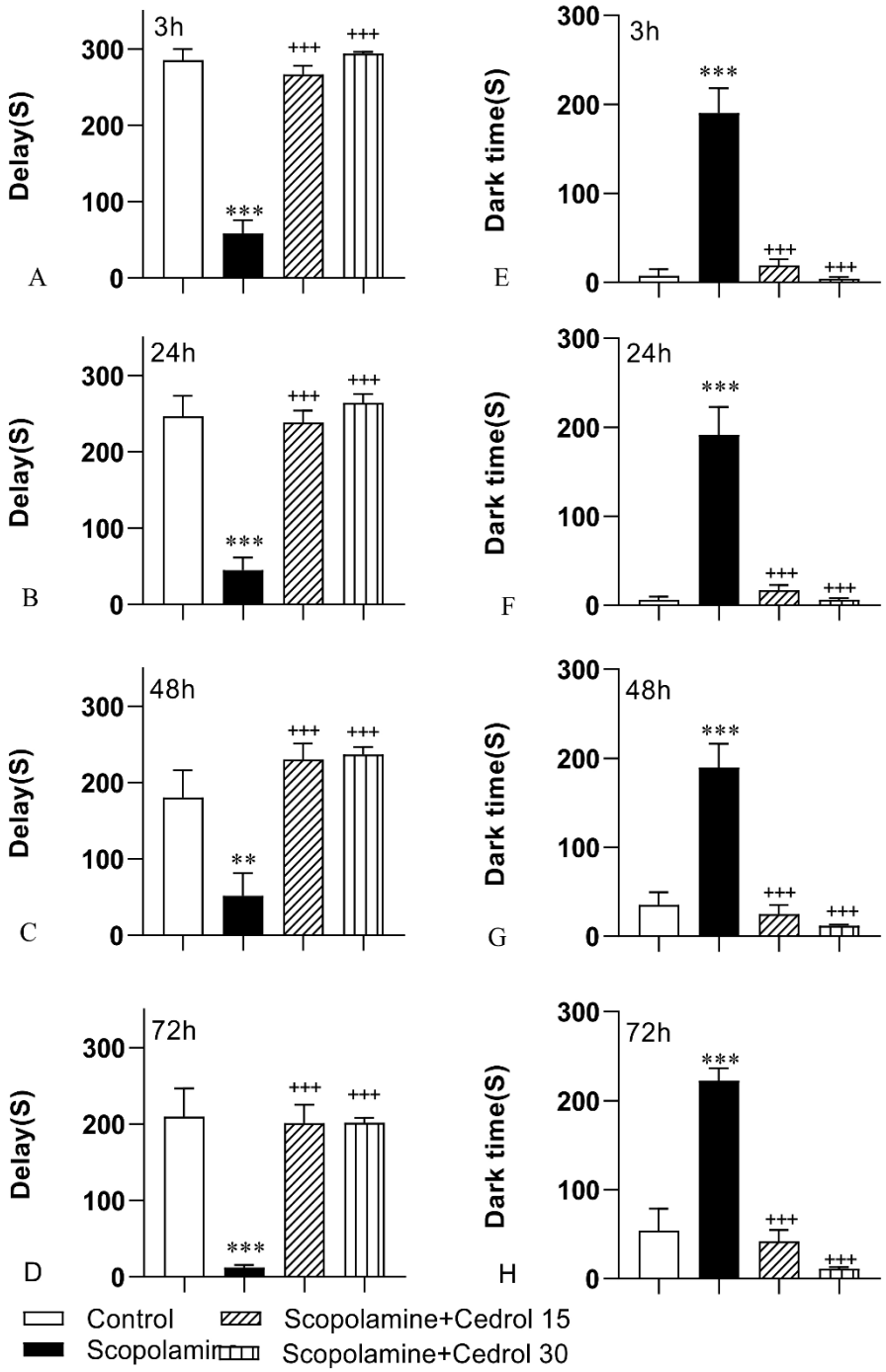

Figure 4. The results of the PAT including delay to enter the dark area (A–D) and the total dark time (E–H). **p<0.01 and ***p<0.001 present the difference between the Scopolamine group and Control group, $^{+++}$p<0.001 presents the difference between Scopolamine and Scopolamine + Cedrol 15 groups or Scopolamine and Scopolamine + Cedrol 30 groups. The data are presented as mean ± SEM (n=10).

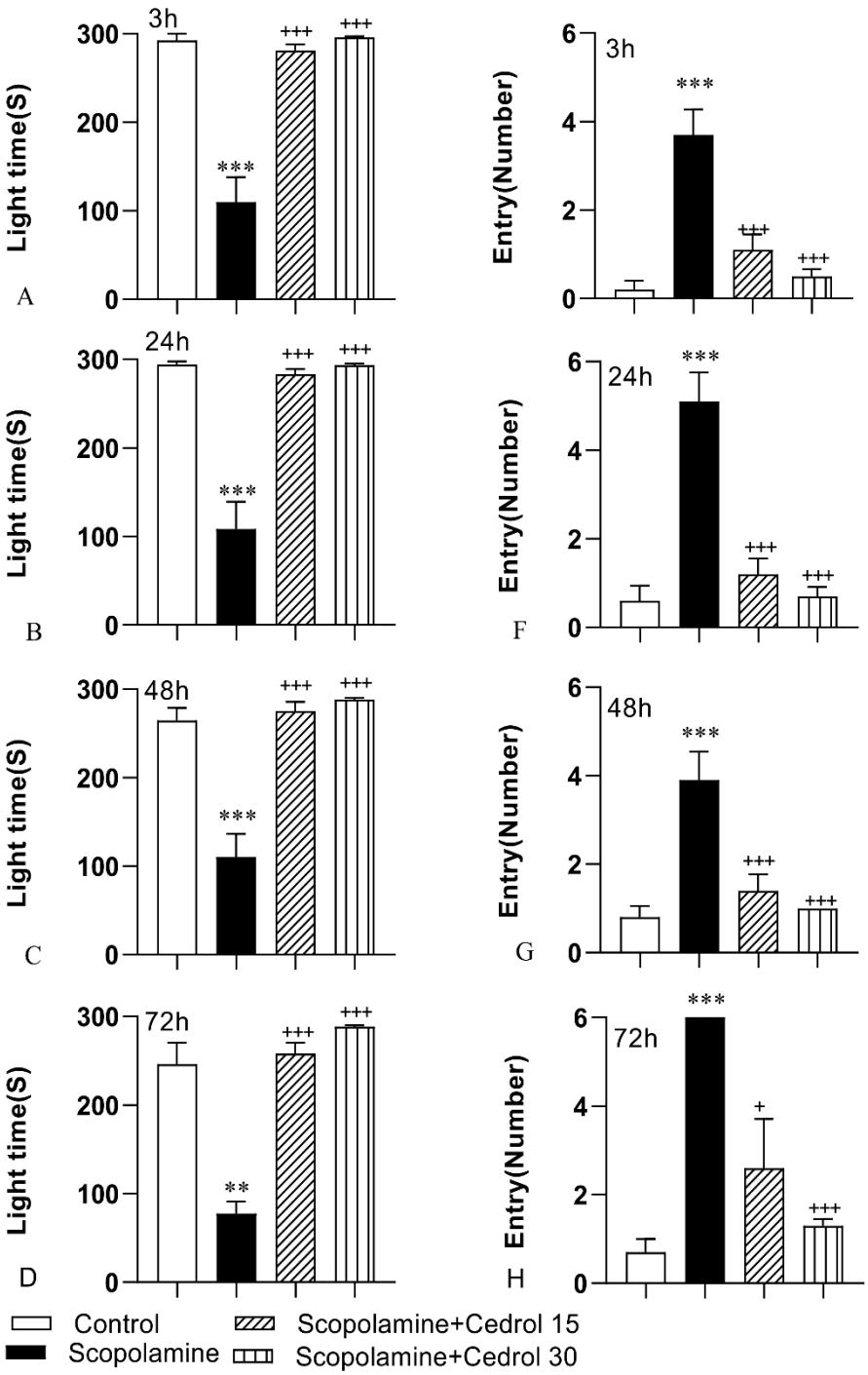

Figure 5. The results of the PA test including the total light time (A–D) and the number of dark area entries (E–H). **p<0.01 and ***p<0.001 present the difference between the Scopolamine group and Control group, +p<0.05 and +++p<0.001 present the difference between Scopolamine and Scopolamine + Cedrol 15 groups or Scopolamine and Scopolamine + Cedrol 30 groups. The data are presented as mean ± SEM (n=10).

Effects of cedrol on hippocampal BDNF levels

Scopolamine administration led to a marked decrease in hippocampal BDNF concentration versus the control group ($p<0.001$; Figure 6). Cedrol intervention at 30 mg/kg markedly elevated BDNF levels relative to the scopolamine group ($p<0.001$). Furthermore, BDNF expression in the Scopolamine + Cedrol 30 mg/kg group was significantly higher than in the Scopolamine + Cedrol 15 mg/kg group ($p<0.001$; Figure 6). No significant variation was detected between the Scopolamine + Cedrol 30 mg/kg group and the control group, whereas BDNF levels in the Scopolamine + Cedrol 15 mg/kg group remained significantly lower than those in controls ($p<0.001$; Figure 6).

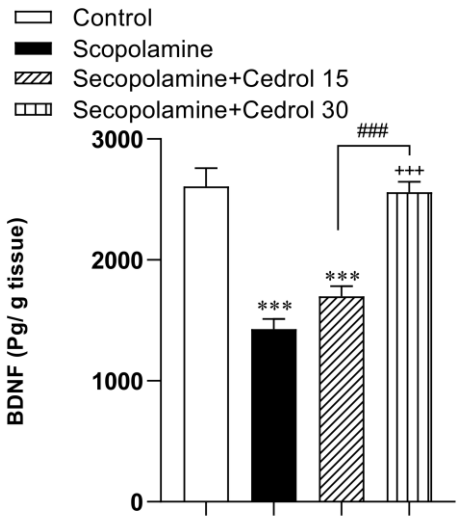

Figure 6. BDNF concentration in the hippocampus. *p<0.05 and **p<0.01 present the difference between different groups and Control group, +++p<0.001 presents the difference between the Scopolamine group and Scopolamine+Cedrol 30 group, ###p<0.001 presents the difference between Scopolamine+cedrol 15 and Scopolamine+Cedrol 30 groups. The data are presented as mean ± SEM (n=7).

Effects of cedrol on oxidative stress parameters

Scopolamine administration caused a notable elevation in MDA levels within both the hippocampus and cortex compared to the control group ($p<0.001$; Figure 7A, B). Cedrol treatment at both 15 and 30 mg/kg doses markedly reduced hippocampal MDA concentrations relative to the scopolamine group ($p<0.001$; Figure 6A). In the cortex, only the higher dose of cedrol produced a significant reduction in MDA levels relative to animals receiving scopolamine ($p<0.001$; Figure 7B). Despite these improvements, MDA concentrations in the hippocampus of cedrol-treated groups and in the cortex of the high-dose cedrol group remained significantly elevated versus the control values ($p<0.001$; Figure 7). Moreover, the reduction in MDA levels achieved with the 30 mg/kg dose of cedrol was significantly greater than that observed with the 15 mg/kg dose ($p<0.05$ to $p<0.001$; Figure 7A, B), indicating a dose-dependent antioxidant effect.

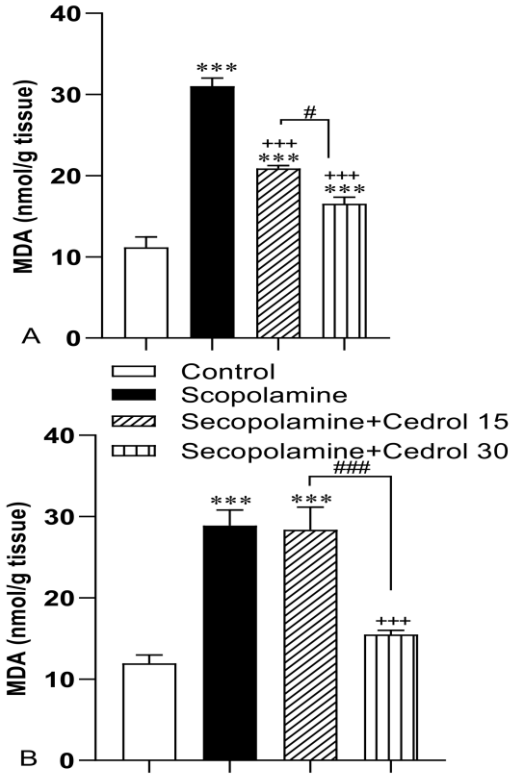

Figure 7. The MDA concentration in the hippocampus (A) and cortex (B). ***p<0.001 presents the difference between different groups and Control group, +++p<0.001 presents the difference between Scopolamine and Scopolamine + Cedrol 15 groups or Scopolamine and Scopolamine + Cedrol 30 groups, #p<0.05 and ###p<0.001 present the difference between Scopolamine + Cedrol 15 and Scopolamine + Cedrol 30 groups. The data are presented as mean ± SEM (n=7).

Scopolamine administration significantly reduced thiol concentrations in both the hippocampus and cortex versus to control animals ($p<0.001$ for both regions). Treatment with cedrol at 30 mg/kg effectively elevated thiol levels in both brain areas ($p<0.001$), whereas the 15 mg/kg dose increased this parameter only in the cortex ($p<0.01$). Despite the improvements, thiol concentrations in both the Scopolamine + Cedrol 15 mg/kg and Scopolamine + Cedrol 30 mg/kg groups remained significantly lower than those observed in the control group ($p<0.01$ to $p<0.001$). Moreover, thiol levels in the hippocampus and cortex were substantially elevated in the Scopolamine + Cedrol 30 mg/kg group versus to the Scopolamine + Cedrol 15 mg/kg group ($p<0.01$ to $p<0.001$; Figure 8).

Scopolamine administration led to a significant reduction in the activities of SOD and catalase in brain tissue versus the control group ($p<0.001$ for both enzymes; Figures 9 and 10). Treatment with cedrol at a dose of 30 mg/kg markedly restored the activity levels of both SOD and catalase ($p<0.001$; Figures 9 and 10), whereas the 15 mg/kg dose did not produce a statistically substantial recovery. Despite partial recovery, SOD activity in both cedrol-treated groups and catalase activity in the low-dose group remained substantially lower than those observed in control animals ($p<0.01$ to $p<0.001$; Figures 8 and 9). Furthermore, the enhancement in antioxidant enzyme activity achieved with the higher dose of cedrol was significantly higher than that observed with the lower dose ($p<0.001$; Figures 9 and 10), indicating a dose-dependent neuroprotective effect.

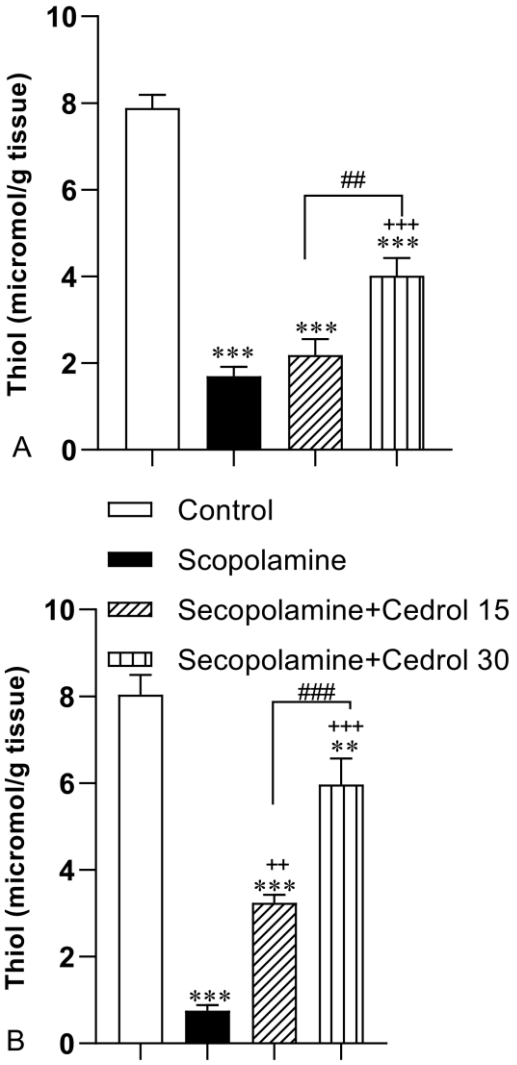

Figure 8. The thiol groups content in the hippocampus (A) and cortex (B). **p<0.01 and ***p<0.001 present the difference between other groups and Control group, $^{+++}$p<0.001 *and* $^{++}$p<0.01 presents the difference between the Scopolamine group and Scopolamine + Cedrol 30 group, $^{\#\#}$p<0.01 and $^{\#\#\#}$p<0.001 present the difference between Scopolamine + Cedrol 15 and Scopolamine + Cedrol 30 groups. The data are presented as mean ± SEM (n=7).

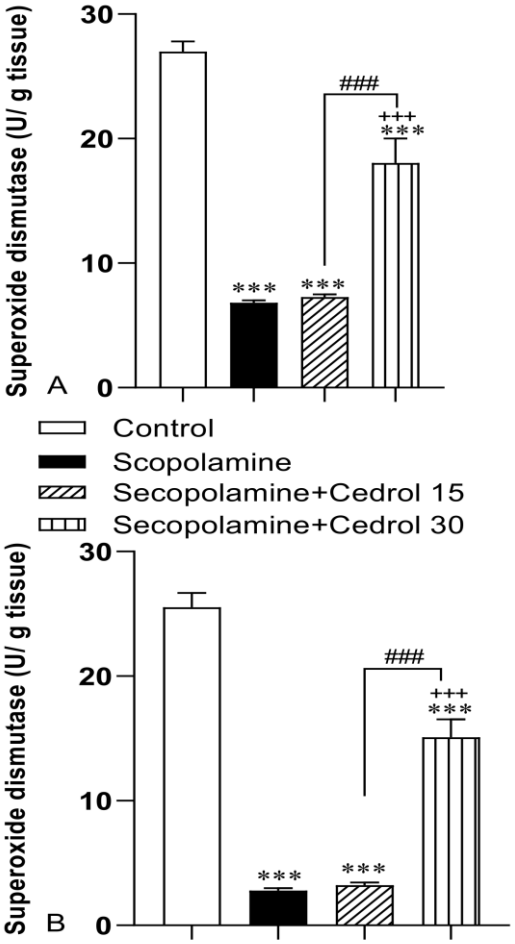

Figure 9. Superoxide dismutase levels in the hippocampus (A) and cortex (B). ***p<0.001 presents the difference between different groups and Control group, $^{+++}$p<0.001 presents the difference between the Scopolamine group and Scopolamine + Cedrol 30 group, $^{###}$p<0.001 presents the difference between Scopolamine + Cedrol 15 and Scopolamine + Cedrol 30 groups.The data are presented as mean ± SEM (n=7).

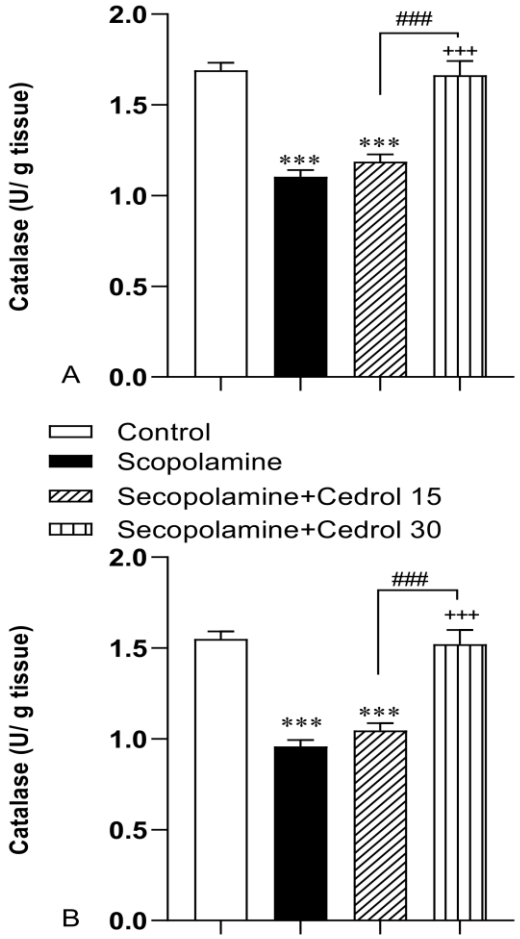

Figure 10. Catalase levels in the hippocampus (A) and cortex (B). ***p<0.001 presents the difference between different groups and Control group, $^{+++}$p<0.001 presents the difference between Scopolamine and Scopolamine + Cedrol 15 groups or Scopolamine and Scopolamine + Cedrol 30 groups, $^{###}$p<0.001 presents the difference between Scopolamine + Cedrol 15 and Scopolamine + Cedrol 30 groups. The data are presented as mean ± SEM (n=7).

Discussion

In the current study, cedrol administration enhanced hippocampal BDNF levels and mitigated oxidative stress in a scopolamine-induced rodent model of cognitive dysfunction. These findings were observed in the context of learning and memory impairments, as assessed by the PAT and MWMT. Consistent with earlier studies, scopolamine significantly disrupted cognitive performance in both paradigms (Mansouri et al. 2021; Tabrizian et al. 2015).

The findings demonstrated that animals treated with scopolamine exhibited significantly extended escape latency and increased path length were required to reach the hidden platform during MWMT acquisition, versus the control animals. Additionally, animals subjected to scopolamine treatment showed reduced time spent and distance traveled in the goal quadrant, indicating impaired spatial memory retention. These behavioral deficits suggest that scopolamine

administration disrupts both learning and mnemonic functions.

Consistent results were observed in the PAT where scopolamine-treated rats displayed a diminished avoidance response, reflected by shorter entry latency relative to controls. Moreover, these showed greater time allocation to the dark area and elevated entry counts, and spent less time in the illuminated compartment, further confirming the role of scopolamine in producing cognitive dysfunction.

Scopolamine, a well-established inhibitor of muscarinic receptors, is widely used to model cholinergic dysfunction and memory deficits in rodents (Rezvani et al. 2013; Tabrizian et al. 2015). In alignment with previous reports, experimental data confirmed that scopolamine administration substantially diminished hippocampal BDNF levels (Lee et al. 2014; Shi et al. 2013; Weon et al. 2016). This reduction is consistent with evidence linking central cholinergic system dysfunction to diminished BDNF and CREB expression in memory-related brain regions (Bekinschtein et al. 2008; Hong et al. 2011). Furthermore, prior investigations have demonstrated that stimulation of muscarinic receptors via pilocarpine enhances BDNF expression in areas associated with memory processing (da Penha Berzaghi et al. 1993).

Scopolamine-induced memory deficits are primarily due to disruption of the central cholinergic system, including elevated AChE activity in the brain (Kwon et al. 2010). Previous reports have also linked scopolamine deleterious effects on cognitive functions to enhanced oxidative stress within neural tissues (Goverdhan et al. 2012). In this investigation, scopolamine administration led to marked oxidative injury, evidenced by increased levels of MDA and diminished thiol groups, catalase, and SOD. The findings are consistent with earlier reports indicating that scopolamine impairs antioxidant defense mechanisms by decreasing the level of SOD, catalase, and glutathione, while promoting lipid peroxidation (Amirahmadi et al. 2022).

Moreover, accumulating evidence suggests a negative correlation among BDNF levels and oxidative stress markers in the brain tissue (Kapczinski et al. 2008; Zhang et al. 2015). Hacioglu et al. (2016) further indicted that BDNF elicits neuroprotective effects against oxidative damage induced by immobilization stress in murine models. Research utilizing scopolamine-based models of cognitive impairment, as well as other experimental and clinical studies, has consistently implicated BDNF depletion as a pivotal factor in the onset and progression of AD (Beheshti et al. 2021; Bekinschtein et al. 2008; Callaghan and Kelly 2012; Cunha et al. 2010; Mansouri et al. 2021). Oxidative stress is also widely recognized as a major contributor to the development of the pathophysiology of neurodegenerative diseases such as AD (Goverdhan et al. 2012; Manoharan et al. 2016; Niedzielska et al. 2016; Shakeri et al. 2020). Notably, antioxidant compounds have demonstrated neuroprotective potential in mitigating such conditions.

In the current study, cedrol administration significantly improved cognitive performance in scopolamine-treated rats. In the MWMT, both 15 and 30 mg/kg doses of cedrol diminished escape times and path trajectories, indicative of facilitated learning. During the probe trial, rats in these groups exhibited prolonged occupancy and increased swim distance within the goal quadrant, reflecting improved memory retention. Similarly, results from the PAT confirmed cedrol beneficial effects, as treated rats exhibited longer latencies to cross into the dark chamber, increased time in the illuminated area, and fewer entries into the dark zone collectively supporting cedrol role in ameliorating scopolamine-induced cognitive deficits.

To this point, no prior reports have explicitly investigated the memory-enhancing impacts of cedrol in animal

models of cholinergic dysfunction. However, evidence from other experimental paradigms suggests that cedrol exerts beneficial effects on nervous system function (Kagawa et al. 2003; Zhang and Yao 2019). Supporting the current findings, research using a rat model of global cerebral ischemia followed by reperfusion has demonstrated that cedrol administration improved functional ability in the PAT and attenuated oxidative damage in brain tissue via improvement of SOD activity and reducing MDA levels. Cedrol treatment also significantly elevated hippocampal BDNF concentrations (Asgharzadeh et al. 2025). In a separate investigation, cedrol was shown to ameliorate ischemic brain injury by modulating microglial activation and suppressing neuroinflammatory signaling via the ERβ–NF-κB pathway, thereby contributing to improved behavioral outcomes (Bi et al. 2024).

Using an elevated plus maze and dark/light box, it was revealed that cedrol had anxiolytic effects in mice and it improved the brain levels of serotonin and decreased dopamine levels (Zhang and Yao 2019). Clinical evidence has demonstrated that inhalation of cedrol enhances hippocampal vascular perfusion (Hori et al. 2012). Additionally, cedrol has exhibited neuroprotective properties in a laboratory-induced neuropathic pain paradigm in rats (Sakhaee et al. 2020). This natural product also suppressed glioblastoma progression and showed anti-tumor effects (Chang et al. 2020). To clarify the potential biological processes responsible for observed impacts of cedrol, oxidative stress parameters were evaluated in brain tissue. The findings revealed that cedrol, particularly at the higher dose, significantly reduced MDA levels and enhanced antioxidant defenses, as evidenced by increased thiol content and elevated levels of catalase and SOD in brain tissue. In previous studies, the benefits of cedrol on the nervous system disorders were reported to be due to its anti-oxidant effects (Sakhaee et al. 2020). The antioxidant potential of cedrol was further supported by findings from Emami et al. who demonstrated its free-radical scavenging activity using the DPPH assay (Emami et al. 2011). Additionally, the beneficial effect of cedrol on neuropathic pain in rats was attributed to its anti-oxidant effect (Sakhaee et al. 2020). In addition, the antiarthritic property of cedrol was shown to be due to its protection against inflammatory signaling cascades and oxidative cellular injury related damage (Forouzanfar et al. 2022). The findings of the current study further demonstrated that application of an elevated dose of cedrol significantly elevated hippocampal BDNF levels. This suggests that the observed improvements in learning and memory may be partially mediated through the upregulation of BDNF expression. Also considering the correlation between BDNF and oxidative stress (Kapczinski et al. 2008; Zhang et al. 2015) cedrol may attenuate oxidative stress by which improve BDNF and finally improve learning and memory abilities. It was reported that administration of cedrol can enhance the hippocampal regional blood flow bilaterally (Hori et al. 2012). A relationship between cerebral blood flow and cognitive function related to memory has been proposed in previous research (Heo et al. 2010). In light of this evidence, the memory-enhancing effects of cedrol detected in the present study may be partially attributed to its influence on hippocampal perfusion; however, this potential mechanism warrants further investigation. Regarding the possible mechanisms, we hypothesize that cedrol improved BDNF levels by restoring the cholinergic system and also suppressed oxidative damage by activating potential pathways such as Nrf2.

This study had some limitations that should be presented in the prospective researches. These are exemplified by investigating a wider range of cedrol doses to thoroughly examine dose-response relationships and potential dose-dependent effects. Furthermore, alternative treatment

protocols could be explored. Elucidating the underlying cellular and molecular mechanisms responsible for its neuroprotective properties is also crucial, as it would pave the way for its potential application in clinical applications.

The findings of our study indicated that cedrol could mitigate cognitive dysfunction induced by cholinergic dysfunction through attenuating oxidative stress and improving BDNF levels. Nonetheless, subsequent studies are justified to elucidate the explicit processes underlying its anti-amnesic effects.

Acknowledgment

This study was a report of a proposal which was approved by the North Khorasan University of Medical Sciences (990226). The research was validated by ethical committee of North Khorasan University of Medical Sciences. The Ethic Approval Code is IR.NKUMS.REC.1400.066.

Conflicts of interest

The authors affirm that there are no financial or personal communication that could be perceived as potential conflicts of interest influencing the content of this study.

Funding

North Khorasan University of Medical Sciences (990226)

Ethical Considerations

The research was validated by ethical committee of North Khorasan University of Medical Sciences. The Ethic Approval Code is IR.NKUMS.REC.1400.066.

Code of Ethics

IR.NKUMS.REC.1400.066.

Authors' Contributions

FS and MH planned the experiments. MHEG and AR wrote the initial version of the manuscript, FM and MHEG carried out the behavioral experiments, FB, AR, and FF Carried out the biochemical tests, MH analyzed the data, BB, MH and AR finalized the manuscript

References

Akbarian M, Mirzavi F, Amirahmadi S, Hosseini M, Alipour M, Feizi H, Rajabian A (2022) Amelioration of oxidative stress, cholinergic dysfunction, and neuroinflammation in scopolamine-induced amnesic rats fed with pomegranate seed. Inflammopharmacol 30(3): 1021-1035. doi: 10.1007/s10787-022-00971-7

Amirahmadi S, Farimani FD, Akbarian M, Mirzavi F, Eshaghi Ghalibaf M H, Rajabian A, Hosseini M (2022) Minocycline attenuates cholinergic dysfunction and neuro-inflammation-mediated cognitive impairment in scopolamine-induced Alzheimer's rat model. Inflammopharmacol 30(6): 2385-2397. doi: 10.1007/s10787-022-01071-2

Asgharzade F, Ahmadzadeh, AM, Pourbagher-Shahri S, Forouzanfar F (2025) Protective effects of cedrol against transient global cerebral ischemia/reperfusion injury in rat'. BMC Complement Med Ther 25: 1-10.

Azizi-Malekabadi H, Hosseini M, Soukhtanloo M, Sadeghian R, Fereidoni M, Khodabandehloo F (2012) Different effects of scopolamine on learning, memory, and nitric oxide metabolite levels in hippocampal tissues of ovariectomized and Sham-operated rats. Arq Neuropsiquiatr 70(6): 447-452. doi: 10.1590/s0004-282x2012000600012

Beheshti F, Akbari HR, Baghcheghi Y, Mansouritorghabeh F, Mortazavi Sani SS, Hosseini M (2021) Beneficial effects of angiotensin converting enzyme inhibition on scopolamine-induced learning and memory impairment in rats, the roles of brain-derived neurotrophic factor, nitric oxide and neuroinflammation. Clin Exp Hypertens 43(6): 505-515. doi: 10.1080/10641963.2021.1901112

Behl C, Moosmann B (2002) Antioxidant neuroprotection in Alzheimer's disease as preventive and therapeutic approach. Free Radic Biol Med 33(2): 182-191. doi: 10.1016/s0891-5849(02)00883-3

Bekinschtein P, Cammarota M, Katche C, Slipczuk L, Rossato JI, Goldin A, Izquierdo I, Medina JH (2008) BDNF is essential to

promote persistence of long-term memory storage. Proc Natl Acad Sci 19;105(7):2711-2716. doi: 10.1073/pnas.0711863105

Bi Y, Xie Z, Cao X, Ni H, Xia S, Bao X, Huang Q, Xu Y, Zhang Q (2024) Cedrol attenuates acute ischemic injury through inhibition of microglia-associated neuroinflammation via ERβ-NF-κB signaling pathways. Brain Res Bull 218:111102. doi: 10.1016/j.brainresbull.2024.111102.

Boskabady MH, Amin F, Shakeri F (2021) The effect of Curcuma longa on inflammatory mediators and immunological, oxidant, and antioxidant biomarkers in asthmatic rats. Evid Based Complement Alternat Med 2021(1):1-13. doi:10.1155/2021/4234326

Callaghan CK, Kelly ÁM (2012) Differential BDNF signaling in dentate gyrus and perirhinal cortex during consolidation of recognition memory in the rat. Hippocampus 22(11): 2127-2135. doi: 10.1002/hipo.22033

Chang KF, Huang XF, Chang JT, Huang YC, Weng JC, Tsai NM (2020) Cedrol suppresses glioblastoma progression by triggering DNA damage and blocking nuclear translocation of the androgen receptor. Cancer Lett 495: 180-190. doi: 10.1016/j.canlet.2020.09.007

Cunha C, Brambilla R, Thomas KL (2010) A simple role for BDNF in learning and memory? Front Mol Neurosci 3:1-10. doi: 10.3389/neuro.02.001.2010

da Penha Berzaghi M, Cooper J, Castrén E, Zafra F, Sofroniew M, Thoenen H, Lindholm D (1993) Cholinergic regulation of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) but not neurotrophin-3 (NT-3) mRNA levels in the developing rat hippocampus. J Neurosci 13(9): 3818-3826. doi: 10.1523/jneurosci.13-09-03818.1993

Dabouri Farimani F, Hosseini M, Amirahmadi S, Akbarian M, Shirazinia M, Barabady M, Rajabian A (2024) Cedrol supplementation ameliorates memory deficits by regulating neuro-inflammation and cholinergic function in lipopolysaccharide-induced cognitive impairment in rats. Heliyon 10(9): e30356. doi: 10.1016/j.heliyon. 2024.e30356

Emami SA, Abedindo BF, Hassanzadeh-Khayyat M (2011) Antioxidant Activity of the Essential Oils of Different Parts of Juniperus excelsa M. Bieb. subsp. excelsa and J. excelsa M. Bieb. subsp. polycarpos (K. Koch) Takhtajan (Cupressaceae). Iran J Pharm Res 10(4): 799-810.

Eshaghi Ghalibaf MH, Rajabian A, Parviz M, Akbarian M, Amirahmadi S, Vafaee F, Hosseini M (2023) Minocycline alleviated scopolamine-induced amnesia by regulating antioxidant and cholinergic function. Heliyon 9(2): e13452. doi: 10.1016/j.heliyon. 2023.e13452.

Forouzanfar F, Pourbagher-Shahri AM, Ghazavi H (2022) Evaluation of Antiarthritic and Antinociceptive Effects of Cedrol in a Rat Model of Arthritis. Oxid Med Cell Longev 2022: 4943965. doi: 10.1155/2022/4943965

Giacobini E, Cuello AC, Fisher A (2022) Reimagining cholinergic therapy for Alzheimer's disease. Brain 145(7): 2250-2275. doi: 10.1093/brain/awac096

Giacobini E, Khachaturian ZS (2019) Revisiting the Cholinergic Hypothesis in Alzheimer's Disease: Emerging Evidence from Translational and Clinical Research. J Prev Alzheimers Dis 6(1): 2-15. doi: 10.14283/jpad.2018.43

Giacobini E, Pepeu G (2018) Sex and Gender Differences in the Brain Cholinergic System and in the Response to Therapy of Alzheimer Disease with Cholinesterase Inhibitors. Curr Alzheimer Res 15(11): 1077-1084. doi: 10.2174/1567205015666180613111504

Goverdhan P, Sravanthi A, Mamatha T (2012) Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress. Int J Alzheimers Dis 2012: 974013. doi: 10.1155/2012/974013

Hacioglu G, Senturk A, Ince I, Alver A (2016) Assessment of oxidative stress parameters of brain-derived neurotrophic factor heterozygous mice in acute stress model. Iran J Basic Med Sci 19(4): 388-393.

Hampel H, Mesulam MM, Cuello AC, Khachaturian AS, Vergallo A, Farlow MR, Snyder PJ, Giacobini E, Khachaturian ZS (2019) Revisiting the Cholinergic Hypothesis in Alzheimer's Disease: Emerging Evidence from Translational and Clinical Research. J Prev Alzheimers Dis 2019;6(1):2-15. doi: 10.14283/jpad.2018.43.

Hartmann D, Drummond J, Handberg E, Ewell

S, Pozzo-Miller L (2012) Multiple approaches to investigate the transport and activity-dependent release of BDNF and their application in neurogenetic disorders. Neural Plast 2012: 203734. doi: 10.1155/2012/203734

Heo S, Prakash RS, Voss MW, Erickson KI, Ouyang C, Sutton B P Kramer AF (2010) Resting hippocampal blood flow, spatial memory and aging. Brain Res 1315: 119-127. doi: 10.1016/j.brainres.2009.12.020

Hong SW, Yang JH, Joh EH, Kim HJ, Kim DH (2011) Gypenoside TN-2 ameliorates scopolamine-induced learning deficit in mice. J Ethnopharmacol 134(3): 1010-1013. doi: 10.1016/j.jep.2011.02.002

Hori E, Shojaku H, Watanabe N, Kawasaki Y, Suzuki M, de Araujo M F, Nagashima Y, Yada Y, Ono T, Nishijo, H (2012) Effects of direct cedrol inhalation into the lower airway on brain hemodynamics in totally laryngectomized subjects. Auton Neurosci 168(1-2): 88-92. doi: 10.1016/j.autneu.2012.01.010

Hosseini Z, Mansouritorghabeh F, Kakhki SH, Hosseini M, Rakhshandeh H, Hosseini A, Hasanpour M, Iranshahi M, Rajabian, A (2022) Effect of Sanguisorba minor on scopolamine-induced memory loss in rat: involvement of oxidative stress and acetylcholinesterase. Metab Brain Dis 37(2): 473-488. doi: 10.1007/s11011-021-00898-y

Jamialahmadi K, Sadeghnia HR, Mohammadi G, Kazemabad A M, Hosseini M (2013) Glucosamine alleviates scopolamine induced spatial learning and memory deficits in rats. Pathophysiol 20(4): 263-267. doi: 10.1016/j.pathophys.2013.04.003

Kagawa D, Jokura H, Ochiai R, Tokimitsu I, Tsubone H (2003) The sedative effects and mechanism of action of cedrol inhalation with behavioral pharmacological evaluation. Planta Med 69(7), 637-641. doi: 10.1055/s-2003-41114

Kapczinski F, Frey BN, Andreazza AC, Kauer-Sant'Anna M, Cunha AB, Post RM (2008) Increased oxidative stress as a mechanism for decreased BDNF levels in acute manic episodes. Braz J Psychiatry 30(3): 243-245. doi: 10.1590/s1516-44462008000300011

Keshavarzi Z, Amiresmaili S, Shahrokhi N, Bibak B, Shakeri F (2021) Neuroprotective effects of auraptene following traumatic brain injury in male rats: The role of oxidative stress. Brain Res Bull 177: 203-209. doi: 10.1016/j.brainresbull.2021.09.021

Kwon SH, Lee HK, Kim JA, Hong S I, Kim HC, Jo TH, Park YI, Lee CK, Kim YB, Lee SY, Jang CG (2010) Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. Eur J Pharmacol 649(1-3): 210-217. doi: 10.1016/j.ejphar.2010.09.001

Lee B, Sur B, Shim J, Hahm DH, Lee H (2014) Acupuncture stimulation improves scopolamine-induced cognitive impairment via activation of cholinergic system and regulation of BDNF and CREB expressions in rats. BMC Complement Altern Med 14: 338. doi: 10.1186/1472-6882-14-338

Manoharan S, Guillemin GJ, Abiramasundari R S, Essa MM, Akbar M, Akbar MD (2016) The Role of Reactive Oxygen Species in the Pathogenesis of Alzheimer's Disease, Parkinson's Disease, and Huntington's Disease: A Mini Review. Oxid Med Cell Longev 2016: 8590578. doi: 10.1155/2016/8590578

Mansouri F, Ghanbari H, Marefati N, Arab Z, Salmani H, Beheshti F, Hosseini M (2021) Protective effects of vitamin D on learning and memory deficit induced by scopolamine in male rats: the roles of brain-derived neurotrophic factor and oxidative stress. Naunyn Schmiedebergs Arch Pharmacol 394(7): 1451-1466. doi: 10.1007/s00210-021-02062-w

Marefati N, Mokhtari-Zaer A, Beheshti F, Karimi S, Mahdian Z, Khodamoradi M, Hosseini M (2019) The effects of soy on scopolamine-induced spatial learning and memory impairments are comparable to the effects of estradiol. Horm Mol Biol Clin Investig 39(3): doi: 10.1515/hmbci-2018-0084

Medina JH (2008) BDNF is essential to promote persistence of long-term memory storage. Proc Natl Acad Sci 105(7): 2711-2716. doi: 10.1073/pnas.0711863105

Nie K, Yu JC, Fu Y, Cheng HY, Chen FY, Qu Y, Han JX (2009) Age-related decrease in constructive activation of Akt/PKB in SAMP10 hippocampus. Biochem Biophys Res Commun 378(1): 103-107. doi: 10.1016/j.bbrc.2008.11.010

Niedzielska E, Smaga I, Gawlik M,

Moniczewski A, Stankowicz P, Pera J, Filip M (2016) Oxidative Stress in Neurodegenerative Diseases. Mol Neurobiol 53(6): 4094-4125. doi: 10.1007/s12035-015-9337-5

Rastegar-Moghaddam SH, Amirahmadi S, Akbarian M, Sharizina M, Beheshti F, Rajabian A, Eshaghi Ghalibaf MH, Azimi M, Mahmoudabady M, Hosseini M (2024) Cardioprotective effect of cedrol in an inflammation systemic model induced by lipopolysaccharide: Biochemical and histological verification. J Cardiovasc Thorac Res 2024, 16(2), 120-128.

Rezvani AH, Cauley M, Xiao Y, Kellar KJ, Levin ED (2013) Effects of chronic sazetidine-A, a selective α4β2 neuronal nicotinic acetylcholine receptors desensitizing agent on pharmacologically-induced impaired attention in rats. Psychopharmacol 226(1): 35-43. doi: 10.1007/s00213-012-2895-6

Sakhaee MH, Sayyadi SAH, Sakhaee N, Sadeghnia HR, Hosseinzadeh H, Nourbakhsh F, Forouzanfar F (2020) Cedrol protects against chronic constriction injury-induced neuropathic pain through inhibiting oxidative stress and inflammation. Metab Brain Dis 35(7): 1119-1126. doi: 10.1007/s11011-020-00581-8

Saura CA, Valero J (2011) The role of CREB signaling in Alzheimer's disease and other cognitive disorders. Rev Neurosci 22(2): 153-169. doi: 10.1515/rns.2011.018

Sayre LM, Perry G, Smith MA (2008) Oxidative stress and neurotoxicity. Chem Res Toxicol 21(1), 172-188. doi: 10.1021/tx700210j

Shakeri F, Bianconi V, Pirro M, Sahebkar A (2020) Effects of plant and animal natural products on mitophagy. Oxid Med Cell Longev 2020:6969402. doi:10.1155/2020/6969402.

Shi Z, Chen L, Li S, Chen S, Sun X, Sun L, Li Y, Zeng J, He Y, Liu X (2013) Chronic scopolamine-injection-induced cognitive deficit on reward-directed instrumental learning in rat is associated with CREB signaling activity in the cerebral cortex and dorsal hippocampus Psychopharmacol 230(2): 245-260. doi: 10.1007/s00213-013-3149-y

Soares JC (2015) The interplay between BDNF and oxidative stress in chronic schizophrenia. Psychoneuroendocrinol 51: 201-208. doi: 10.1016/j.psyneuen.2014.09.029

Tabrizian K, Yaghoobi NS, Iranshahi M, Shahraki J, Rezaee R, Hashemzaei M (2015) Auraptene consolidates memory, reverses scopolamine-disrupted memory in passive avoidance task, and ameliorates retention deficits in mice. Iran J Basic Med Sci 18(10): 1014-1019.

Tang KS (2019) The cellular and molecular processes associated with scopolamine-induced memory deficit: A model of Alzheimer's biomarkers. Life Sci 233: 116695. doi: 10.1016/j.lfs.2019.116695

Terry AV, Buccafusco JJ (2003) The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 306(3): 821-827. doi: 10.1124/jpet.102.041616

Weon JB, Jung YS, Ma CJ (2016) Cognitive-Enhancing Effect of Dianthus superbus var. Longicalycinus on Scopolamine-Induced Memory Impairment in Mice. Biomol Ther 24(3): 298-304. doi: 10.4062/biomolther.2015.083

Zhang K, Yao L (2018) The anxiolytic effect of *Juniperus virginiana* L. essential oil and determination of its active constituents. Physiol Behav 189: 50-58. doi: 10.1016/j.physbeh.2018.01.004

Zhang K, Yao L (2019) Anxiolytic Effect of Cedrol on Behavior and Brain Neurotransmitter Levels of Female Mice. Biol Pharm Bull 42(9): 1575-1580. doi: 10.1248/bpb. b19-00380

Zhang XY, Chen DC, Tan YL, Tan SP, Wang ZR, Yang FD, Okusaga OO, Zunta-Soares GB, Soares JC (2015) The interplay between BDNF and oxidative stress in chronic schizophrenia. Psychoneuroendocrinol 51: 201-208. doi: 10.1016/j.psyneuen.2014.09.029