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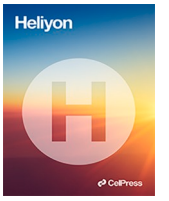

Research article

Cedrol supplementation ameliorates memory deficits by regulating neuro-inflammation and cholinergic function in lipopolysaccharide–induced cognitive impairment in rats

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ABSTRACT

Background: Cedrol, a sesquiterpene alcohol, is found in a high amount in several conifers. It possess several beneficial health effects, including antioxidant and anti-inflammatory properties. Objective: This study evaluates the neuroprotective role of cedrol against lipopolysaccharide (LPS)-induced neuroinflammation and memory loss in rats.
Methods: Wistar rats were treated with cedrol (7.5, 15, and 30 mg/kg, oral, two weeks). During the last week, the rats (except for the control group) were treated with LPS (intraperitoneal injection, 1 mg/kg) to induce memory impairment. After that, the animals were subjected to behavioral studies (Morris water maze and passive avoidance) and biochemical assessments.
Results: Our results showed a significant decrease in learning and memory function in LPS-induced rats which were reversed by cedrol. Also, there was a significant increase in the cerebral levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and malondialdehyde (MDA) as well as acetylcholinesterase (AChE) activity in LPS-treated rats. Besides, a significant reduction in total thiol and superoxide dismutase levels was observed in LPS-treated rats. However, cedrol significantly decreased the brain level of AChE, TNF-α, and IL-1β. Administration of cedrol also restored the oxidative stress markers.
Conclusion: the beneficial effects of cedrol against LPS-induced memory impairment could be due to antioxidant activities and modulation of neuro-inflammatory mediators.

1. Introduction

Alzheimer's disease (AD) is the most common age-related disease [1]. Accumulating evidence demonstrated that AD patients present some degree of neurocognitive symptoms, including memory and learning impairment [1,2]. It is well known that insufficient

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release of acetylcholine (ACh) leads to cognitive dysfunction in AD patients [2]. In AD brains, up-regulation of toll-like receptor 4 (TLR4) and its ligand were shown to be associated with loss of cholinergic neurons [3]. TLR4 promotes nuclear factor kappa B (NF-κB) -related pro-inflammatory pathways [3,4]. Systemic administration of lipopolysaccharide (LPS), as a TLR4 ligand, was found to elevate the expression of downstream NF-κB target genes (such as tumor necrosis factor-alpha (TNF-α)), chemokine (C–C motif) ligand 2 (CCL2), interleukin-1 beta (IL-1β)) in the brain tissue [3,4]. LPS also induces degenerative changes in brain tissue that include excess free radical generation and antioxidants deplession [4–6]. Therefore, LPS-treated rodents are being used extensively as an experimental model of AD [4,5,7,8]. The pro-inflammatory cytokines are suggested to trigger neuro-inflammation, leading to neurodegeneration [5,9]. During inflammation, the immune system is activated primarily by glial cells, such as microglia and astrocytes [9].

Besides, oxidative stress plays an important role in the pathogenesis of neurodegenerative disordes [2]. Hence, the antioxidant and anti-inflammatory agents are suggested to exert cognitive-enhancing effects [10–13]. Cedrol, a sesquiterpene alcohol, is naturally found in high content in the essential oil of Cedrus atlantica, Juniperus virginiana, and Cupressus sempervirens [14–16]. This phytochemical has been reported to possess several beneficial health effects which include antitumor, anticlotting, anti-inflammatory [17,18], anti-microbial, anticancer [19], and antioxidant activity [14,16,20]. Cedrol affects the central nervous system [21–23] and is thought to induce anxiolytic and sedative effects [24]. In this context, it demonstrated a stimulatory effect on cerebral blood flow in hippocampus [25]. The present study aimed to evaluate the possible mechanisms of action underlying the memory-improving potential of cedrol in LPS-induced neuro-inflammation.

2. Material and methods

2.1. Drugs and animals

Cedrol was obtained from Tinab Shimi Khavarmianeh Company (Mashhad, Khorasan Province, Iran). LPS (Escherichia coli serotype 055: B5) was purchased from Sigma company (USA). Other chemicals were purchased from Sigma Aldrich Company (Merck KGaA, Darmstadt, Germany).

2.2. Experimental animals

Wistar rats (male, n = 50, 10–12 weeks old, weighting 220 ± 20 g) were purchased from the animal house of Mashhad University of Medical Sciences, Iran. During the experiment, the temperature of the rearing environment was 22–24 °C, the humidity was 55 ± 5 %, and 12 h: 12 h light-dark cycle with free access to water and food. All animals were acclimatized before starting any procedures. Experimental procedures were approved by the Animal Care and Use Committee of Mashhad University of Medical Sciences (Number: IR.MUMS.MEDICAL.REC.1401.029), and the principles outlined in the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1978).

2.3. Experimental design

Fig. 1 illustrates a diagram timeline of the experiments. The rats were randomly divided into control, LPS, and LPS-Cedrol groups. The LPS group received a fresh solution of LPS (1 mg/kg in saline (pH 7.4), intraperitoneal injection) for seven days. Cedrol was first dissolved in dimethyl sulfoxide (DMSO) 3 % and then diluted with physiological saline (NaCl 0.9 %) to provide the final concentrations of 7.5, 15, and 30 mg/kg. The doses of LPS and cedrol were selected on previous reports in the literature [26–28]. Normal saline

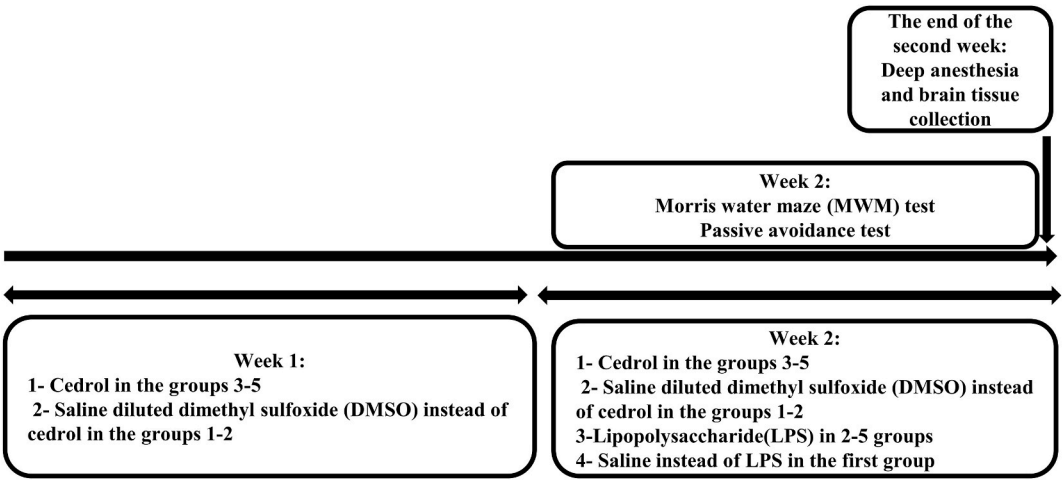

Fig. 1. Timeline diagram of the experimental protocol.

solution (0.9 % w/v, 2 ml/kg) was given to the control group. The LPS-Cedrol groups were treated with cedrol (7.5–30 mg/kg, oral), whereas the LPS and control groups received the vehicle for two weeks. During the second week, the rats were given LPS (except for the control group). Rats were subjected to behavioral tests after completion of the treatment schedule [28].

3. Behavioral test

3.1. Morris water maze test

Morris water maze test was done using a round pool (150 cm in diameter, 50 cm in height) filled with water up to 30 cm. The pool was hypothetically divided into four quadrants (I - IV). A circular platform (10 cm in diameter, 28 cm in height) was located in the middle of the first quadrant (target area). There was also a set of photographic signs around the pool. The water temperature was kept at 23–25 °C throughout the experiment. The test lasted for six days and it was included the training phase (5 days) and the probe test (6th day). In the training phase, the rats were placed in the water facing the pool wall from four entry points, and the time the rat searched for the platform and the distance the rats traveled to reach the platform was recorded. Each rat was tested 4 times per day at an interval of 15–20 s. In the probe test, the platform was removed, and the rat was allowed to move freely for 60 s. The time spent and the distance traveled in the target area were recorded [26,27].

3.2. Passive avoidance test

The simple non-spatial learning ability was assessed using a device with two equal-sized light and dark sections. In this apparatus, a guillotine door acted as the separation of the two sections. Assessment of passive avoidance ability includes three phases. During the first stage of the experiment which lasted two consecutive days, the rat was placed in the apparatus and accustomed to it. To perform the acquisition trial (second phase), the rats were placed in the apparatus. The door was closed automatically as soon as the rat entered the dark compartment, and an electric foot shock (2 mA for 2 s) was delivered. Finally, the third stage (retention trials) was performed 3, 24, 48, and 72h after the training trial using the same training program. The delay value was estimated by measuring the time before the animal entered the dark compartment. Furthermore, the time spent in the black and light sections (sec) within 300 s was noted at 3, 24, 48, and 72h after receiving the punishment [28,29].

3.3. Biochemical test

3.3.1. Tissue collection and preparation

After conducting the behavioural tasks, the animals were subjected to a deep anesthesia. Then, the barins were dissected on ice to get the hippocampus. Rat' hippocampus has cashew-shaped structure lying just beneath the neocortex. It consists of two parts located symmetrically in the medial temporal areas of the hemispheres [30]. The isolated tissues were homogenized in the ice-cold phosphate buffer saline (PBS, 0.1 M, pH 7.4), and centrifuged at 12000×*g* for 10 min. The supernatants were separated for the biochemical assessments [28,29].

3.3.2. Assessment of cytokine levels using enzyme-linked immunosorbent assay (ELISA)

The concentrations of TNF-α and IL-1β in the hippocampus were detected according to the instructions of the ELISA kit (Karmania, Kerman, Iran).

3.3.3. Acetylcholinesterase (AChE) assessment

AChE enzyme assessment was performed using a solution of acetylcholine iodide. For this purpose, each sample was added to a cuvette that contained 5, 5′-dithiobis- (2-nitrobenzoic acid) (DTNB) and PBS solution. After that, this was incubated for about 15 min. The reaction was initiated by adding five μl of the substrate acetylcholine iodide. Finally, the absorbance was measured using the UV–vis spectrophotometer at 412 nm. The enzyme activities are expressed as nmol substrate/tissue weight (g) [11,29].

3.3.4. Measurement of malondialdehyde (MDA) level

The MDA concentration, a crucial important oxidative marker, was measured as previously described [31]. In short, 1 ml of thiobarbituric acid (TBA) reagent was added to each supernatant. The samples were incubated in boiling water for 40 min. The absorbance was detected at 532 nm to get the thiobarbituric acid reactive species proportional to lipid peroxidation levels.

4. Antioxidants

4.1. Total thiol groups

The total thiol (sulfhydryl) groups was measured using the previously published studies [26,31]. Thiol content was detected by adding the DTNB (10 mmol/l) reagent to each sample. The absorbance of the solution was assessed at 412 nm wavelength. The thiol contents are presented as μmol/tissue wight (g).

4.2. SOD activity

The SOD activity was evaluated based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction by SOD enzyme. The reaction mixture in tubes contained PBS, pyrogallol, and MTT solution. The solution was incubated for 5 min at room temperature. Then, the absorbance was assessed at 570 nm wavelength, using a spectrophotometer. The amount of enzyme that developed 50 % inhibition is defined as one unit [29].

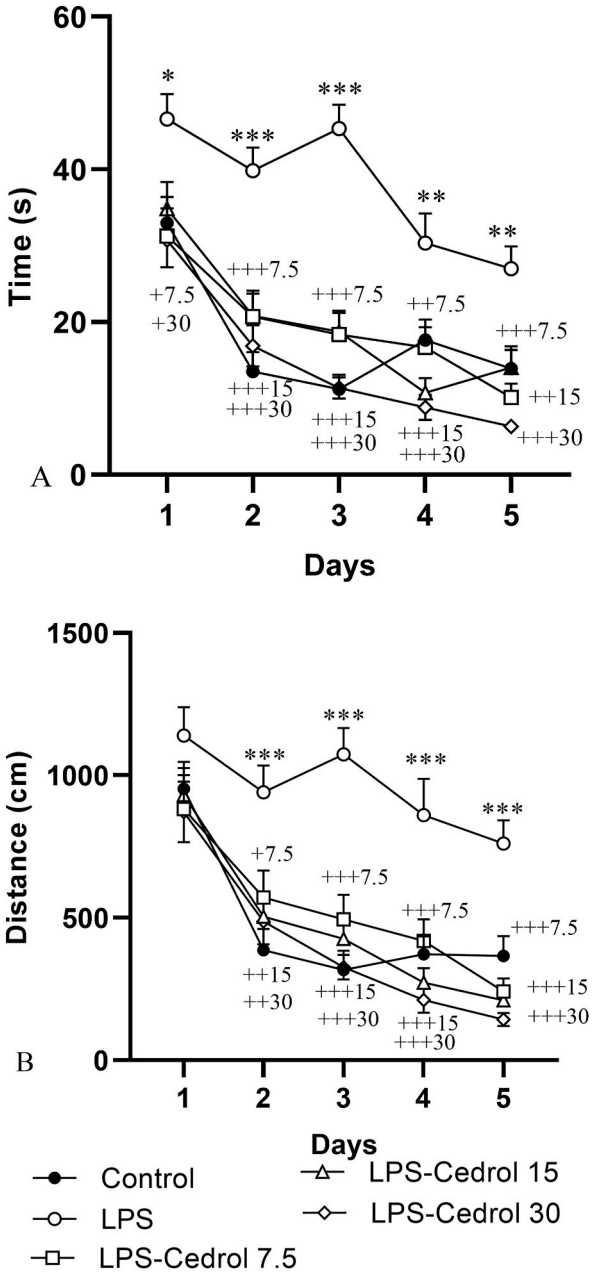

Fig. 2. Cedrol improves memory and learning impairment in lipopolysaccharide (LPS)-treated rats. (A and B) the learning trial of the Morris water maze (MWM). (A) Latency time to find the platform. (B) Traveled distance to find the platform. Data are presented as mean ± SEM (n = 10/group). *P < 0.05, **P < 0.01, ***P < 0.001 LPS group compared with Control group.+P < 0.05, $^{++}$ P < 0.01, $^{+++}$ P < 0.001 Cedrol-LPS groups compared with LPS group.

4.3. Statistical analysis

All values are expressed as mean ± SEM. SPSS version 23 was used to do the statistical analysis. For the Morris water maze test: repeated measures of two-way ANOVA followed by the Bonferroni test were performed. For all other parameters, one-way ANOVA followed by Tukey's test was done. Differences were considered statistically significant at $p < 0.05$.

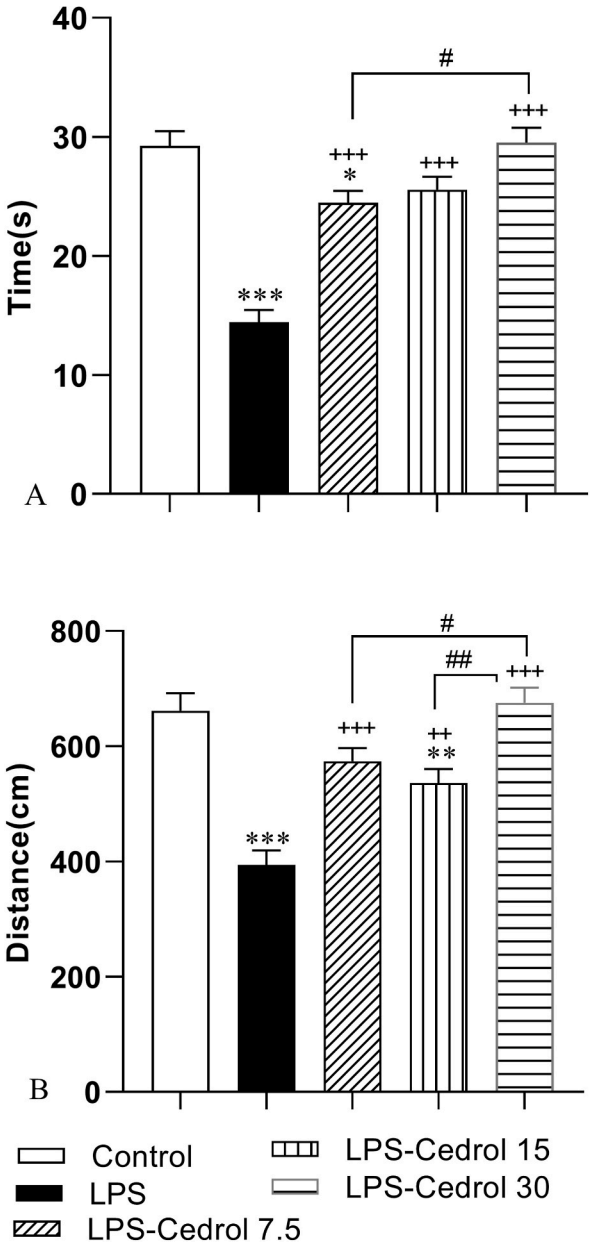

Fig. 3. Cedrol improves memory and learning impairment in lipopolysaccharide (LPS)-treated rats. (A and B) the probe test of the Morris water maze (MWM). (A) Time spent in the target zone. (B) Traveled distance in the target zone. Data are presented as mean ± SEM (n = 10/group). *P < 0.05, **P < 0.01, ***P < 0.001 LPS group compared with Control group. $^{++}$ P < 0.01, $^{+++}$ P < 0.001 Cedrol-LPS groups compared with LPS group.$^{\#}$P < 0.05, $^{\#\#}$P < 0.01 difference among the Cedrol-LPS groups.

5. Results

5.1. Cedrol improves the spatial memory ability of LPS-treated rats in MWM

Fig. 2 (A, B) depicts the detailed findings of the MWM test. The LPS group showed an increase in the escape latency ($P < 0.05$-$P<0.001$) and traveling distance ($P < 0.001$ for all days) during the training phase compared to the control group. However, oral administration of cedrol significantly decreased the escape latency ($P < 0.05$-$P<0.001$) and traveling distance compared with the LPS group ($P < 0.05$-$P<0.001$). There was no significant difference among different groups treated with cedrol.

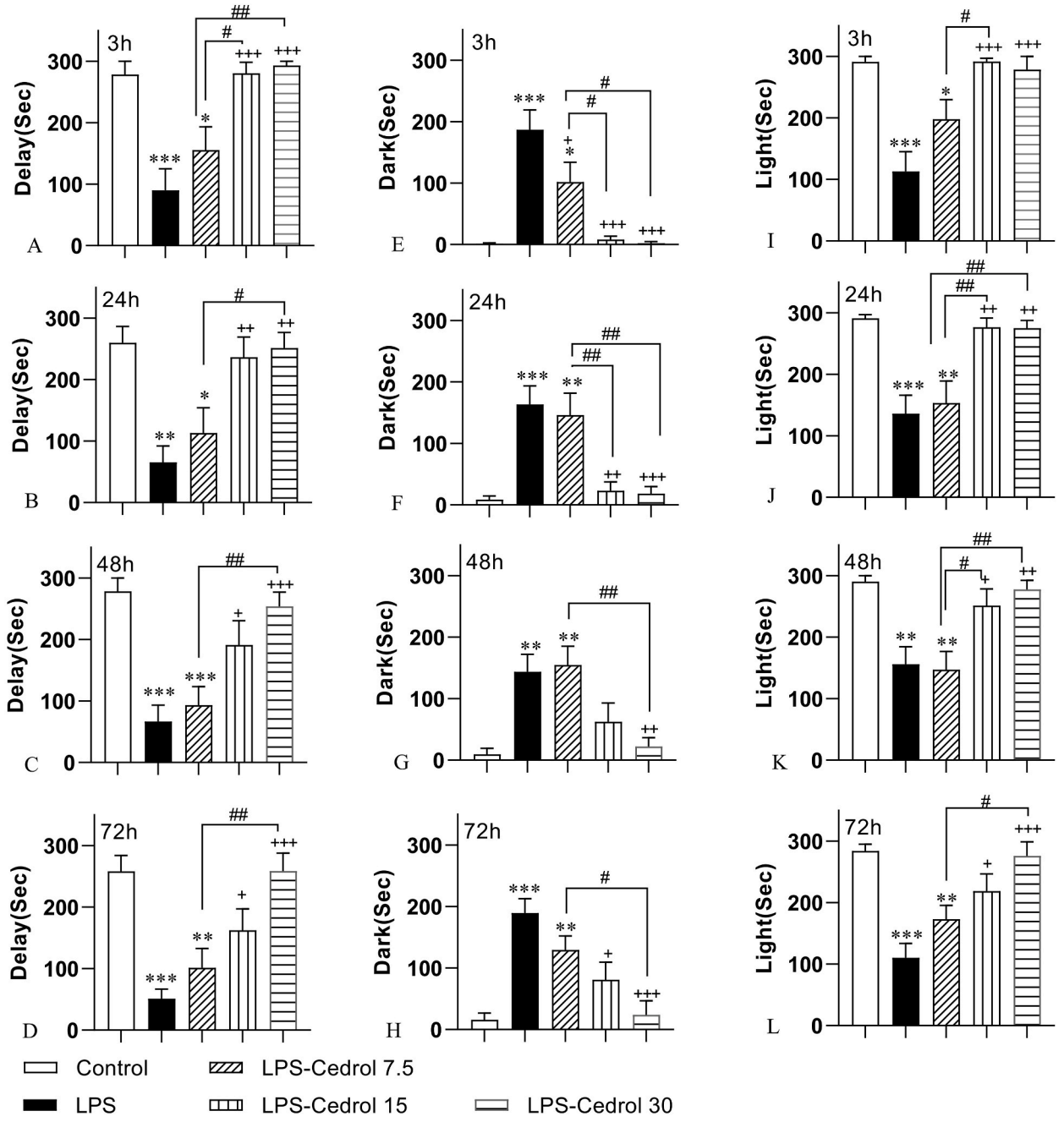

Fig. 4. Cedrol improves memory and learning impairment in lipopolysaccharide (LPS)-treated rats. The passive avoidance (PA) assessment includes delay to enter the dark time (A–D), dark stay time (E–H), and light stay time (I–L). Data are presented as mean ± SEM (n = 10/group). *P < 0.05, **P < 0.01, ***P < 0.001 LPS group compared with Control group. $^{++}$P < 0.01, $^{+++}$P < 0.001 Cedrol-LPS groups compared with LPS group. $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 difference among the Cedrol-LPS groups.

Data analysis of the probe test showed a significant decrease in the time spent and distance traveled by the rats of the LPS group in the target quadrant compared to the control group (Fig. 3 (A, B), $P < 0.001$ for both values). However, treatment with cedrol at all doses remarkably increased both the same values in LPS-Cedrol 7.5 ($P < 0.001$ for both time and distance), LPS-Cedrol 15 ($P < 0.001$ for time and $P < 0.01$ for distance), and LPS-Cedrol 30 ($P < 0.001$ for both time and distance) groups. The results also showed a significant increase in both the time and distance values in the LPS-Cedrol 30 group compared to the LPS-cedrol 7.5 group ($P < 0.05$). A significant increase in the distance value was also found in LPS-Cedrol 30 compared to the LPS-Cedrol 15 and LPS-Cedrol 7.5 groups ($P < 0.01$ and $P < 0.05$). Regarding time spent in the target zone, a significant decrease was found in the LPS-Cedrol 7.5 group compared to the control group (Fig. 3A, $P < 0.05$). Similarly, the distance traveled in the target zone was found to be lower in LPS-Cedrol 15 group than the control group ($P < 0.01$, Fig. 3B).

5.2. Cedrol improves the passive avoidance memory of LPS-treated rats in PA

As shown in Fig. 4 (A-L), the delay in entering the dark in the LPS group was significantly decreased compared with the control group, 3 h, 24 h, 48 h, and 72 h post-shock delivery ($P < 0.001$; $P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively). Compared to the LPS group, treatment with cedrol significantly increased the delay value in LPS-Cedrol 15 and LPS-Cedrol 30 groups, at 3 h ($P < 0.001$ for

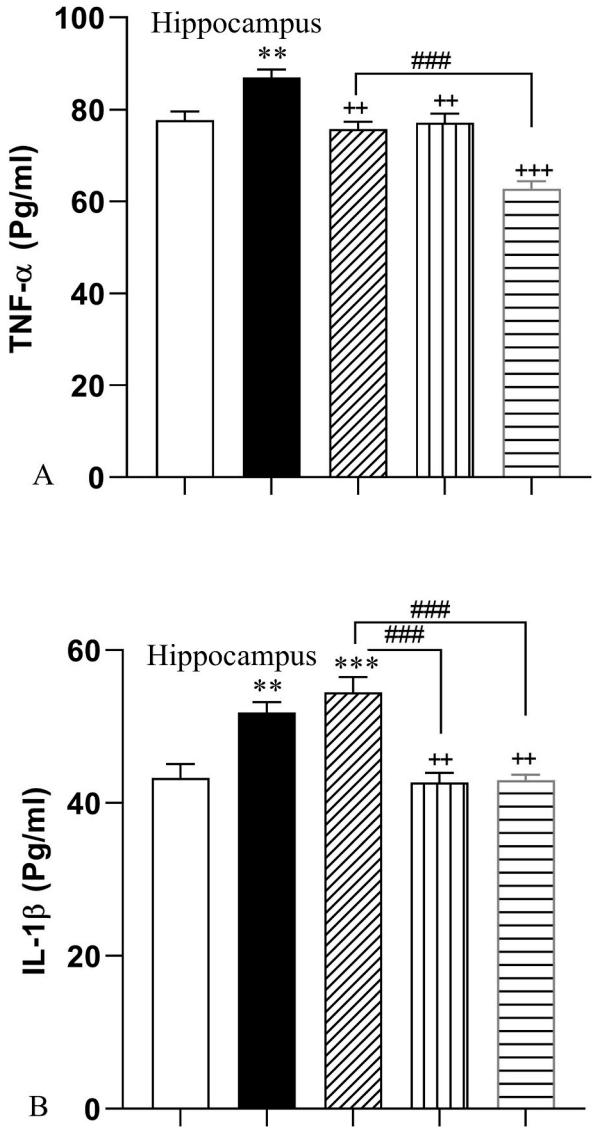

Fig. 5. Cedrol modifies pro-inflammatory cytokine (A and B, tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β, respectively) levels in lipopolysaccharide (LPS)-treated rats. Data are presented as mean ± SEM (n = 10/group). **P < 0.01, ***P < 0.001 LPS group compared with Control group. $^{++}$ P < 0.01, $^{+++}$ P < 0.001 Cedrol-LPS groups compared with LPS group. $^{###}$P < 0.001 difference among the Cedrol-LPS groups.

both groups), 24 h ($P < 0.01$ for both groups), 48 h ($P < 0.05$ and $P < 0.01$, respectively), and 72 h ($P < 0.05$ and $P < 0.001$, respectively) post-shock delivery times (Fig. 4 (A-D)). However, the delay value in LPS-Cedrol 7.5 was still higher than that of the normal control group, at 3 h ($P < 0.05$), 24 h ($P < 0.05$), 48 h ($P < 0.001$), and 72 h ($P < 0.01$) post-shock delivery times. Furthermore, the delay value in the LPS-Cedrol 30 group was significantly higher relative to the LPS-Cedrol 7.5 group at 3 h ($P < 0.01$), 24 h ($P < 0.05$), 48 h ($P < 0.01$), and 72 h ($P < 0.01$) post-shock delivery times. A higher level of the delay value was found in LPS-Cedrol 15 than the LPS-Cedrol 7.5 group, at 3 h ($P < 0.01$) post-shock delivery times.

LPS-treated rats also exhibited a remarkable increase in the time spent in the dark area compared to the normal control group at 3 h, 24 h, 48 h, and 72 h post-shock delivery times (Fig. 4 (E-H), $P < 0.001$, $P < 0.001$, $P < 0.01$, and $P < 0.001$, respectively). However, compared with the LPS group, treatment with cedrol remarkably decreased the dark value in the LPS-Cedrol groups, 3 h ($P < 0.001$ for both LPS-Cedrol 15 and LPS-Cedrol 30 groups), 24 h ($P < 0.01$ and $P < 0.001$ for LPS-Cedrol 15 and LPS-Cedrol 30, respectively), 48 h ($P < 0.01$ only in LPS-Cedrol 30), and 72 h ($P < 0.05$ and $P < 0.01$ for LPS-Cedrol 15 and LPS-Cedrol 30, respectively) post-shock delivery. Notably, the dark time in LPS-Cedrol 7.5 was still higher than that of the control group, 3 h ($P < 0.05$), 24 h ($P < 0.01$), 48 h ($P < 0.01$), and 72 h ($P < 0.01$) post-shock delivery. Furthermore, the dark time in the LPS-Cedrol 30 group was significantly

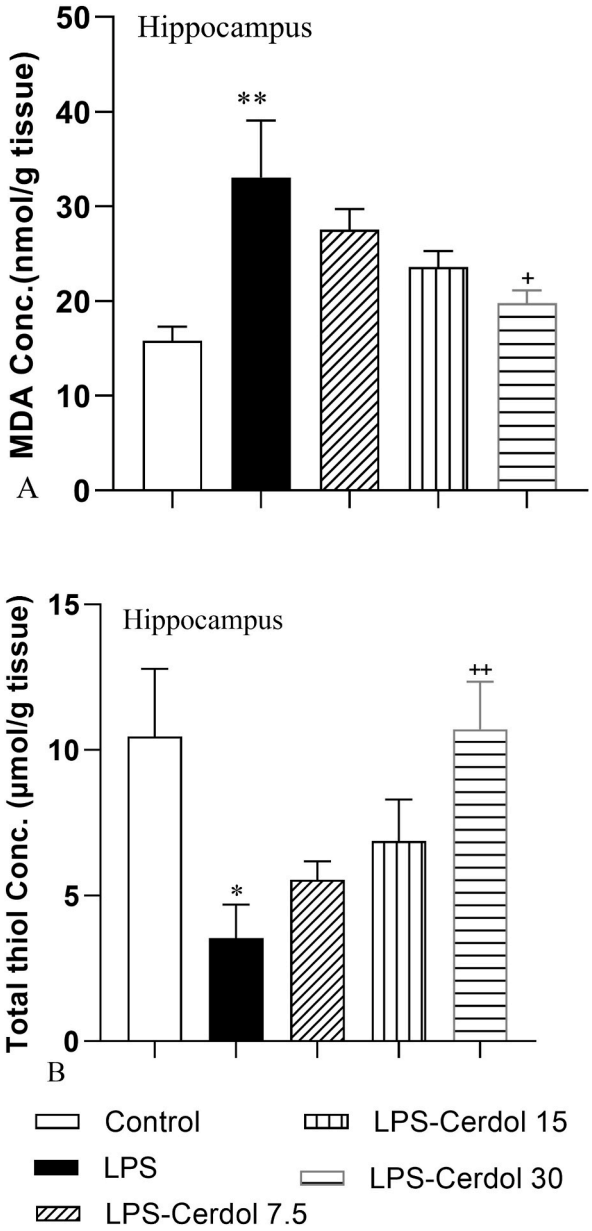

Fig. 6. Cedrol modifies malondialdehyde (A) and thiol content (B) in lipopolysaccharide (LPS)-treated rats. Data are presented as mean ± SEM (n = 10/group). *$P < 0.05$, **$P < 0.01$, LPS group compared with Control group.+$P < 0.05$, $^{++}$ $P < 0.01$ Cedrol-LPS groups compared with LPS group.

lower relative to the LPS-Cedrol 7.5 group, 3 h ($P < 0.05$), 24 h ($P < 0.01$), 48 h ($P < 0.01$), and 72 h ($P < 0.05$) post-shock delivery. Similarly, the dark time in the LPS-Cedrol 15 group was significantly lower relative to the LPS-Cedrol 7.5 group, 3 h ($P < 0.05$) and 24 h ($P < 0.01$) post-shock delivery times.

The results of time spent in the light area are presented in Fig. 4 (I-L). According to the results, LPS-treated rats showed a remarkable decrease in light stay time relative to the control group at 3 h, 24 h, 48 h, and 72 h post-shock delivery times ($P < 0.001$; $P < 0.001$, $P < 0.01$, and $P < 0.001$, respectively). However, compared with the LPS group, treatment with cedrol remarkably increased the light time, at 3 h ($P < 0.001$ for both LPS-Cedrol 15 and LPS-Cedrol 30), 24 h ($P < 0.01$ for LPS-Cedrol 15 and LPS-Cedrol 30, respectively), 48 h ($P < 0.05$ and $P < 0.01$ for LPS-Cedrol 15 and LPS-Cedrol 30, respectively), and 72 h ($P < 0.05$ and $P < 0.001$ for LPS-Cedrol 15 and LPS-Cedrol 30, respectively) post-shock delivery times. Notably, the light time in LPS-Cedrol 7.5 was still lower than that of the control group, at 3 h ($P < 0.05$), 24 h ($P < 0.01$), 48 h ($P < 0.01$), and 72 h ($P < 0.01$) post-shock delivery times. Furthermor, the light time in LPS-Cedrol 30 group was higher relative to the LPS-Cedrol 7.5 group at 24 h ($P < 0.01$), 48 h ($P < 0.01$), and 72 h ($P < 0.05$) post-shock delivery times. Regarding time spent in the light, a higher level was found in LPS-Cedrol 15 group relative to LPS-

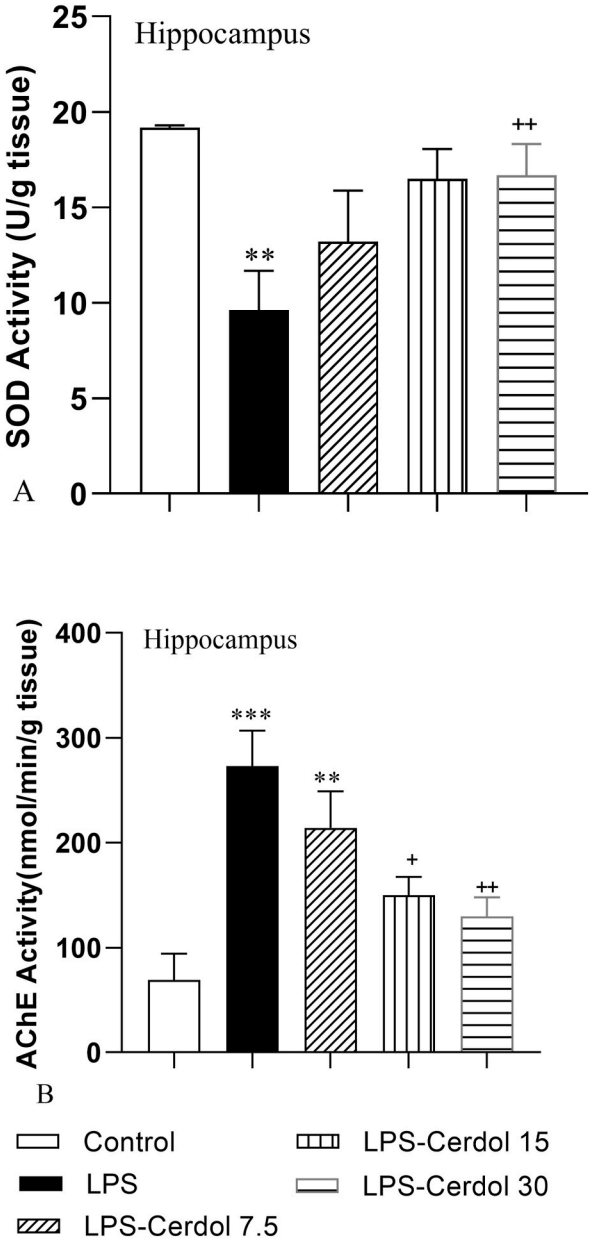

Fig. 7. Cedrol modifies SOD (A) and AChE activity (B) in lipopolysaccharide (LPS)-treated rats. Data are presented as mean ± SEM (n = 10/group). **$P < 0.01$, ***$P < 0.001$ LPS group compared with Control group.+$P < 0.05$, $^{++}$ $P < 0.01$ Cedrol-LPS groups compared with LPS group.

Cedrol 7.5 group, at 3 h ($P < 0.05$), 24 h ($P < 0.01$), and 48h ($P < 0.05$) post-shock delivery times.

6. Biochemical parameters

6.1. Cedrol modulates pro-inflammatory cytokines

As Fig. 5 (A, B) exhibits, the administration of LPS caused a significant increase in the levels of TNF-α and IL-1β when compared with the normal rats ($P < 0.01$ for both). However, treatment with cedrol (at all doses: $P < 0.01$ and $P < 0.001$) significantly reduced TNF-α levels compared to the LPS group (Fig. 5A). Notably, a significant reduction in TNF-α level was observed in LPS-Cedrol 30 compared with the LPS-Cedrol 7.5 group ($P < 0.001$). Comapred to LPS group, treatment with cedrol at 15 and 30 mg/kg significantly reduced IL-1β level ($P < 0.01$ for both). Notably, the level of IL-1β in both the LPS-Cedrol 30 and LPS-Cedrol 15 groups was significantly lower compared to the LPS-Cedrol 7.5 group (Fig. 5B, $P < 0.001$ for both LPS-Cedrol 30 and LPS-Cedrol 15 Vs. LPS-Cedrol 7.5).

6.2. Cedrol restores oxidative stress markers

Brain thiol level of LPS-treated animals was significantly lower than that of the control group (Fig. 6B, $P < 0.05$). In contrast, the brain MDA level of the LPS group ($P < 0.01$) was significantly higher than that of the control group (Fig. 6A). However, treatment with cedrol at 30 mg/kg significantly increased the thiol content while decreasing the MDA level, compared to the LPS group ($P < 0.05$ and $P < 0.01$, respectively; Fig. 6A and B). Fig. 7A demonstrates a significant reduction in brain SOD activity of the LPS group, compared to the control group ($P < 0.01$). However, SOD activity in LPS-Cedrol 30 group was higher than that of the LPS group ($P < 0.01$).

6.3. Cedrol modifies AChE enzyme activity

AChE activity in rat' brain was measured to study the effect of LPS and cedrol administration on cholinergic transmission. The brain AChE activity of the LPS group was significantly higher than that of the control group (Fig. 7B, $P < 0.001$). Treatment with cedrol decreased AChE activity in the LPS-Cedrol 30 and LPS-Cedrol 15 groups compared to the LPS group ($P < 0.05$ and $P < 0.01$, respectively; Fig. 7B).

7. Discussion

The present study demonstrated the neuroprotective role of cedrol against LPS-induced neuroinflammation accompanied by improved cholinergic function. Our results suggest that LPS administration induces neurodegenerative alterations in rat brain, reflected by deficits of learning and memory, increased MDA production, and depletion of the antioxidants.

Neuroinflammation induced by LPS has been reported to be accompanied by learning and memory impairment [10,11,26,32,33]. A higher level of IL-1β, and TNF-α in the hippocampus of the LPS group which was seen in the current study confirmed a neuroinflammation status follwoing LPS injection. Our results also showed that LPS-induced neuroinflammation was accompanied by a cognitive impairment confirmed by the resuls of MWM and PA tests. The results showed that the rats of the LPS group traveled longer times and distances to find the platform during the learning phase of MWM. They also spent a shorter time and traveled a shorter distance in the target area of the probe test impling that they didn't remember the platform' location. According to the results of PA test, the rats of LPS group didn't remember the location of the shock and they showed a short delay in entering the dark at all times after the shock. They also spent a longer time in the dark part and spent a shorter time in the light part of the PA test. These observations are consistent with earlier studies in which neuroinflammation induced by LPS was accompanied by learning and memory impairment [4,6,26,34].

Our results showed that learning and memory impairment induced by neuroinflamamtion was accompanied by AChE overactivity. Interestingly, ACh participates in the cholinergic anti-inflammatory system [35]. The cholinergic anti-inflammatory mechanisms are also supposed to regulate IL-6, IL-1β, and TNF-α [35].

Activation of the inflammatory responses following LPS injection was shown to damage the synapses that contribute to neurodegeneration. These results confirm this assumpsion that cholinergic dysfunction contributes to neuroinflammation-associated cognitive disturbance [36]. The previous studies also demonstrated that the loss of cholinergic neurons in the LPS models is accompanied by impaired behavioral functions [4,6,11].

Further biochemical assessments exhibited that LPS dramatically exacerbated oxidative stress in the brain tissue, evidenced by greater MDA levels and lower levels of thiol and SOD in the hippocampus of LPS group relative to the control group. These data support the previous evidence [27,37,38]. The oxidative injury occurs when levels of peroxides and reactive oxygen species (ROS) exceed endogenous antioxidant defenses [1,2]. Many studies showed a significant increase in ROS levels following LPS administration [3]. ROS damages polyunsaturated fatty acids in the brain tissue. The brain is especially prone to oxidative damage due to its lack of antioxidant defense mechanisms [1]. The pro-inflammatory cytokines was also suggested to promote oxidative stress [9,34,38]. Considering our results, it seems that learning and memory disturbance which was obsereved in the current study is linked to oxidative stress.

This study further suggested that cedrol, dose-dependently prolonged the time spent and distance traveled in the target zone of probe trial. The cedrol-treated rats also spent a shorter time and traveled a shorter distance to find the plaform than LPS group in this test. It also, dose-dependently modified PA parameters including delay, dark, and light stay time. Accordingly, we demonstrated, for

the first time, the cedrol administration, at all doses, protected the animals from LPS-induced behavioral deficits by augmenting PA and spatial memory.

Considering the critical role of AChE in maintaining ACh homeostasis and synaptic neurotransmission [1,2], repression of AChE activity by cedrol suggests its potential effect in the improvement of cognitive dysfunstion seen in the present study.

According to the biochemical data, a higher level of SOD and thiols was observed in the groups treated with the highest dose relative to the LPS group. The highest dose of cedrol also attenuated peroxidation of the lipid membrane, which was evident from lower levels of MDA in the treatment groups. Several studies investigated the free radical scavenging and antioxidant activities of essential oils containing cedrol [14,16,20].

Oxidative stress was suggested to influence cognitive process [1,2,4]. Therefore, amelioration of antioxidant capacity observed in cedrol-treated groups could be another mechanism supporting the neuroprotective and memory-improving effects of cedrol. Further biochemical measurements demonstrated that the cedrol repressed the production of TNF-α and IL-1β in brain tissue. Interestingly, previous studies have shown immunomodulatory and anti-inflammatory effects of cedrol [17,18]. A previous study demonstrated that cedrol inhibited human neutrophil chemotaxis [17]. Cedrol was also found to inhibit the secretion of pro-inflammatory cytokines (IL-1 and TNF-α) and cyclooxygenase in an animal model of arthritis accompanied by improvement of histopathological alterations, bone and cartilage damage [18]. Therefore, the ability of the cedrol to inhibit both pro-inflammatory cytokines could likewise add to its neuroprotective potential. Elevated levels of pro-inflammation mediators (IL-6, TNFα, IL-1β) have been linked to memory loss, and increased risk of dementia [2,6,9,34]. Microglia activation and inflammatory responses facilitate free radical accumulation, ultimately leading to neurodegeneration [5,9]. Hence, evidence suggests that the agents regulating the activity of microglia and pro-inflammatory cytokines may mitigate neuronal injury [9].

It was previously shown that learning and memory impairment induced by LPS is accompanied by neuroinflamamtion, barin tissue oxidative stress, and an increased level of amyloid beta (Aβ) in the hippocampus [32,33,39]. Protection against Aβ might be also suggested as an explation for learning and memory-improving effects of cedrol seen in the present study. However, more precise studies are required to support this idea.

In conclusion, the results of this study suggested that cedrol exerts its effects by facilitating cholinergic transmission and quenching oxidative stress, which in turn attenuates neuroinflammation-associated neurodegeneration in the brain, and thus preserves memory and learning function. Hence, cedrol may be employed as a natural agent in the prevention and management of inflammation-mediated neurodegenerative diseases.

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Data availability statement

The authors confirm that the data supporting the findings of the present study are available within the article [and/or] its supplementary materials.

CRediT authorship contribution statement

Faezeh Dabouri Farimani: Data curation. **Mahmoud Hosseini:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Sabiheh Amirahmadi:** Data curation. **Mahsan Akbarian:** Data curation. **Matin Shirazinia:** Data curation. **Moselm Barabady:** Data curation. **Arezoo Rajabian:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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