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Original Article

The Effects of Aqueous Extract of *Boswellia Serrata* on Memory Impairment Induced by Lipopolysaccharide

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ABSTRACT

Background and Aims: The therapeutic effects of the olibanum, the resin of *Boswellia serrata (B. serrata*) from the Burseraceae family in inflammatory disease have been reported. There are more than 200 active ingredients in this resin, including Boswellic acid. It is proposed that aqueous extract of *B. serrata* can improve memory impairment induced by cerebral inflammation result in the administration of lipopolysaccharide (LPS).

Materials and Methods: In this study, after the treatment of rats with LPS, brain toxicity induction was performed, and finally, the behavioral tests were evaluated. Following cerebral inflammation induction and treatment, behavioral performance biochemistry tests and molecular methods were assessed in all groups.

Results: LPS administration increased the duration and distance to find the platform in the Morris water maze test compared to the control group in 5 days ($p<0.05$ to $p<0.001$). Furthermore, LPS reduced the peripheral, central, and total locomotion compared to the control group ($p< 0.001$) in the open field test. Pretreatment with both doses of aqueous extract of *B. serrata* enhanced performances of the rats in Morris water maze ($p<0.05$ to $p<0.01$) and open field test ($p<0.01$ to $p<0.001$). LPS also increased hippocampus Interleukin-6, malondialdehyde levels ($p<0.001$).

Conclusions: Aqueous extract of *B. serrata* can be a useful drug in memory impairment caused by LPS-induced inflammation.

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Introduction

Boswellia serrata (*B. serrata*), commonly known as frankincense or olibanum-tree, is a tree in the *Burseraceae* family [1]. They are native to Arab countries and India. This plant has long been noticed as an herbal compound with a beneficial role for the treatment of inflammatory diseases such as arthritis, chronic colitis, as well as healing of wounds and improvement of the female endocrine system (the study of the co-administration of *B. serrata* and Dracocephalum on the elderly memory) [2, 3]. The anti-inflammatory effects of olibanum are attributed to terpenoid acids, particularly *B. serrata*, and other terrenes derivatives [4, 5].

The extensive spread experiments conducted to investigate *B. serrata*'s anti-inflammatory mechanism have demonstrated that they are selective inhibitors of 5-lipoxygenase, preventing leukotriene synthesis [6, 7]. Also, another inhibitory effect of *B. serrata* has been observed for the biosynthesis of glycosaminoglycan. Some evidence obtained from animal studies indicates the advantageous effects of *B. serrata* on memory function [8, 9].

According to findings, *B. serrata* can play a positive role in brain development, formation of axons and dendrites, and better neuronal communications. Lipopoly-saccharide (LPS) is a gram-negative bacteria-derived endotoxin, which induces the production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), Interleukin (IL)-1 beta, and IL-6 followed by impairment in synaptic plasticity, learning process, and memory [10,11]. Various researches indicated that *B. serrata* reduced anxiety symptoms. Besides, *B. serrata* can reduce the levels of inflammatory cytokines through the effect of the nuclear factor kappa enhancer binding protein (NF-κB) pathway that led to inhibition of hyperactivity and anxiety [12, 13].

Similarly, Sayed et al. indicated that frankincense has an anti-inflammatory effect. Using it triggered to diminish the level of IL-6. Also, in a behavioral test, an open arm's presence in an elevated plus-maze increased [14]. The present study was aimed to investigate the effects of aqueous extract of *B. serrata* on LPS-induced memory impairment.

Materials and Methods

Animals and drugs

In this experiment, 60 male Wistar rats weighing between 200 and 250 g were prepared. Animals were kept under controlled situations, including temperature at 22±2˚C and lighting conditions with 12-h light: dark cycle. Additional food and water were available for each rat [15]. All experiments were admired by the Research Committee of Nourdanesh Institute of Higher Education, Meymeh, Iran. The oleo-gum resin of *B. serrata* was taken, and then 100 g of powder was added to 400 ml of ethyl acetate. Subsequently, it was shacked for 48 hours until the particles were completely dissolved. After filtration, we used the rotary equipment to remove the solvent. The residues were then maintained at 20 °C until use. The percent yield of the procedure was about 30% [15].

Groups and treatments

In this research, animal were divided into 6 groups (n=10). Group 1: control group saline –

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diluted Dimethyl sulfoxide (1mg/kg); group 2: LPS (1mg/kg) negative control group; group 3: LPS (1mg/kg)+aqueous extract (0.5 mg/kg); group 4: LPS (1mg/kg)+aqueous extract (1 mg/kg); group 5: aqueous extract (5 mg/kg) and group 6: Vitamin E 5 mg/kg+LPS (1mg/kg) were treated groups. In relation to conducting behavioral tests, the day after the injection the rats were subjected to behavioral tests such as Morris water maze (MWM) test, open-field and shuttle box one day after the injection.

Behavioral study

MWM apparatus and procedures

MWM test is suitable for the analysis of the spatial memory and learning of rats. A circular pot carried out the test with a diameter of 136 cm and a height of 30 cm, which is supposedly divided into four quadrants, north, south, right, and left [16]. At the center of the Northwest quadrant, a platform with a height of 28 cm and diameter of 10 cm is placed and the pot reaches a height of 1.5 cm above the surface of the platform with water with temperature of 23-25˚C. MWM testing took five days according to protocols [17].

Open-field test

This test is designed to test in vitro spatial memory in the rat. In this test, the animal is placed in a box environment without causing pleasant or unpleasant behavior. This box structurally consists of white wood, had a floor of 100×100 cm divided by red lines into 25 equal units of 20×20 cm and 50 cm high. A camera is mounted on top of the box to monitor the animal's behavior closely. According to test protocols, the animal's behavior is examined for its presence in different areas of the box (in the middle or around) according to test protocols [18,19].

Biochemical assessment

After learning and memory tests, animals were sacrificed, and the hippocampus tissues were dissected and kept at -80˚C for biochemical evaluations. The hippocampus samples were then homogenized in volumes of 9 g/L ice-cold normal saline (1:9 w/v). The supernatant was collected after centrifugation homogenates at 4000 rpm/min for 10 min at 40˚C. The supernatants were used for the evaluation of activities of malondialdehyde (MDA), glutathione (GSH), using a spectrophotometer (Jenway 6105 UV/Vis, UK); following the protocols provided with the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, PR China). IL-6 levels were measured using a specific protocol for rats inside the kit (bioscience Co., San Diego, CA, USA) [20].

Histopathological study

At the end of the injection period and behavioral tests, the animals were killed, and the brains were maintained in 10% formalin. After five days of formalin storage, cannulated sections were examined for hemotoxin eosin and toluidine blue staining. The samples were fixed with ethanol and dried with xylene after leaving formalin. Finally, the samples were embedded in paraffin for tissue sections and staining. The paraffin blocks were cut from1–3 mm posterior from bregma by microtome (Leica Biosystems, Milan, Italy). Different sections of each brain sample were prepared at 2 µm intervals and prepared for staining with hemotoxin-eosin and toluidine blue. Optical microscopy (40 x) was used for microscopic examination (Olympus BX51, Japan). Images were captured digitally from different hippocampus subfields, including CA1 of both hemispheres [21].

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Western blotting

For western blot analysis, the hippocampus was dissected from the brains of the rats. Tissues were homogenized at 4˚C in the lysis buffer. Then, lysates were sonicated on ice using a probe sonicator (UP100H, Germany). After centrifugation at 10000 g for 10 min at 4˚C, supernatants were collected and transferred to clean microtubes, and the protein concentrations were determined using a Bio-Rad protein assay kit.

All protocols were performed according to the reference and kit [22]. The primary antibodies were polyclonal BAX (Cell signaling, cat# 2772), monoclonal BCL2 (Cell signaling, cat# 2870), monoclonal Caspase 3-cleaved (Cell signaling, cat#9664), monoclonal Caspase 9-cleaved (Abcam, cat#7237) were considered to be involved in cell death and also apoptosis pathway [23, 24].

Statistical analysis

The time and distance data in MWM in five days were compared using repeated-measures analysis of variance (ANOVA) with Tukey's post-hoc test. The biochemical analysis data, probe day data, and shuttle box were reported by one-way ANOVA followed by Tukey's post-test. The differences level among groups were considered statistically significant when $p<0.05$. All data were presented as means±standard error of the mean (SEM).

Results

MWM results

LPS administration for five days increased elapsed time and traveled path to find the platform compared to the control group ($p<0.05$ to $p<0.001$). The time and distance mentioned were significantly reduced after the injection of aqueous extract of *B. serrata* 0.5 mg/kg and 1 mg/kg compared to the LPS group ($p<0.01$ to $p<0.001$) (Fig. 1 and 2). After the removal of the platform on probe day, the animals in the LPS-receiving group also spent less time and distance in the target quadrant ($p<0.001$), whereas the results in the *B. serrata* groups were opposite ($P<0.001$) (Fig. 3).

Open-field test results

On the first day, LPS alone or in combination with aqueous extract of *B. serrata* did not show a significant effect on total locomotion (Fig. 3A), and on the last day, LPS (1 mg/kg) reduced the peripheral, central and total locomotion's compared to control group ($p<0.001$). Aqueous extract of *B. serrata* (0.5 mg/kg and 1 mg/kg) and Vitamin E plus LPS significantly increased the peripheral and total locomotion ($p<0.001$) (Fig. 3B). Also, treatment with *B. serrata* (1 mg/kg) significantly increased central locomotion compared to melatonin treated rats ($p<0.001$).

Effect of *B. serrata* on lipid peroxidation

As shown in Fig. 4A, exposure to LPS significantly increased the MDA level compared to control ($p<0.001$). Treatment with *B. serrata* (0.5 and 1 mg/kg) and vitamin E reduced MDA content ($p<0.001$). In the Och treated group, GSH content was decreased in the hippocampus ($p<0.001$) (Fig. 4B). Treatment with *B. serrata* (0.5 and 1 mg/kg) and vitamin E significantly increased GSH content compared to LPS treated rats ($p<0.001$).

Effect of *B. serrata* on inflammatory markers

The role of inflammation in the pathogenesis of certain diseases, such as memory loss, has been

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documented. The results showed that the IL-6 level was increased in the hippocampus of LPS (1 mg/kg) treated rats compared to the control (P<0.001). As shown in Fig. 5, it was indicated that *B. serrata* (1 mg/kg) significantly decreased IL-6 level compared to the LPS group (p<0.001).

Effect of *B. serrata* on apoptotic factors (Bax/Bcl-2, Caspase 3 and Caspase 9)

As indicated in Fig. 6, protein expression of Bax/Bcl2 was increased in the LPS group (p<0.001). Besides, the protein levels of cleaved caspases 3 and 9 were up-regulated by LPS. Co-treatment of LPS with *B. serrata* (1 mg/kg) or vitamin E significantly decreased the ratio of Bax/Bcl2 (p<0.001 and p<0.001, respectively). Furthermore, *B. serrata* (0.5 and 1 mg/kg) or vitamin E plus LPS inhibited the activation of caspases 3 and 9 (Fig. 7A, 7B).

Histology

LPS (1 mg/kg) reduced the number of degenerating neurons in the CA1 subfields (Fig. 8), compared to the control group. Also, *B. serrata* (0.5 and 1 mg/kg) increased the number of positive neurons in the CA1 subfields, p<0.01, and p<0.001) in comparison with the control group.

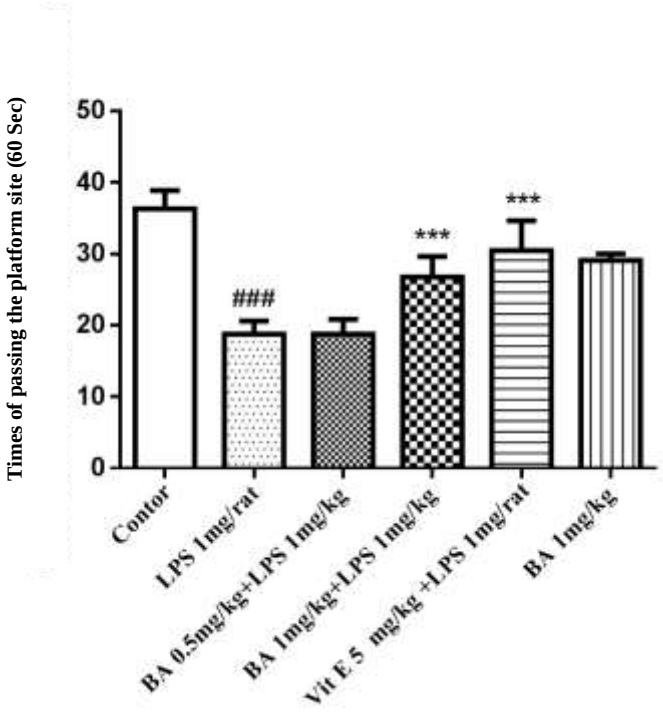

Fig. 1. Effect of lipopolysaccharide (LPS) and *B. serrata* (BA) on time spent in the target quadrant in the probe test of Morris water maze on day 14. Data are presented as mean±SEM. ###P < 0.001 compared to control, ***P< 0.001compared to LPS group.

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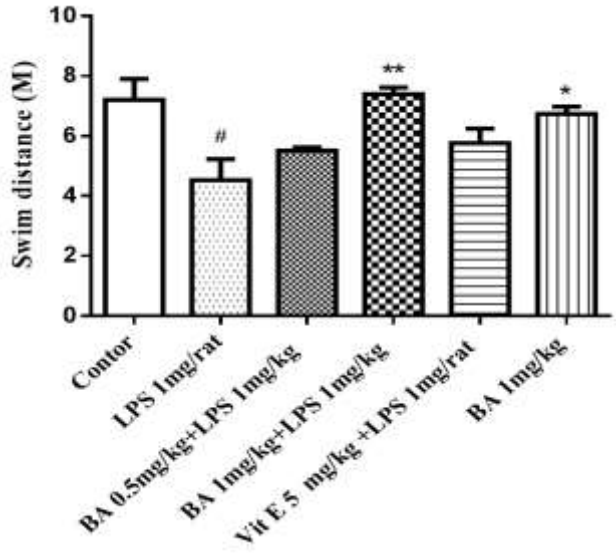

Fig. 2. Effect of lipopolysaccharide (LPS) and *B. serrata* (BA) on swim distance in the target quadrant in the probe test of Morris water maze on day 14. Data are presented as mean ± SEM. #P< 0.05 compared to control, **P< 0.01 and *P< 0.05 compared to LPS group.

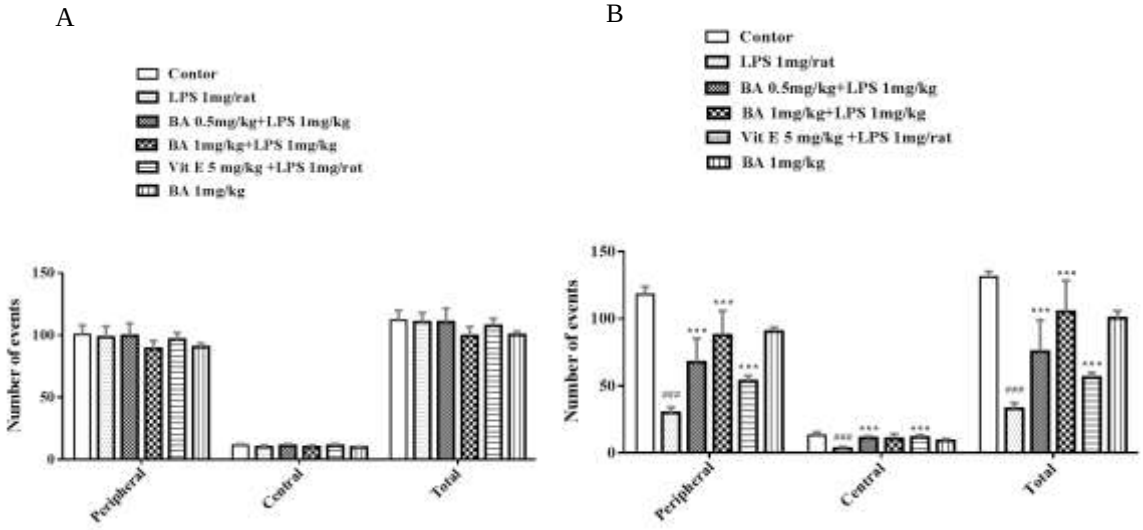

Fig. 3. Effect lipopolysaccharide (LPS) and *B. serrata* (BA) (0.5 and 1mg/kg) and Vit E on total locomotion, central locomotion, and peripheral locomotion in the open-field test at the first day of the experiment (A) and the end of experiment (B). Data are presented as mean±SEM. ***P<0.001 compared to control and ###P<0.001 compared to LPS group.

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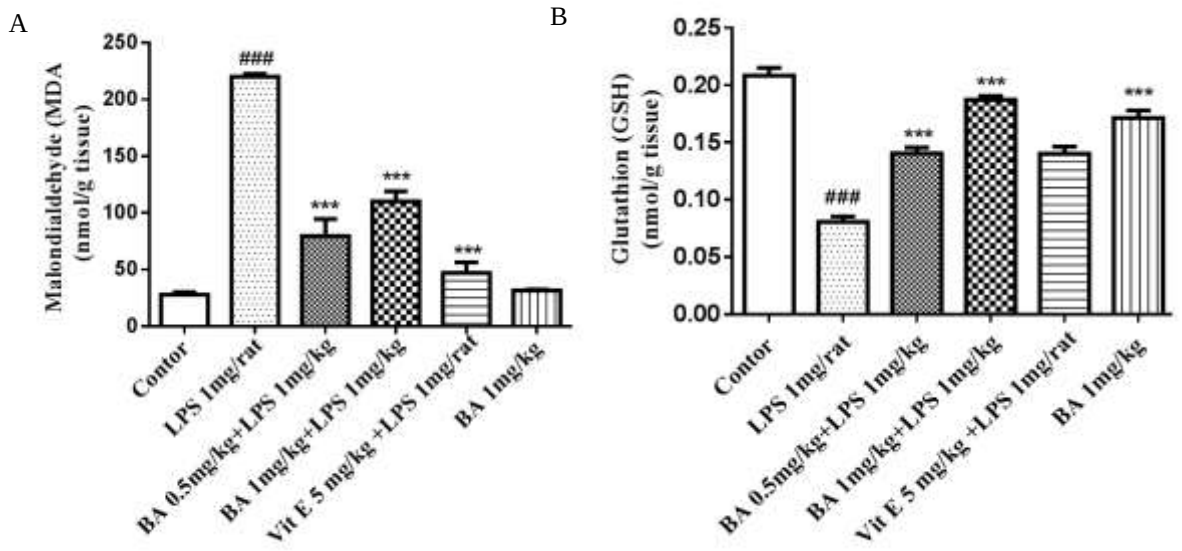

Fig. 4. Effect of *B. serrata* (BA) on lipid peroxidation (A) and glutathione content (B) in the hippocampus following exposure to lipopolysaccharide (LPS). Data are expressed as mean±SEM. ###P< 0.001 compared to control, ***P< 0.001 compared to BA group (A). ###P< 0.001 compared to control and ***P< 0.001 compared to LPS group.

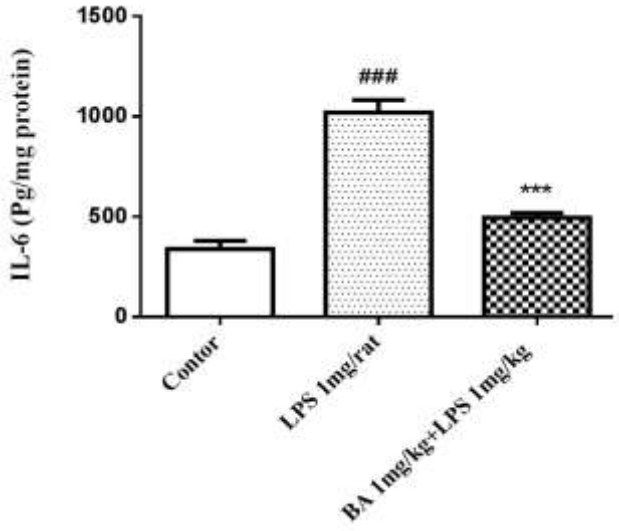

Fig. 5. Effect of *B. serrata* (BA) (0.5 and 1mg/kg) in the Interlukine (IL)-6 hippocampus. Data are presented as mean ±SEM. ###P < 0.001 compared to control and ***P< 0.001 compared to lipopolysaccharide (LPS) group.

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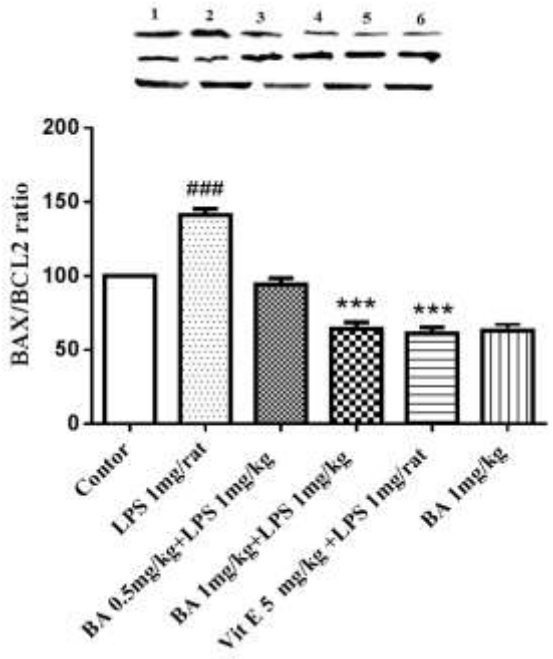

Fig. 6. Effects of *B. serrata* (BA) (0.5, 1 mg/kg) and lipopolysaccharide (LPS) has given alone or concurrently on apoptotic factors in the rats' hippocampus through western blotting analysis. The blots and the bars exhibit the densitometry analysis of western blots for the Bax/Bcl-2 ratio. ###P< 0.001 vs. control, ***P< 0.001 vs. LPS-administered rats.

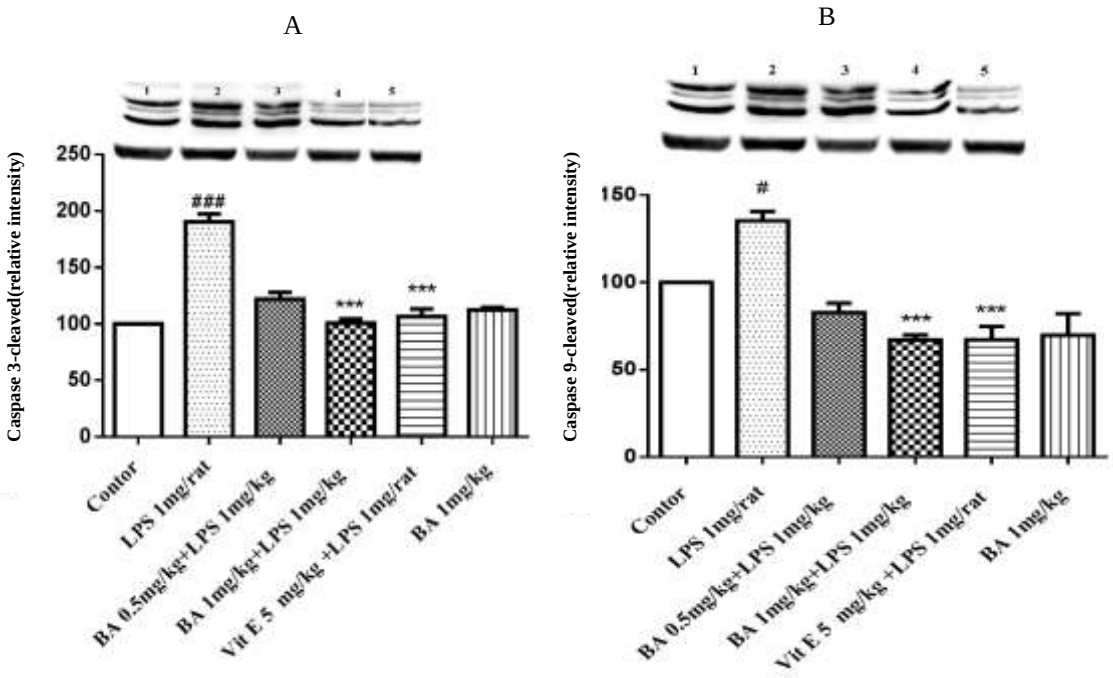

Fig. 7. Effects of *B. serrata* (BA) (0.5, 1 mg/kg) and lipopolysaccharide (LPS) has given alone or concurrently on apoptotic factors in the rats' hippocampus through western blotting analysis. The blots exhibit the densitometry analysis of western blots for caspase 3 (A) and caspase 9 (B) proteins, consecutively. #P< 0.05 and ###P< 0.001 vs. control, ***P< 0.001 vs. LPS-administered rats.

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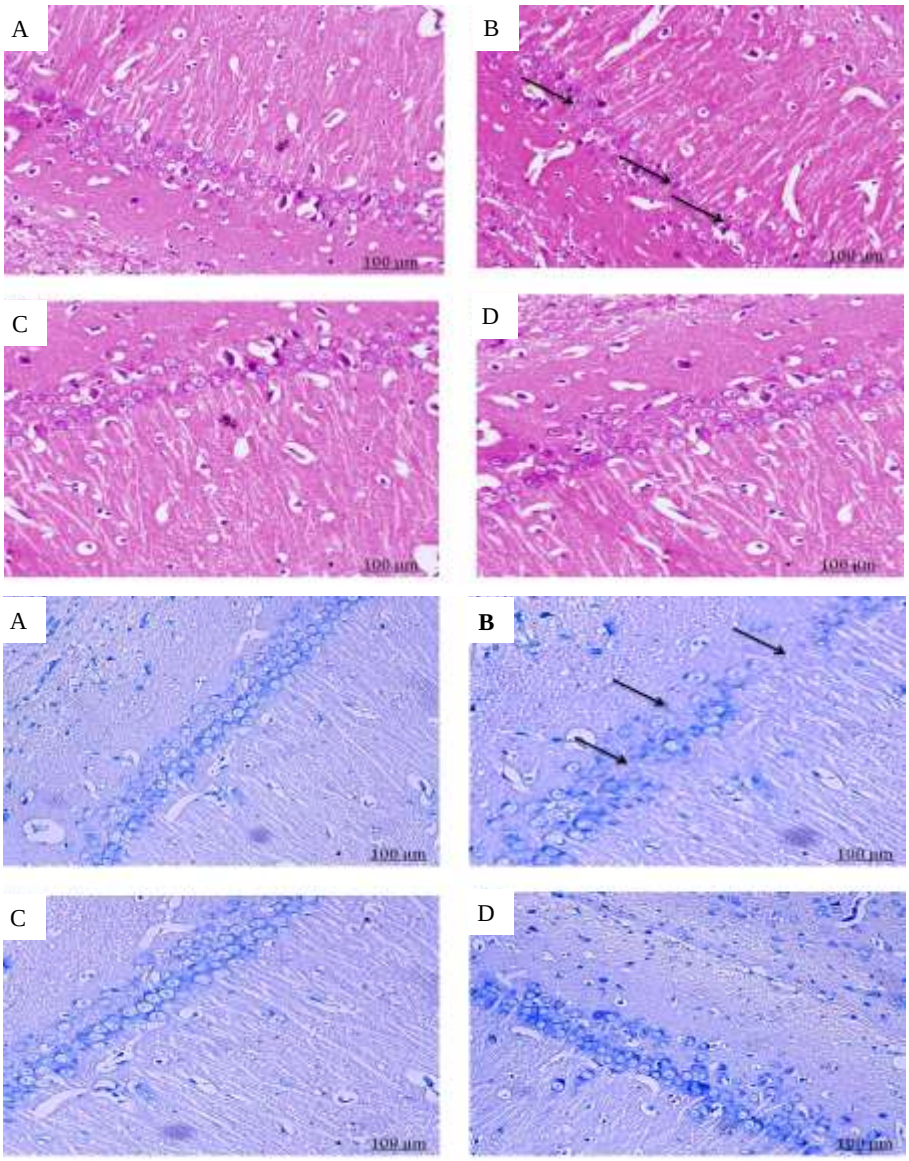

Fig. 8. Photomicrographs of Hemotoxin Eosin and Toluidine blue staining in the rat hippocampus. *B. serrata* decreased the degenerating neurons in the CA1 subfield of the hippocampus A: Cotrol group, B: lipopolysaccharide (LPS) (1 mg/kg) group, C: LPS+*B. serrata* (1 mg/kg) group, and D: LPS+Vitamin E (5 mg/kg) group. Arrowheads point to representative degenerating neurons—scale bars: 100 µm.

Discussion

The results of current research showed that, *B. serrata* (0.5 and 1 mg/kg) improve learning and memory impairment induced by LPS. According to previous studies, LPS as a cell wall of gram negative bacteria lead to learning and memory impairment through induction of neuroinflammation. A possible mechanism of LPS that suggested by our data that are coordinate with previous studies were an increase in inflammatory (IL-6) and oxidative stress (MDA) factors. As a result of such changes, LPS lead to learning and memory loss in behavioral tests.

B. serrata is a major component of olibanum which is a resin of *B. serrata* which is an indigenous Arab plant, East African and Indian,

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used for therapeutic purposes. Long-standing studies have suggested that olibanum has been used as a useful compound in treating neuroinflammation disease. These anti-inflammatory properties are linked to inhibition of the lipoxygenase, a key enzyme in leukotriene synthesis. Clinical therapeutic effects of frankincense on the treatment of stroke and decreasing edema have also been mentioned in previous studies. Administration of 500 mg/kg of frankincense in capsule form to patients with stroke every 6 hours for one-month results in restoring muscle strength in the left limbs [25]. Daily administration of 100 mg frankincense extract in lactating rats improved hippocampal function through increased dendritic fractions and increased hippocampal neuronal cell volume in neonates [26]. Dosage of 50 mg for 21 to 42 days consistently improved memory impairment due to streptozotocin injection. As delay time to enter to dark compartment increased and frequency of entry into the dark chamber decreased [27].

Based on Hosseini-Sharifabad et al., oral administration of olibanum due to the presence of boswellic acid at a daily dose of 100 mg/kg for four weeks to 24-month-old rats resulted in augmented growth of dendritic branches, increased volume of the hippocampal pyramidal layer, and reduce the speed of changes to dendrite win analysis. In their study, it was found that the resin of *B.serrata* improved spatial memory performance in the water maze test, which was attributed to the protective effect of boswellic acid against oxidative stress in the brain [28]. As a result of our finding, boswellic acid has beneficial effects on learning and memory loss induced by LPS in rats because this useful material of olibanum decrease delay time and distance to reach the escape platform in MWM. As well as in probe day, the rats spent more time and distance in the goal quarter. According to the data obtained from the plan, we propose that boswellic acid by blocking the production of inflammatory cytokines such as IL-6, as well as by strengthening the brain's antioxidant system with increase the levels of superoxide dismutase, catalase, and total thiol groups. Also, boswellic acid weakened the oxidative system and prevented toxic metabolites like nitric oxide metabolite. This effective constituent of olibanum leads to increasing the level of factors affecting neurogenesis leading to improved harmful effects of LPS administration.

In the study of Ebrahimpour et al., *B. serrata* [40, 80 and 160 mg/kg, i.p.) improve learning and memory in water maze task in trimethyltin-induced neuronal toxicity model through inhibition of acetylcholinesterase, increased glutathione levels, reduced malondialdehyde levels in the cerebral cortex [29]. Besides, boswellic acid can reduce apoptosis of hippocampus memory-related cells by blocking the production of free radicals [28]. After memory impairment induced by scopolamine, *B.serrata* extract due to its resin's main composition, boswellic acid leads to suppressing the generation of leukotrienes and inflammatory cytokines, inhibition of 5-lipooxygenase activity and reduction of prostaglandin E2 formation and cerebral edema discount [9].

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In parkinsonian rats, boswellic acid delays the processes associated with aging of the brain with inhibition of expression of inflammatory cytokines like IL-6, IL-1, and TNF-α. Also, boswellic acid suppresses inducible nitric oxide synthesizes, and COX2 acts as a neuroprotection factor for improving motor dysfunction in Parkinson's disease [30]. Sayed and collogues showed that administration of 3-acetyl-11-keto-β-boswellic acid (AKBA) 5 mg/kg for seven days showed anti-apoptotic, and anti-amyloidogenic effects in LPS-injected mice. Evidence suggests that the mechanism of the effect of AKBA is through decreased expression of brain-related genes like phosphorylated inhibitory protein for NF-κB, IκB-α (P-IκB-α), inflammatory microRNA-155, and reduced oxidative stress content as carbonyl protein content. In addition, AKBA caused an increase in the SOCS-1 expression level. This experiment also showed that AKBA could decrease apoptosis and amyloidogenesis and act as a therapeutic drug for relieving the symptoms of the neuroinflammatory disorder like Alzheimer [14].

Conclusion

The current study proposed that spatial learning and memory enhanced during the administration of two doses of *B. serrata* (5 and 10 mg/kg) in the LPS-treatment groups. This finding is in agreement with the results reported by the researcher. Results were reported by the researcher that high antioxidant agents.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors didn't declare any acknowledgments.

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