Neuroprotective and memory enhancing effects of Zataria multiflora in lipopolysaccharide-treated rats

Zohreh Arab¹, Mahmoud Hosseini¹,², Narges Marefati³, Farimah Beheshti⁴,⁵, Akbar Anaegoudari⁶, Hamid Reza Sadeghnia⁷, Mohammad Hossein Boskabady⁸

¹Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; ²Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; ³Department of Physiology and Medical Physics, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran; ⁴Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran; ⁵Department of Physiology, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran; ⁶Department of Physiology, School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran; ⁷Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran; ⁸Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

The study was aimed to evaluate the effects of hydro-ethanol extract Zataria multiflora on the brain tissue oxidative damage, and hippocampal interleukin-6 (IL-6) as well as learning and memory capacity in Lipopolysaccharide (LPS) - challenged rats. The rats were randomized into five groups as follow: Control group: Rats were treated with saline, LPS group: Rats were treated with LPS 1.00 mg kg⁻¹, ZM50, ZM100 and ZM200 groups in which the rats were treated with Z. multiflora extract (50.00, 100 or 200 mg kg⁻¹ per day, respectively). The treatments including extract or vehicle were administered intraperitoneally and given three days before the behavioral tests and were continued within a6-day behavioral experiment. Injection of LPS was daily done before the behavioral tests. Finally, the brains were collected for biochemical evaluations. Although LPS administration prolonged the latency in Morris water maze and shortened the latency to enter the dark chamber in passive avoidance test, ZM extract restored these changes to approach control group values. Also, LPS increased IL-6, malondialdehyde (MDA) and nitric oxide (NO) metabolites levels and lowered thiol, superoxide dismutase (SOD) and catalase (CAT) levels in the brain, however, Z. multiflora extract reduced IL-6, MDA and NO metabolites concentrations, but increased thiol content, SOD, and CAT levels. The results of this study showed that Z. multiflora ameliorated learning and memory dysfunction in LPS - challenged rats by alleviating of inflammatory responses and brain tissue oxidative damage.

© 2022 Urmia University. All rights reserved.

Introduction

Many people suffer from different levels of learning and memory impairments. As previously reported, inflammatory cytokines and oxidative stress markedly contribute to learning and memory deficits.¹ Scientific evidence indicates that the immune system plays an important role in adjusting brain functions such as learning and memory.² Environmental and psychological stimulators activate the immune system to promote memory consolidation under normal conditions. Inflammation provokes the secretion of different cytokines such as interleukin-6 (IL-6) which is an important signaling molecule in the central nervous system and exerts detrimental effects on learning and memory.³ In this context, increased plasma levels of IL-6 correlate with cognitive failure. It is reported that systemic administration of lipopolysaccharide (LPS) induces neuro-inflammation in the brain by releasing cytokines such as IL-6 from immune cells leading to impairment of learning and memory functions.²,⁵ The LPS, as a chemical compound extracted from Gram-negative bacteria, induces some inflammatory responses within the brain of rodents by regulating pro-inflammatory cytokines.⁵ Moreover, LPS increases the production of nitric oxide (NO) by enhancing the release of pro-inflammatory cytokines from macrophages and leucocytes. The NO is a small gaseous molecule

¹Correspondence:
Mahmoud Hosseini, PhD
Division of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
E-mail: hosseinim@mums.ac.ir

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.
Materials and Methods

Animals and grouping. Adult male Wistar rats (8-10 weeks old and 250 ± 10.00 g) were provided by laboratory animal center and housed under standard conditions of temperature, light and food. The study was done according to the ethics, principles, and guidelines approved by the Committee of Animal Research of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.fm.REC.1397.139). The rats were randomly divided into the following five groups (n = 10 in each group): (1) Control group in which the animals were treated by vehicle (2) LPS-treated group in which the animals received 1.00 mg kg⁻¹ LPS; (3) ZM50 group in which the animals were treated with 50.00 mg kg⁻¹ of Z. multiflora extract once a day; (4) ZM100 in which the animals received 100 mg kg⁻¹ of Z. multiflora extract (5) ZM200 in which the animals were treated with 200 mg kg⁻¹ Z. multiflora extract. The extract was dissolved in saline diluted tween (final concentration 0.20%) once a day. The LPS (E. coli 055:BS) was prepared from Sigma (St. Louis, USA) and dissolved in saline and intraperitoneally injected. In LPS and ZM groups, LPS was daily injected 2 hr before the behavioral tests within six days of the experiments. In ZM groups, daily treatment by ZM extract (50.00 ,100 and 200 mg kg⁻¹) was done during three days before starting the behavioral tests and was continued to be daily administered intra-peritoneally 30 min before each LPS injection within six days of the behavioral experiments. In LPS group, the rats were administered with 1.00 mL kg⁻¹ of saline diluted tween (final concentration 0.20%) instead of ZM extract.

Preparation of the extract. The plant was purchased from a local market and an identification number was allocated by herbarium of Ferdowsi University of Mashhad (No: 35314). The plant was dried and then grounded by a mortar. To prepare the extract, 100 g of the powders of the plant was mixed with 875 mL of 70.00% ethanol. The mixture was located on a shaker at room temperature. After 72 hr, the mixture was filtered using a filter paper. A reduced pressure method was used to remove the solvent. The extract was then dried. The yielded extract was 33.20 g which was kept in a refrigerator until it use. To prepare the appropriate doses of the extract, the extract was dissolved in a saline diluted tween solution.

Morris water maze (MWM) test. To study the spatial memory in rats, the MWM apparatus was used. The apparatus was a pool (1.36 m diameter and 0.60 m depth) which was filled with water (23.00 - 25.00 °C) up to 0.30 m. It was partitioned into four quadrants (i.e. north, east, south and west). A circular-shaped platform was placed below the surface of water exactly in the center of the southeast quadrant. Some visual signs, used as cues for animals’ navigation, were attached on the wall of the laboratory room. On the test day, the rats were randomly unleashed on the surface of water in one of the four quadrants. Then, the rats were permitted to search for the hidden platform within 60 sec. The parameters recorded by camera (time latency to find the platform and the length of the swimming path) were computed by a video tracking system (Radian, Tehran, Iran). The animals found the platform in 60 sec were permitted to stay on it for 20 sec. The rats failed to find the platform were conducted to the platform by the experimenter. The tests were repeated on a daily basis for five consecutive days. On day six (i.e. probe day), the platform was removed and the animals were allowed to swim for 60 sec. The time spent as well as the distance travelled in the target quadrant was measured.

Passive avoidance test. To evaluate negative reinforcement, we used passive avoidance apparatus (Noavarane Sanaie Amoozeshi, Mashhad, Iran). The apparatus consisted of two chambers (light and dark rooms) separated by a gate. The floor of the dark room was comprised of an electrifiable grid. A set of infrared photocells were used to record the activity of the rats automatically. On the first and second days, the rats were accustomed to the apparatus for 5 min once a day. On the
acquisition day, the animals were put in light chamber and the gate was opened. The gate was closed when the animals were entered the dark room. Then, a punishing electric shock (50.00 Hz, 2 sec, 2.00 mA) was given to the animals legs through the chamber floor. To assess the retention, the latency to enter the dark chamber was calculated 3, 24, 48 and 72 hr after administration of the punishing electric shock.\textsuperscript{17}

**Biochemical assessments.** Following the completion of behavioral tests, the rats were anesthetized using intraperitoneal injection of 1.60 g kg\textsuperscript{-1} urethane (Sigma) and sacrificed and then their brains were harvested. The hippocampus and cortex were homogenized in phosphate buffered saline solution. The homogenates were then centrifuged and the supernatants were used for biochemical assessments. The levels of oxidative stress indicators including thiol, malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were measured. Concentrations of NO metabolites and IL-6 were also determined. The total thiol content and MDA concentrations were assessed based on a previously described method.\textsuperscript{6} The activity of SOD was defined according to the method reported by Madesh and Balasubramanian.\textsuperscript{18} The CAT activity was estimated based on the method outlined by Aebi.\textsuperscript{19} The compounds used for measurement of MDA, thiol, SOD and CAT were purchased from Merck Company (Darmstadt, Germany). The NO metabolites concentration was estimated using the Griess reaction method\textsuperscript{2} and a kit purchased from Promega Corporation (Madison, USA). The IL-6 was measured using an ELISA kit (Ebioscience Co., San Diego, USA) based on a previously described method.\textsuperscript{20}

**Statistical analysis.** Findings were reported as mean ± SEM. The data of time and distance during the five days of the MWM were analyzed using repeated measures analysis of variance (ANOVA) followed by Tukey post-hoc comparison test using SPSS Software (version 20.0; SPSS Inc., Chicago, USA). Probe trial data, passive avoidance test data and biochemical findings of different groups were compared by one-way ANOVA followed by Tukey post-hoc comparison test. A $p$ value less than 0.05 was considered statistically significant.

**Results**

**MWM results.** The findings of MWM test indicated that LPS administration prolonged the escape latency and travelled distance as compared to the control group ($p < 0.05$, $p < 0.001$). The latency and travelled distance to reach the stand in ZM50, ZM100 and ZM200 groups were significantly shorter in comparison to LPS group ($p < 0.05$, $p < 0.001$). There was no significant difference between ZM50, ZM100, ZM200 groups and control group. Moreover, there were no significant differences between ZM100 and ZM200 groups in terms of latency and travelled distance to find the platform. Significantly, ZM200 group spent less time and travelled shorter distance compared to ZM50 ($p < 0.05$ for both), (Figs. 1A and 1B). On the probe day, the rats of the LPS group spent less time and travelled a shorter distance in the target quadrant compared to the rats in control group ($p < 0.001$ for both cases). The animals in ZM200 group spent more time in the target quadrant compared to the rats in control group ($p < 0.05$). The animals in ZM50 and ZM100 groups travelled shorter distance in the target quadrant than the rats in control group ($p < 0.05$ for both groups). The animals in ZM50, ZM100 and ZM200 groups spent more time and travelled longer distance in the target quadrant than rats in LPS group ($p < 0.001$, for all groups). There were no significant differences among the control, ZM50 and ZM100 groups in the time spent in the target quadrant. In addition, no significant difference was found between control and ZM200 groups in terms of travelled distance. The ZM200 group spent longer distance in the target quadrant compared to ZM50 group ($p < 0.001$), (Figs. 1C and 1D).

**Passive avoidance test results.** The results of the passive avoidance test showed that the latency to enter the dark room 3, 24, 48 and 72 hr after receiving electrical shock, was significantly reduced in the LPS group in comparison to the control group ($p < 0.01$ and $p < 0.001$). The latency to enter the dark compartment 3, 24, 48 and 72 hr after receiving a shock, in ZM50, ZM100 and ZM200 groups were significantly longer than that of the LPS group ($p < 0.05$, $p < 0.001$, respectively). Nevertheless, there were no significant differences in latency to enter the dark compartment 3, 24, 48 and 72 hr after receiving a shock when a comparison was done between ZM50, ZM100, and ZM200 groups with the control group (Fig. 2A). The total time spent in the dark compartment 3, 24, 48 and 72 hr after receiving a shock in LPS group, was statistically longer than that of the control group ($p < 0.05$, $p < 0.001$, $p < 0.001$ and $p < 0.001$ respectively). The total time spent in the dark compartment 3, 24, 48 and 72 hr after receiving a shock in ZM50, ZM100, and ZM200 groups were significantly lower than that of LPS group ($p < 0.05$, $p < 0.001$). However, no significant differences were observed in terms of the total time spent in the dark chamber when was done between ZM50, ZM100, and ZM200 groups with the control group (Fig. 2B). The results also showed a significant effect for LPS on the total time spent in light compartment 3, 24, 48 and 72 hr after the shock. The total time spent in light compartment by LPS-group animals was lower than that of the control group 3, 24, 48 and 72 hr after receiving the shock ($p < 0.05$, $p < 0.001$ and $p < 0.01$ respectively); Nevertheless, in this regard, ZM50, ZM100 and ZM200 groups had higher values compared to LPS group ($p < 0.05$, $p < 0.001$). No significant difference was observed among control, ZM50, ZM100, and ZM200 groups (Fig. 2C).
Biochemical assessment of hippocampal tissue.

Intraperitoneally injection of LPS caused a significant increase in MDA concentration in hippocampal tissue of LPS group compared to control group \( p < 0.05 \). Pretreatment with the extract caused a significant decrease in MDA concentration in hippocampal tissue of ZM100 and ZM200 groups compared to LPS group \( p < 0.05 \). No significant dose dependent effect was found between the three doses (Fig. 3A). The LPS administration resulted in a reduction of the total thiol content in hippocampal tissue of LPS and ZM50, ZM100 and ZM200 groups compared to the control group \( p < 0.05 \). The administration of 200 mg kg\(^{-1}\) of the extract enhanced the total thiol content in the hippocampal tissue of ZM200 group compared to LPS group \( p < 0.001 \). In addition, the total thiol level in the hippocampal tissue of ZM100 and ZM200 groups were significantly higher than that of ZM50 group \( p < 0.05 \) and \( p < 0.001 \), respectively, (Fig. 3B). Based on our findings, the SOD activity in the hippocampal tissue of LPS and ZM50 groups was lower compared to the control group \( p < 0.001 \). The SOD activity in ZM200 group was higher compared to LPS group \( p < 0.05 \). In addition, we found no significant difference in SOD activity among the control, ZM100 and ZM200 groups. Moreover, the SOD activity in the hippocampal tissue was significantly higher in ZM200 group compared to ZM50 group \( p < 0.01 \). We didn’t find any difference between treatment doses in this test (Fig. 3C).

Based on the results, hippocampal tissue IL-6 level was higher in LPS, ZM50, and ZM100 groups than the control group \( p < 0.01 \) and \( p < 0.001 \), respectively. Hippocampal tissue IL-6 level was lower in ZM50, ZM100, and ZM200 groups when compared to LPS group \( p < 0.001 \). Also, IL-6 was lower in ZM100 and 200 groups compared to ZM50 group \( p < 0.001 \), (Fig. 3D). Nitric oxide metabolite concentration in the hippocampal tissue of LPS and treatment groups was higher than those of the control group \( p < 0.001 \). Administration of ZM decreased the concentration of these metabolites in the hippocampal tissue of ZM100 and ZM200 groups compared to LPS and ZM50 groups \( p < 0.001 \). Nitric oxide metabolite levels did not vary significantly between LPS and ZM50 groups. In all extract treated groups, NO metabolites were higher than that in the control group \( p < 0.001 \), (Fig. 3E). Hippocampal tissue CAT activity of LPS group was lower compared to the control group \( p < 0.001 \). In ZM50, ZM100 and 200 groups CAT activity was higher than that in LPS group \( p < 0.001 \) but in ZM50 and ZM100 groups, CAT activity didn’t reach to the control group \( p < 0.001 \). Also, CAT level was higher in ZM200 group compared to ZM50 group \( p < 0.001 \), (Fig. 3F).

Biochemical assessment of the cortical tissue.

When compared to the control group, LPS administration increased cortical tissues’ MDA concentration in LPS group \( p < 0.05 \). Treatment with 50.00, 100, and 200 mg kg\(^{-1}\) of the plant extract caused a significant reduction in the MDA.
According to the results, SOD activity in the cortical tissue of LPS group was lower than that of the control group ($p < 0.05$). The activity of SOD in ZM100 group was higher than that of LPS group ($p < 0.05$). There were no significant differences in SOD activity in cortical tissue among LPS, ZM50 and ZM200 groups (Fig. 4C). The results also showed that in cortical tissue, NO$_2$ and NO$_3$ levels were higher in LPS and the extract-treated groups compared to the control group ($p < 0.001$ for all cases). NO$_2$ and NO$_3$ levels were significantly lower in ZM100 and ZM200 groups compared to LPS and ZM50 groups ($p < 0.001$ for all cases). There were no differences in the level of NO metabolites between LPS and ZM50 groups (Fig. 4D). The CAT activity in the cortical tissue of LPS, ZM50 and ZM100 groups were lower than that of the control group ($p < 0.001$ for all cases). Also, the CAT level was higher in ZM groups compared to LPS group ($p < 0.01$ and $p < 0.001$). Moreover, the CAT activity in ZM100 and ZM200 groups was higher than that of ZM50 group ($p < 0.05$ and $p < 0.001$ respectively). We found no significant differences in the CAT activity between the control and ZM200 groups (Fig. 4E).

**Discussion**

The findings of present study, including decrement of the latency to enter the dark room, increment of the total time spent in the dark room, and the reduced total time spent in the light room in LPS group compared to the control group, confirmed that LPS disturbed passive avoidance memory in rats. Formerly researchers observed a disturbed passive avoidance learning 24 h after administration of LPS.\textsuperscript{21} In addition, in our research; LPS attenuated spatial memory performance of the rats. In this regard, the rats of LPS group spent more time and travelled longer distance to find the hidden platform compared to the control group. On probe day, the rats of LPS group spent shorter time and traveled lower distance in the target quadrant than the control group.

Microglia, as basic operators of the immune system in the brain release pro-inflammatory cytokines, affects the neuronal function.\textsuperscript{22} Some brain regions such as hippocampus, which are implicated in learning and memory, appeared to be sensitive to inflammation.\textsuperscript{23} In experimental models, it was also presented that the infusion of inflammatory mediators including IL-6 and other immune system stimuli such as LPS, can unsettle the learning and memory process.\textsuperscript{22} The destructive effects of LPS on the learning and memory are attributed to synthesis and release of inflammatory cytokines from macrophages and other cell types. For instance, the injection of LPS could intensify the inflammatory responses in the hippocampus and cortex.\textsuperscript{1} Previously, it was also documented that LPS leads to over-production of pro-inflammatory cytokines such as IL-6.\textsuperscript{24} Data reported
by the above-noted studies are consistent with the results of the current work in which LPS injection increased the level of IL-6 in the hippocampus of LPS group compared to control group.

Moreover, oxidative stress was demonstrated to disturb learning and memory.\textsuperscript{25} In our study, the increased level of MDA but decreased levels of CAT, SOD, and total thiols were found after LPS injection in the brain of LPS group as compared to the control group. Similar to our results, it has also been previously reported that LPS injection is followed by an imbalance in oxidant- anti oxidant system in both the hippocampus and cortex.\textsuperscript{26}

Nitric oxide as a reactive oxygen species (ROS) acts as a neuronal cytotoxic agent and induces learning and memory dysfunction, when overproduced.\textsuperscript{27} It was also reported that peroxynitrite produced by the reaction between NO and superoxide, promotes lipid peroxidation and protein oxidation.\textsuperscript{28} Also, the results of researches verified the effect of LPS on overproduction of NO in the brain tissue.\textsuperscript{29} In this work, the levels of NO metabolites (NO\textsubscript{2}/NO\textsubscript{3}) in the brain of LPS group were higher than that of the control group. Therefore, it might be concluded that the inflammation responses along with enhancement of NO levels and oxidative stress caused by LPS, considerably contribute to the learning and memory dysfunctions in the current research. In addition, NO is produced in a high level during neuroinflammation which may mediate learning and memory impairments seen in the present study.\textsuperscript{30}

**Fig. 3.** A) Malondialdehyde (MDA) concentration; B) total thiol content; C) superoxide dismutase (SOD) activity; D) levels of interleukin-6 (IL-6); E) level of nitric oxide (NO) metabolites and F) catalase (CAT) in hippocampal tissue. Data are expressed as mean ± SEM (n = 8 - 10 in each group). *p < 0.05, **p < 0.01 and ***p < 0.001 show significant differences compared to the control group; *p < 0.05 and **p < 0.01 and ***p < 0.001 show significant differences compared to LPS group. $p < 0.05$ and $$$p < 0.001$ show significant differences compared to LPS-ZM50 group.
In this study, the administration of *Z. multiflora* extract before LPS injection was protected from LPS-induced learning and memory impairments in both behavioral tests in rats. In the behavioral experiments, the rats of ZM groups spent less time and travelled shorter distances to find the platform compared to LPS group. On the probe day, the rats of ZM groups searched for the platform in the target quadrant were better than the animals in LPS group. In addition compared to LPS group, an increase in the latency to enter the dark room, a decrease in the time spent in the dark room, and an increase in time spent in the light room in passive avoidance test were observed in ZM groups which confirmed an improving effect of *Z. multiflora* on LPS-caused memory dysfunction. Passive avoidance test has been frequently used as a tool to challenge contextual learning and memory and is often considered as nonspatial memory which may be a nonhippocampal mediated memory. In addition, spatial memory has been frequently evaluated by MWM which is mainly considered to be hippocampal dependent.

Considering the results of current study, *Z. multiflora* seems to be prevented from both hippocampal and non-hippocampal dependent learning and memory impairments induced by LPS. Interestingly, the effect of the extract on spatial learning and memory seems to be dose dependent and the highest dose had the best effect. However, the effect of *Z. multiflora* on nonhippocampal mediated memory was not dose dependent.

The *Z. multiflora* was revealed to have antioxidant, anti-inflammatory, and immunoregulatory properties. It was shown that the extract of *Z. multiflora* significantly reduced serum levels of IL-8 in chronic obstructive pulmonary disease (COPD) animals. In the current study, all three doses of the plant extract were prevented from increasing of IL-6 levels in brain tissue in a dose dependent manner. Considering this result, it seems that
the plant extract reduced neuroinflammation to improve learning and memory of LPS injected rats.

Moreover, it has been indicated that Z. multiflora had beneficial effects on lipid peroxidation and boosts antioxidant power. Researchers showed that Z. multiflora suppressed free radicals production and supported the body against oxidative stress. In another study, Z. multiflora was found to have a potent protective effect against testicular toxicity caused by cisplatin due to its antioxidant activities. Findings of the current research also exhibited that the administration of Z. multiflora extract before LPS, prevented from increasing of the MDA concentration in cortical and hippocampal tissue. According to the results of the present work, Z. multiflora extract (200 mg kg⁻¹) enhanced the total thiol level in hippocampal and cortical tissue. Moreover, the activity of SOD and CAT in the hippocampal and cortical regions in ZM groups were higher than that of LPS group. Therefore, the anti-oxidant effects of the plant extract might be considered as a possible mechanism (s) for learning and memory improving effects of Z. multiflora which was seen in the current research. A close relationship between neuroinflammation and oxidative stress has also been suggested.

It has been reported that after activation by neuroinflammation, glial cells were able to act as source of free radicals. It might be suggested that inhibition of neuroinflammation by the extract of Z. multiflora was followed by a decrease in the glial cells activity, inhibition of subsequent free radical production, reduction in brain tissue oxidative damage, and finally learning and memory improving effects.

Our findings also displayed that NO metabolites concentration was significantly lower in the brain of ZM groups compared to LPS group, and the effects of the plant extract on NO metabolites was dose dependent. Similar to our results, it has been previously reported that Z. multiflora could prevent an overproduction of NO especially during inflammation. Beside the anti-inflammatory effect, the plant extract prevented an overproduction of NO to decrease learning and memory impairing effects of LPS which was seen in the present study. It is also notable to mention that a decrease in NO production by Z. multiflora is accompanied by a decrease in brain tissue oxidative damage which might be considered to elucidate the beneficial effect of the Z. multiflora in the present study.

Learning and memory enhancing effects of Z. multiflora methanolic extract and its essential oil have been attributed to its anticholinesterase activity, which may also have a role in the results which seen in the present study. It however, needs to be more examined in the future studies. To support this idea, it has been reported that neuroinflammation is accompanied by an increase in acetyl cholinesterase activity.

It has also been previously reported that neuroinflammation was accompanied by an increased level of amyloid beta protein in the hippocampus. Essential oil of Z. multiflora, was reportedly, able to improve performances of the rats in MWM amyloid beta - induced learning and memory impairment model. It was also able to decrease amyloid beta in the hippocampus. The current findings showed that Z. multiflora was probably able to prevent amyloid beta production to improve learning and memory which may have a role in the positive effects of the plant extract. This was reported in current research, but it needs to be investigated more in the future. The neuroinflammation is followed by a decrease in the level of brain derived neurotrophic factor (BDNF) in the brain.

The compound(s), responsible for beneficial effects of the plant extract, was not evaluated in the current study, however, some ingredients including thymol, carvacrol, apigenin, and luteolin may have a role. For example, learning and memory improving effects of thymol in a rat model of amyloid beta memory impairment has been reported to be accompanied by a decrease in amyloid beta. Carvacrol has also been reported to have neuroprotective, anti-neuroinflammation, and anti-oxidant effects in the brain and improved memory of the rats in Parkinsonian model. Leuteolin has also been shown to have anti-inflammatory effects. It has also been able to improve learning and memory, and protects the brain against neurotoxicity. Leuteolin was also reported that increased neurogenesis in the hippocampus. It was also reported that luteolin decreased neuroinflammation while improved insulin signaling in the hippocampus to attenuate symptoms of Alzheimer in rats. Each of these components may be essential in learning and memory improving effects of Z. multiflora which was seen in the present study, however, it needs to be more investigated.

Our data indicated that pre-treatment with Z. multiflora extract prevented LPS-caused learning and memory impairments. According to the results of the current study, this beneficial effect was probably mediated through reducing LPS-caused inflammation and oxidative stress.

Acknowledgments

The results were described in this article was part of a MSc. thesis (No: A-1344). The authors appreciate the Vice Chancellor for Research and Technology of Mashhad University of Medical Sciences for their financial support (Project No: 961613).

Conflict of interests

The authors have no conflict of interests to declare.
References

1. Anaegoudari A, Shafei MN, Soukhtanloo M, et al. Lipopolysaccharide-induced memory impairment in rats is preventable using 7-nitroindazole. Arq Neupropsiquiatr 2015; 73(9): 784-790.

2. Anaegoudari A, Norouzi F, Abaresth A, et al. Protective effects of Nigella sativa on synaptic plasticity impairment induced by lipopolysaccharide. Vet Res Forum 2018; 9(1): 27-33.

3. Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun 2011; 25(2): 181-213.

4. Bialuk I, Taranta A, Winnicka MM. IL-6 deficiency alters spatial memory in 4- and 24-month-old mice. Neurobiol Learn Mem 2018; 155: 21-29.

5. Lee B, Shim I, Lee H. Gypenosides attenuate lipopolysaccharide-induced neuroinflammation and memory impairment in rats. Evid Based Complement Alternat Med 2018; 2018: 4183670. doi: 10.1155/2018/4183670.

6. Hosseini M, Anaegoudari A, Beheshti F, et al. Protective effect against brain tissues oxidative damage as a possible mechanism for beneficial effects of L-arginine on lipopolysaccharide induced memory impairment in rats. Drug Chem Toxicol 2018; 41(2): 175-181.

7. Boskabady MH, Gholami Mhtaj L. Effect of the Zataria multiflora on systemic inflammation of experimental animals model of COPD. Biomed Res Int 2014; 2014: 802189. doi: 10.1155/2014/802189.

8. Saei-Dehkhordi SS, Tajik H, Moradi M, et al. Chemical composition of essential oils in Zataria multiflora Boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem Toxicol 2010; 48(6): 1562-1567.

9. Shafiee A, Javidinia K. Composition of essential oil of Zataria multiflora. Planta Med 1997; 63(4): 371-372.

10. Majlessi N, Choopani S, Kamalinejad M, et al. Amelioration of amyloid β-induced cognitive deficits by Zataria multiflora Boiss. essential oil in a rat model of Alzheimer’s disease. CNS Neurosci Ther 2012; 18(4): 295-301.

11. Heydari M, Mokhtari-Zaer A, Amin F, et al. The effect of Zataria multiflora hydroalcoholic extract on memory and lung changes induced by rats that inhaled paraquat. Nutr Neurosci 2021; 24(9): 674-687.

12. Sajed H, Sahebkar A, Iranshahi M. Zataria multiflora Boiss. (Shirazi thyme)--an ancient condiment with modern pharmaceutical uses. J Ethnopharmacol 2013; 145(3): 686-698.

13. Ji YP, Shi TY, Zhang YY, et al. Essential oil from Fructus Alpinia zerumbet (fruit of Alpinia zerumbet (Pers.) Burt.et Smith) protected against aortic endothelial cell injury and inflammation in vitro and in vivo. J Ethnopharmacol 2019; 237: 149-158.

14. Chamanara M, Abdollahi A, Rezayat SM, et al. Thymol reduces acetic acid-induced inflammatory response through inhibition of NF-kB signaling pathway in rat colon tissue. Inflammopharmacology 2019; 27(6): 1275-1283.

15. Boskabady MH, Gholami Mhtaj L. Lung inflammation changes and oxidative stress induced by cigarette smoke exposure in guinea pigs affected by Zataria multiflora and its constituent, carvacrol. BMC Complement Altern Med 2015; 15:39. doi: 10.1186/s12906-015-0574-y.

16. Baghchehgi Y, Hosseini M, Beheshiti F, et al. Thymoquinone reverses learning and memory impairments and brain tissue oxidative damage in hypothyroid juvenile rats. Arq Neupropsiquiatr 2018; 76(1): 32-40.

17. Ramezani M, Darbandi N, Khodagholi F, et al. Myricetin protects hippocampal CA3 pyramidal neurons and improves learning and memory impairments in rats with Alzheimer’s disease. Neural Regen Res 2016; 11(12): 1976-1980.

18. Madesh M, Balasubramanian K. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J Biochem Biophys 1998; 35(3): 184-188.

19. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-126.

20. Abaresti A, Hosseini M, Beheshti F, et al. The effects of captopril on lipopolysaccharide induced learning and memory impairments and the brain cytokine levels and oxidative damage in rats. Life Sci 2016; 167: 46-56.

21. Jain NK, Patil CS, Kulkarni SK, et al. Modulatory role of cyclooxygenase inhibitors in aging- and scopolamine or lipopolysaccharide-induced cognitive dysfunction in mice. Behav Brain Res 2002; 133(2): 369-376.

22. Czerniawski J, Miyashita T, Lewandowski G, et al. Systemic lipopolysaccharide administration impair retrieval of context-object discrimination, but not spatial, memory: Evidence for selective disruption of specific hippocampus-dependent memory functions during acute neuroinflammation. Brain Behav Immun 2015; 44: 159-166.

23. Schöbitz B, de Kloet ER, Sutanto W, et al. Cellular localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. Eur J Neurosci 1993; 5(11): 1426-1435.

24. Yu K, Ma Y, Li X, et al. Lipopolysaccharide increases IL-6 secretion via activation of the ERK1/2 signaling pathway to up-regulate RANKL gene expression in MLO-Y4 cells. Cell Biol Int 2017; 41(1): 84-92.

25. Richardson JS. Free radicals in the genesis of Alzheimer’s disease. Ann N Y Acad Sci 1993; 695: 73-76.

26. Shal B, Khan A, Naveed M, et al. Effect of 25-methoxy hispidol A isolated from Poncirus trifoliate against...
bacteria-induced anxiety and depression by targeting neuroinflammation, oxidative stress and apoptosis in mice. Biomed Pharmacother 2019; 111: 209-223.

27. Yamada K, Komori Y, Tanaka T, et al. Brain dysfunction associated with an induction of nitric oxide synthase following an intracerebral injection of lipopolysaccharide in rats. Neuroscience 1999; 88(1): 281-294.

28. Ischiropoulos H, Zhu L, Beckman JS. Peroxynitrite formation from macrophage-derived nitric oxide. Arch Biochem Biophys 1992; 298(2): 446-451.

29. Hou CC, Lin H, Chang CP, et al. Oxidative stress and pyrogenic fever pathogenesis. Eur J Pharmacol 2011; 667(1-3): 6-12.

30. Ghasemi M, Fatemi A. Pathologic role of glial nitric oxide in adult and pediatric neuroinflammatory diseases. Neurosci Biobehav Rev 2014; 45: 168-182.

31. Wang JH, Fu Y, Wilson FAW, et al. Ketamine affects memory consolidation: differential effects in T-maze and passive avoidance paradigms in mice. Neuroscience 2006; 140(3): 993-1002.

32. Burwell RD, Saddoris MP, Buco DJ, et al. Cortico-hippocampal contributions to spatial and contextual learning. J Neurosci 2004; 24(15): 3826-3836.

33. Khazdair MR, Ghorei V, Alavinezhad A, et al. Pharmacological effects of Zataria multiflora Boiss and its constituents focus on their anti-inflammatory, antioxidant, and immunomodulatory effects. Fundam Clin Pharmacol 2018; 32(1): 26-50.

34. Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of the potential herbal sources of future drugs effective in oxidant-related diseases. Inflamm Allergy Drug Targets 2009; 8(1): 2-10.

35. Karimi S, Hosseiniemehr SJ, Mohammadi HR, et al. Zataria multiflora ameliorates cisplatin-induced testicular damage via suppression of oxidative stress and apoptosis in a mice model. Iran J Basic Med Sci 2018; 21(6): 607-614.

36. Barron H, Hafizi S, Andreazza AC, et al. Neuroinflammation and oxidative stress in psychosis and psychosis risk. Int J Mol Sci 2017; 18(3):651. doi: 10.3390/ijms18030651.

37. Mossakowski AA, Pohlan J, Bremer D, et al. Tracking CNS and systemic sources of oxidative stress during the course of chronic neuroinflammation. Acta Neuropathol 2015; 130(6): 799-814.

38. Kavoosi G, Teixeira da Silva JA, Saharkhiz MJ. Inhibitory effects of Zataria multiflora essential oil and its main components on nitric oxide and hydrogen peroxide production in lipopolysaccharide-stimulated macrophages. J Pharm Pharmacol 2012; 64(10): 1491-1500.

39. Sharififar F, Mirtajadini M, Azampour MJ, et al. Essential oil and methanolic extract of Zataria multiflora Boiss with anticholinesterase effect. Pak J Biol Sci 2012; 15(1): 49-53.

40. Al-Fadl ED, Elzahhar PA, Tramarin A, et al. Tackling neuroinflammation and cholinergetic deficit in Alzheimer’s disease: Multi-target inhibitors of cholinesterases, cyclooxygenase-2 and 15-lipoxygenase. Eur J Med Chem 2019; 167: 161-186.

41. Cai Z, Hussain MD, Yan LJ. Microglia, neuroinflammation, and beta-amyloid protein in Alzheimer’s disease. Int J Neurosci 2014; 124(5): 307-321.

42. Lima Giacobbo B, Doorduin J, Klein HC, et al. Brain-derived neurotrophic factor in brain disorders: focus on neuroinflammation. Mol Neurobiol 2019; 56(5): 3295-3312.

43. Asadbegi M, Yaghmaei P, Salehi I, et al. Investigation of thymol effect on learning and memory impairment induced by intrahippocampal injection of amyloid beta peptide in high fat diet- fed rats. Metab Brain Dis 2017; 32(3): 827-839.

44. Lins LCRF, Souza MF, Bispo JMM, et al. Carvacrol prevents impairments in motor and neurochemical parameters in a model of progressive parkinsonism induced by reserpine. Brain Res Bull 2018; 139: 9-15.

45. Wang P, Luo Q, Qiao H, et al. The neuroprotective effects of carvacrol on ethanol-induced hippocampal neurons impairment via the antioxidative and antiapoptotic pathways. Oxid Med Cell Longev 2017; 2017: 4079425. doi: 10.1155/2017/4079425.

46. Hamzehloei L, Rezvani ME, Rajaei Z. Effects of carvacrol and physical exercise on motor and memory impairments associated with Parkinson’s disease. Arq Neuropsiquiatr 2019; 77(7): 493-500.

47. Haddadi H, Rajaei Z, Alaei H, et al. Chronic treatment with carvacrol improves passive avoidance memory in a rat model of Parkinson’s disease. Arq Neuropsiquiatr 2018; 76(2): 71-77.

48. Gu JX, Cheng XJ, Luo X, et al. Luteolin ameliorates cognitive impairments by suppressing the expression of inflammatory cytokines and enhancing synapse-associated proteins GAP-43 and SYN levels in streptozotocin-induced diabetic rats. Neurochem Res 2018; 43(10): 1905-1913.

49. Zhou WB, Miao ZN, Zhang B, et al. Luteolin induces hippocampal neurogenesis in the Ts65Dn mouse model of Down syndrome. Neural Regen Res 2019; 14(4): 613-620.

50. Park S, Kim DS, Kang S, et al. The combination of luteolin and L-theanine improved Alzheimer disease-like symptoms by potentiating hippocampal insulin signaling and decreasing neuroinflammation and norepinephrine degradation in amyloid-β-infused rats. Nutr Res 2018; 60: 116-131.