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

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Article Info	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 a 6-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.

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.[2,4] 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

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generated from L-arginine by three isoforms of NO synthase (NOS) including inducible (iNOS), neuronal NOS (nNOS) and endothelial NOS (eNOS).[6] Although NO has a neuroprotective effect under the physiological concentrations, high concentrations of NO can promote apoptosis and cell death.[1]

Zataria multiflora is a medicinal herb growing in Iran, Pakistan and Afghanistan. It can be recognized by small leaves, fibrous root, and highly narrow branches varying between 40 and 80 cm in height.[7] This plant contains different compounds including thymol, carvacrol, apigenin, luteolin, 6-hydroxyluteolin glycosides, as well as di-, tri-, and tetramethoxylated. These compounds could be responsible for the therapeutic effects of *Z. multiflora*.[8,9] In Iran, *Z. multiflora* is traditionally used for its antiseptic, analgesic and carminative properties.[9] The *Z. multiflora* also has beneficial effects on learning ability, memory, and cognitive functions.[10] It was also previously shown that *Z. multiflora* improved learning and memory accompanied with lung inflammation in paraquat exposed rats.[11]

According to a recent research, *Z. multiflora* possesses antimicrobial, antioxidant and immune-regulatory properties. Anti-inflammatory effects of *Z. multiflora* are attributed to its suppressing effect on pro-inflammatory cytokines levels and its enhancing effect on anti-inflammatory cytokines.[12] The effects of *Z. multiflora* on neuroinflammation related learning and memory has not been reported. Considering the evidence about the harmful effects of inflammation and oxidative stress on memory, the research was aimed to examine the possible protective effects of *Z. multiflora* on learning and memory impairment due to neuroinflammation caused by LPS.

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:B5) 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.[13,14]

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.[15]

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 (Radiab, 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.[16]

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.[17]

Biochemical assessments. Following the completion of behavioral tests, the rats were anesthetized using intraperitoneal injection of 1.60 g kg^{-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.[6] The activity of SOD was defined according to the method reported by Madesh and Balasubramanian.[18] The CAT activity was estimated based on the method outlined by Aebi.[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[1] 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.[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 hour after receiving the shock (*p* < 0.05, *p* < 0.001 and *p* < 0.001, *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).
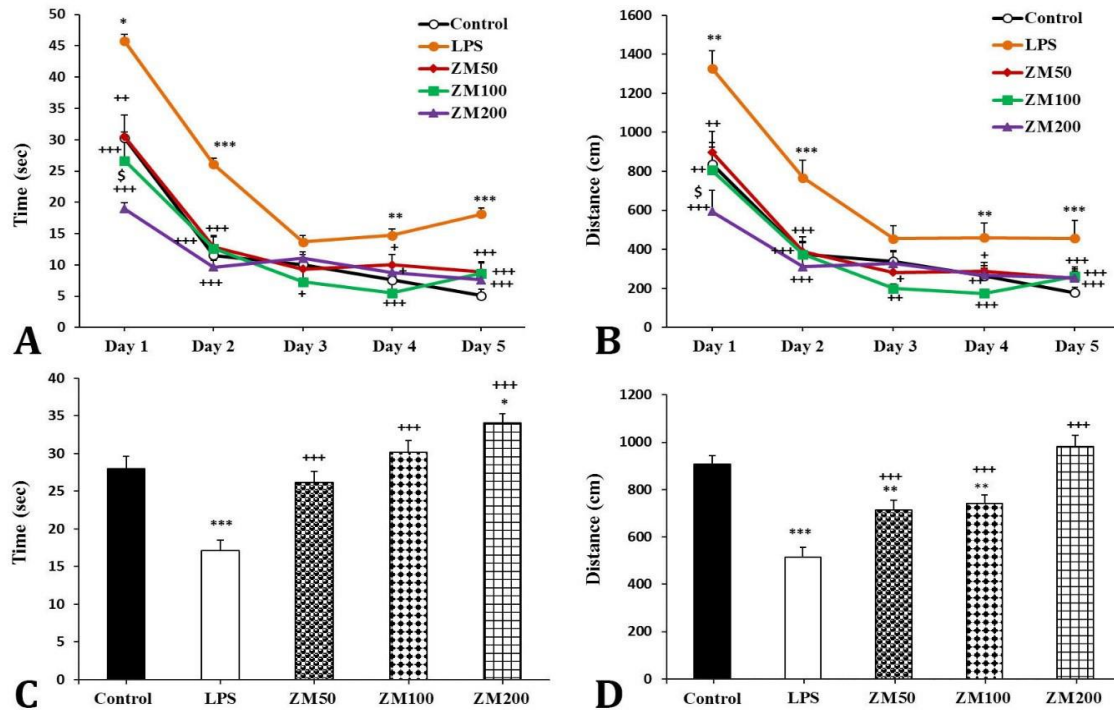
Fig. 1. A) Latency; **B)** travelled distance for reaching platform; **C)** time spent and **D)** distance travelled in the target quadrant on probe day measured by Morris water maze test. The results are expressed as mean ± SEM (n = 10 in each group). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ show significant differences as compared to the control group. + $p < 0.05$, ++ $p < 0.01$, and +++ $p < 0.001$ shows significant differences as compared to LPS group. $ $p < 0.05$ shows significant differences as compared to LPS-ZM50 group.

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 ($p < 0.05$). Pre-treatment 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 ($p < 0.01$ and $p < 0.001$). 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 than that of the control group ($p < 0.01$). 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

level in cortical tissue of ZM50, ZM100, and ZM200 groups compared to LPS group ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively). No difference was found between the three treatment doses (Fig. 4A). The total thiol content in the cortical tissue of ZM50 and ZM100 groups were lower than the control group ($p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively). Administration of 200 mg kg[1] the extract also enhanced the total thiol content in the cortical tissue of ZM200 group compared to LPS group ($p < 0.01$). There were no significant differences in the cortical tissues' thiol content among LPS, ZM50 and ZM100 groups. Also, there were not any significant differences in the thiol content in cortical tissue between the control and ZM200 groups. Total thiol level in ZM groups was not different between the three doses (Fig. 4B).
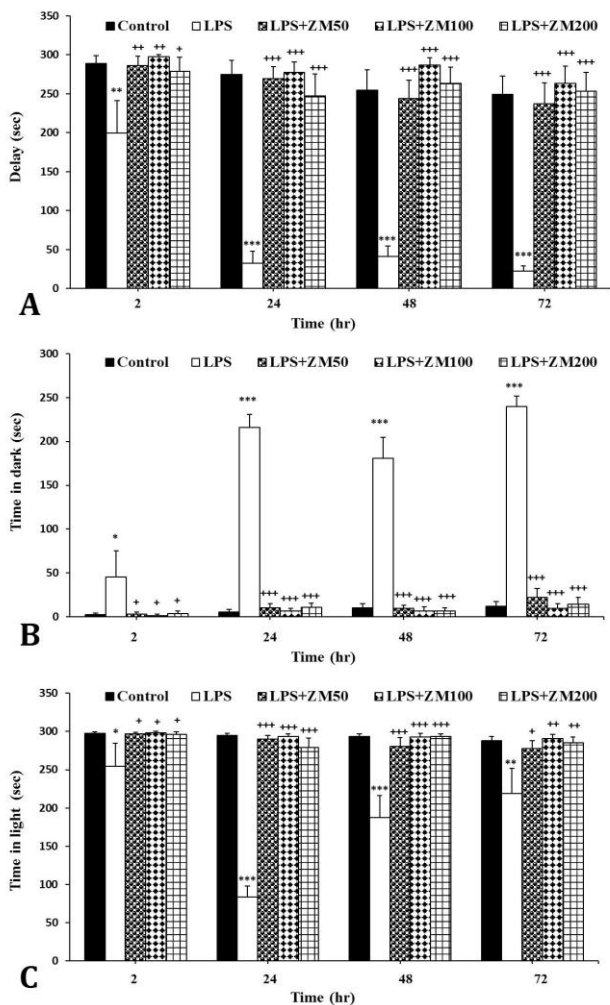

Fig. 2. A) Latency to enter the dark compartment; **B)** the total time spent in the dark compartment and **C)** the total time spent in the light compartment computed 3, 24, 48 and 72 hr after receiving shock, in passive avoidance test. Data are presented as mean ± SEM (n = 10 in each group). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ show significant differences compared to the control group. [+] $p < 0.05$, [++] $p < 0.01$, and [+++] $p < 0.001$ show significant differences compared to LPS group.

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.[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.[22] Some brain regions such as hippocampus, which are implicated in learning and memory, appeared to be sensitive to inflammation.[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.[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.[1] Previously, it was also documented that LPS leads to over-production of pro-inflammatory cytokines such as IL-6.[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.[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 hippo-campus and cortex.[26]

Nitric oxide as a reactive oxygen species (ROS) acts as a neuronal cytotoxic agent and induces learning and

memory dysfunction, when overproduced.[27] It was also reported that peroxynitrite produced by the reaction between NO and superoxide, promotes lipid per-oxidation and protein oxidation.[28] Also, the results of researches verified the effect of LPS on overproduction of NO in the brain tissue.[29] In this work, the levels of NO metabolites (NO_2/NO_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.[30]
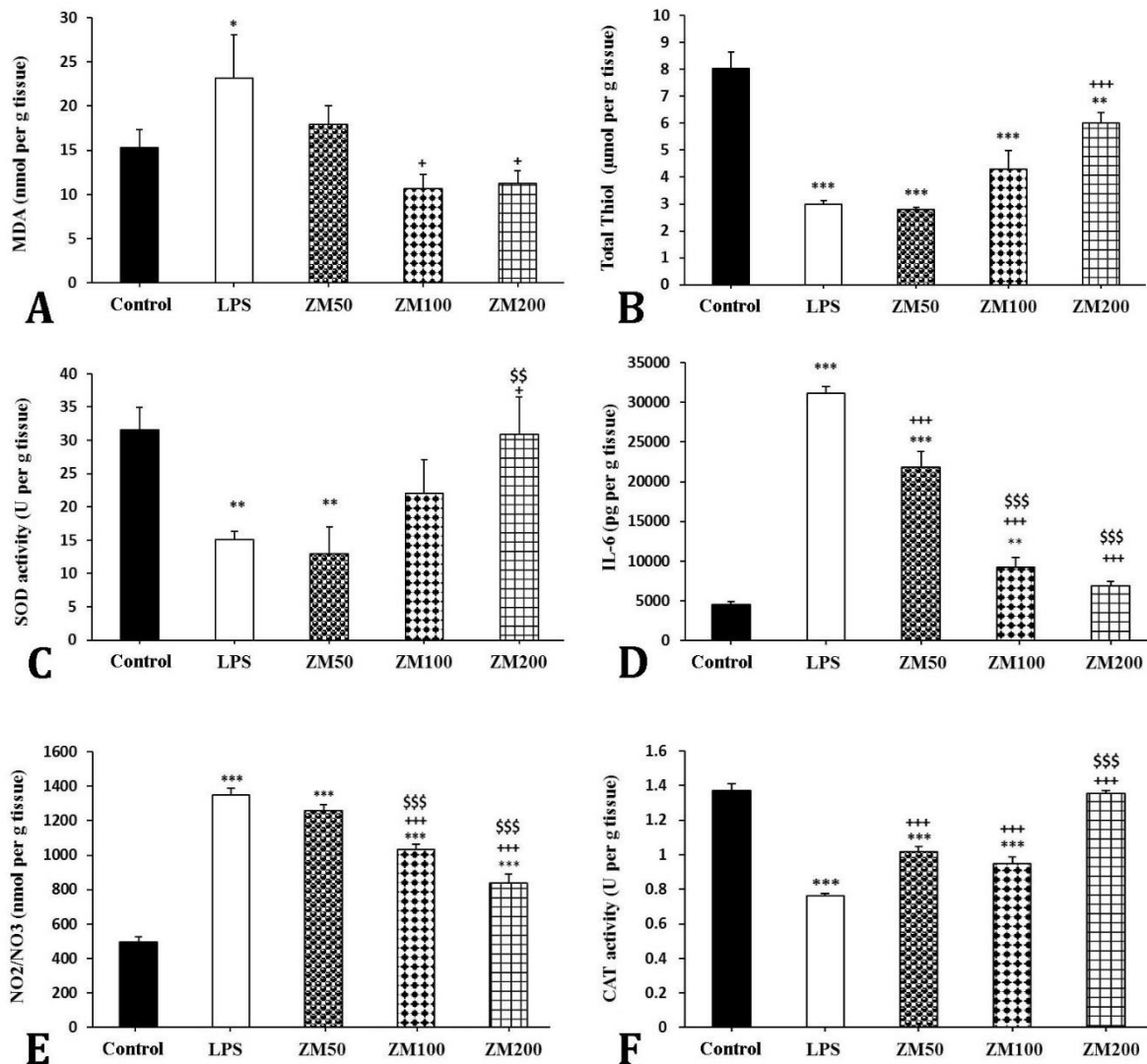

Fig. 3. A) Malondialdehyde (MDA) concentration; **B)** total thiol content; **C)** superoxide dismutase (SOD) activity; **D)** levels of interleukin-6 (IL-6); **E)** level of nitric oxide (NO) metabolites and **F)** catalase (CAT) in hippocampal tissue. Data are expressed as mean ± SEM (n = 8 - 10 in each group). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ show significant differences compared to the control group; + $p < 0.05$ and +++ $p < 0.001$ show significant differences compared to LPS group. $$ $p < 0.05$ and $$$ $p < 0.001$ show significant differences compared to LPS-ZM50 group.
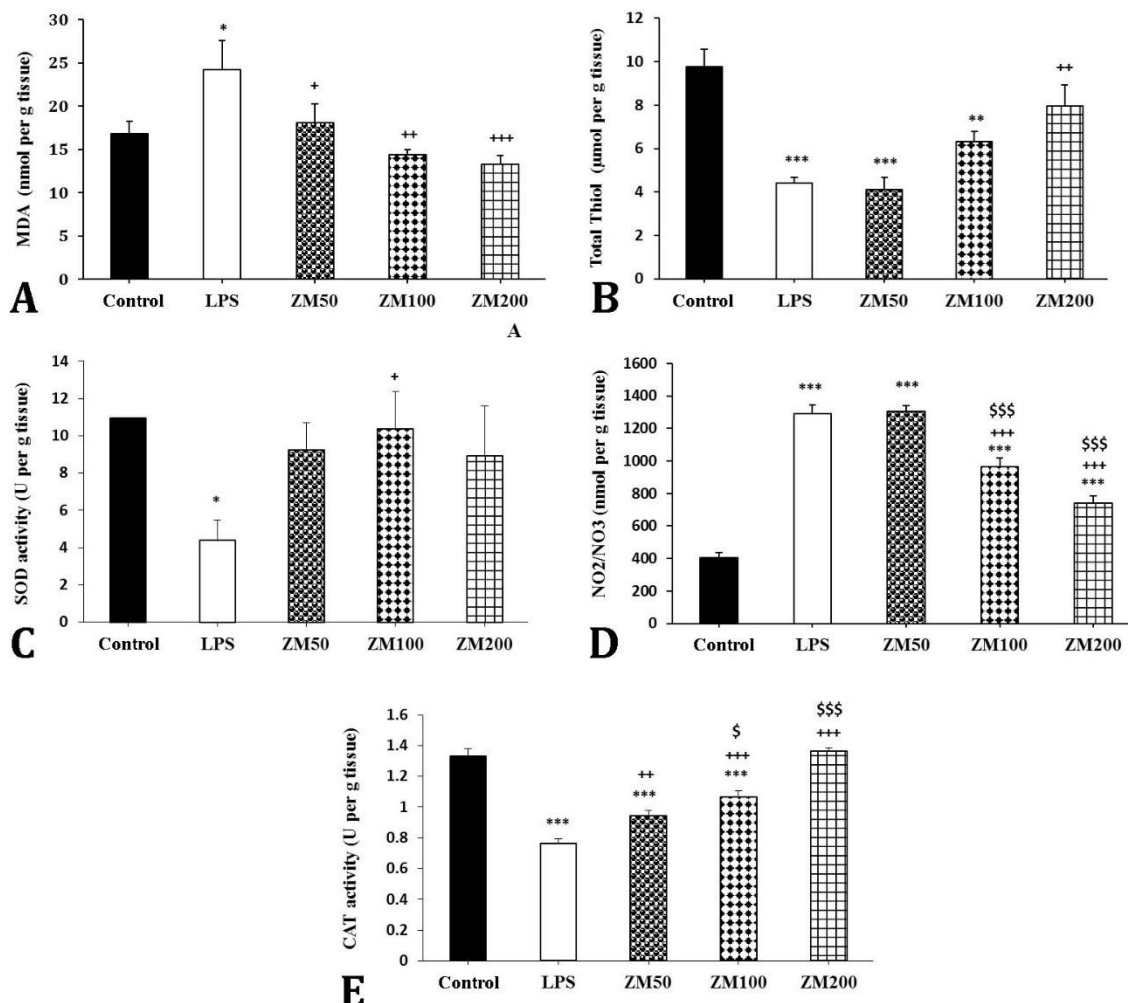
Fig. 4. A) Malondialdehyde (MDA) concentration; **B)** total thiol content; **C)** superoxide dismutase (SOD) activity; **D)** level of nitric oxide (NO) metabolites and **E)** catalase (CAT) activity in cortical tissue. Data are expressed as mean ± SEM (n = 10 in each group). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ show significant differences compared to the control group; + $p < 0.05$, ++ $p < 0.01$ and +++ $p < 0.001$ show significant differences compared to LPS group. $ $p < 0.05$ and $$$ $p < 0.001$ show significant differences compared to LPS-ZM 50 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.[31] In addition, spatial memory has been frequently evaluated by MWM which is mainly considered to be hippocampal dependent.[32] 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.[33] It was shown that the extract of *Z. multiflora* significantly reduced serum levels of IL-8 in chronic obstructive pulmonary disease (COPD) animals.[7] 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.[34] Researchers showed that *Z. multiflora* suppressed free radicals production and supported the body against oxidative stress.[8,35] In another study, *Z. multiflora* was found to have a potent protective effect against testicular toxicity caused by cisplatin due to its antioxidant activities.[35] 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.[36] It has been reported that after activation by neuroinflammation, glial cells were able to act as source of free radicals.[37] 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.[38] 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,[39] 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.[40]

It has also been previously reported that neuro-inflammation was accompanied by an increased level of amyloid beta protein in the hippocampus.[41] 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.[10] 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.[42]

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.[43] 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.[44-47] 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.[48] Leuteolin was also reported that increased neurogenesis in the hippocampus.[49] It was also reported that leuteolin decreased neuroinflammation while improved insulin signaling in the hippocampus to attenuate symptoms of Alzheimer in rats.[50] 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

Conflict of interests

The authors have no conflict of interests to declare.

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