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The effects of aminoguanidine on hippocampal cytokines, amyloid beta, brain-derived neurotrophic factor, memory and oxidative stress status in chronically lipopolysaccharide-treated rats

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ABSTRACT

Introduction: In the present study, the effects of aminoguanidine (AMG) on hippocampal cytokines, amyloid beta (Aβ), brain-derived neurotrophic factor, oxidative stress status and memory in chronically lipopolysaccharide (LPS) treated rats were investigated.

Methods: The rats were divided into five groups and were treated: (1) Control (Saline), (2) LPS (1 mg/kg), (3–5) LPS- AMG50, LPS-AMG100, and LPS-AMG150 (AMG 50, 100 and 150 mg/kg 30 min before LPS injection). The treatment started five weeks prior to the behavioral experiments and continued during the behavioral tests (LPS injection two hours before each behavioral evaluation). Finally, the tissue was removed for biochemical measurements.

Results: The escape latency in Morris water maze test and the latency to enter the dark compartment in passive avoidance test in LPS group were significantly greater than the control group ($P < 0.001$), while, in LPS-AMG 100 and LPS-AMG150 groups they were less than LPS group ($P < 0.001$). Malondialdehyde (MDA), NO metabolites of hippocampal and cortical tissues and interleukin-6 (IL-6), Aβ and tumor necrosis factor-α (TNFα) concentration in the hippocampus of LPS group were higher than control group ($P < 0.001$-$P < 0.05$). However, in LPS-AMG 100 and LPS-AMG150 group they were lower than LPS group ($P < 0.001$-$P < 0.05$). The thiol content and the activities of catalase (CAT) and superoxide dismutase (SOD) in both cortical and hippocampal tissues of LPS group were reduced compared to the control group ($P < 0.001$-$P < 0.05$). These factors enhanced in LPS-AMG 100 and LPS-AMG150 groups compared to LPS ($P < 0.001$-$P < 0.05$). The hippocampal content of brain-derived neurotrophic factor (BDNF) in LPS group was significantly lower compared to the control group ($P < 0.001$). All treated groups had higher BDNF content in comparison to LPS group ($P < 0.01$-$P < 0.001$).

Conclusion: The findings indicated that the protective effects of AMG against LPS-induced memory were accompanied by decreasing of inflammatory cytokines, Aβ, oxidative stress and increasing of anti-inflammatory mediators and BDNF.

1. Introduction

One of the famous progressive neurodegenerative disorders is Alzheimer's disease (AD) which is associated with cognitive dysfunction particularly in the aged population. The contribution of inflammation to the pathogenesis of several nervous system complications including AD has long been established. Results of some studies revealed that the immune system affects the mental functions including learning, memory and neuronal plasticity [1,2]. Also, it was illustrated that inflammation has a critical role in learning and memory impairment [3]. Additionally, it was reported that the systemic inflammation is associated with oxidative injury and has an essential role in cognitive

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dysfunction [4]. Brain inflammation is linked to an impaired spatial learning and memory function accompanying with an oxidative stress status [5]. Lipopolysaccharide (LPS) as a particle of gram negative bacteria has frequently utilized to induce neuroinflammation status in rodents [6] which is accompanied with microglia activation and a high level of cytokines.

Tissue injury by microglia activation can elevate pro-inflammatory cytokines levels in the hippocampus. Cytokines can induce neuronal dysfunction and suppress neurogenesis, memory, learning and long-term potentiation (LTP) [7]. Also, many studies illustrated that LPS-induced inflammation leads to learning and memory impairments by increasing pro-inflammatory cytokines and free radical production [8]. There is also a valid confirmation that oxidative damage contributes to the central nervous system (CNS) disorder pathogenesis and in learning and memory impairments due to neuroinflammation [9]. LPS–receptor or Toll–like receptors (TLRs) are found in cells with or without immune function [10]. LPS induced oxidative stress status is characterized by the alteration of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and excessive reactive oxygen species (ROS accumulation in tissue, leading to cellular injury by the impairment of vital macromolecules, resulting in altered membrane fluidity and mitochondrial function [11]. Thus, it is proposed that the protection against the oxidative injury and employing agents with antioxidant and anti-inflammatory properties can invert the memory impairment.

On the other hand, brain-derived neurotrophic factor (BDNF) was indicated to be the most abundant neurotrophin in the hippocampus. The roles of BDNF in the brain are including neuronal survival and differentiation, synaptic strengthening and morphology regulation [12]. BDNF can protect neural cells from ischemia, trauma, and neurotoxicity [13].

Aminoguanidine (AMG) has several biological effects, such as inhibiting inducible nitric oxide synthase [14,15]. AMG was proved to inhibit inflammation and reduce neurodegeneration in experimental autoimmune encephalomyelitis [16]. AMG was also demonstrated to have a neuroprotective potential in AD models [17]. Recent reports indicated that in mice with dementia induced by streptozotocin, AMG prevented from cognitive deficits and nitrosative stress, alongside with reducing the astroglial activation in the hippocampus [17]. AMG, as an iNOS [14] inhibitor was proved to inhibit inflammatory processes and ameliorated neuronal damage in the hippocampus [17]. AMG also reduced the transient cerebral ischemia-induced memory impairment in Y-maze and contextual fear condition [18]. Intraperitoneally(i.p.) administration of AMG also altered the acquisition and consolidation of the spatial task deficiencies caused by amyloid beta (Aβ) in radial maze test [14].

In this sense, the aim of the current research is to explore the effects of AMG on hippocampal cytokines, Aβ, BDNF, oxidative stress status and memory in chronically LPS treated rats.

2. Materials and methods

2.1. Animals

Fifty male Wistar rats (240 ± 10 g) were obtained from the local animal house located at Mashhad University of Medical Sciences. Then they were accommodated in groups of 5 in separately ventilated cages and maintained under standard conditions (temperature 22 ± 2 °C, humidity of 54 ± 2% and 12 h light/dark cycle). Food and water were freely available. The experimental procedures were approved by The Ethical Committee of Animal Research.

2.2. Chemicals and animal groups

LPS and AMG (Sigma-Aldrich Chemical Co) were freshly dissolved in sterile saline before injection. The rats were divided randomly into five groups as the following protocol: (1) Control which received saline instead of both LPS and AMG, (2) LPS group which received LPS (1 mg/kg/day; i.p.) during 4 weeks and also 120 min before behavioral tests. These animals received 1 ml/kg saline instead of AMG, (3) LPS-AMG 50 mg/kg (LPS-AMG 50), (4) LPS-AMG 100 mg/kg (LPS-AMG 100) and (5) LPS-AMG 150 mg/kg (LPS-AMG 150) groups which underwent a daily injection of LPS (1 mg/kg/day; i.p.) during 4 weeks and also 120 min before behavioral tests and received 50, 100 and 150 mg/kg of AMG dissolved in saline (i.p.) 30 min before LPS administration. The drugs including LPS and AMG were obtained from Sigma Company (Sigma Aldrich Co, St. Louis, MO). Other chemicals which were utilized to measure oxidative stress criteria were obtained from Merck Company (Merck, Darmstadt, Germany).

2.3. Behavioral tests

2.3.1. Morris water maze (MWM) test

The MWM apparatus was consisted of a black circle pool (with a depth of 60 cm and a diameter of 150 cm) filled with water (23–24 °C temperature) to 30 cm deep. As previously described [19], the rats were required to find the location of a plexiglas platform which was located 2 cm beneath the water. The rats underwent four trials per day from different release positions for five consecutive days. A video-tracking system was employed to record the latency of finding the platform. During the acquisition phase, if the rat was unsuccessful to escape on the platform within 60 s, it would lead to climb on the platform. In order to perform a probe test on the 6th day, the platform was removed, and the rat was allowed to search the maze for 60 s and the time spent in the platform area was measured.

2.3.2. Passive avoidance apparatus (PA)

The training apparatus consisted of two compartments (dark and lighted sections) that were separated by a guillotine door. An electric shock was delivered to the dark section by an isolated stimulator. At the commencement of the test, each rat was positioned in the apparatus for 5 min to become habituated to it for two days. On the third day, an acquisition trial was performed; each rat was placed in the light compartment, the removable door was opened, and the rat was allowed to enter the dark compartment. The door was then lowered, and an unavoidable scrambled single electric shock (50 Hz and 1.5 mA for 2 s) was delivered. After exposure to the foot shock, the rat was taken away from the PA apparatus to its home cage. The retention of PA performance was tested 1, 24 and 48 h afterward. The rat was relocated gain in the lighted (safe) compartment with admittance to the dark section without any shock. Therefore, the latency to enter the dark compartment was calculated up to a maximum of 300 s [20].

2.4. Biochemical assessments

After the behavioral tests were completed, the animals were euthanized. The brains were dissected, and hippocampal tissues were detached on an ice-cold surface. The hippocampal tissues were homogenized using phosphate buffer solution (pH 7.4). The homogenates were centrifuged at 1500 rpm for 10 min to be utilized for measuring the malondialdehyde (MDA), total thiol and nitric oxide (NO) metabolites concentration and also SOD and CAT activity. Interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-α, BDNF and Aβ were measured in hippocampal tissues as well.

2.4.1. Measurement of IL-6, IL-10, TNF-α, BDNF, and Aβ

Specific ELISA kits (Ebioscience Co, San Diego, CA, USA) and the instructions provided by the manufacturer were utilized to determine IL-6, IL-10, TNF-α, BDNF and Aβ (MyBioSource Co, San Diego, CA, USA) concentrations in the hippocampal tissues. The measured absorbance of the samples in a microplate reader (Biotek, USA) was compared with an established standard curve in the same measurement,

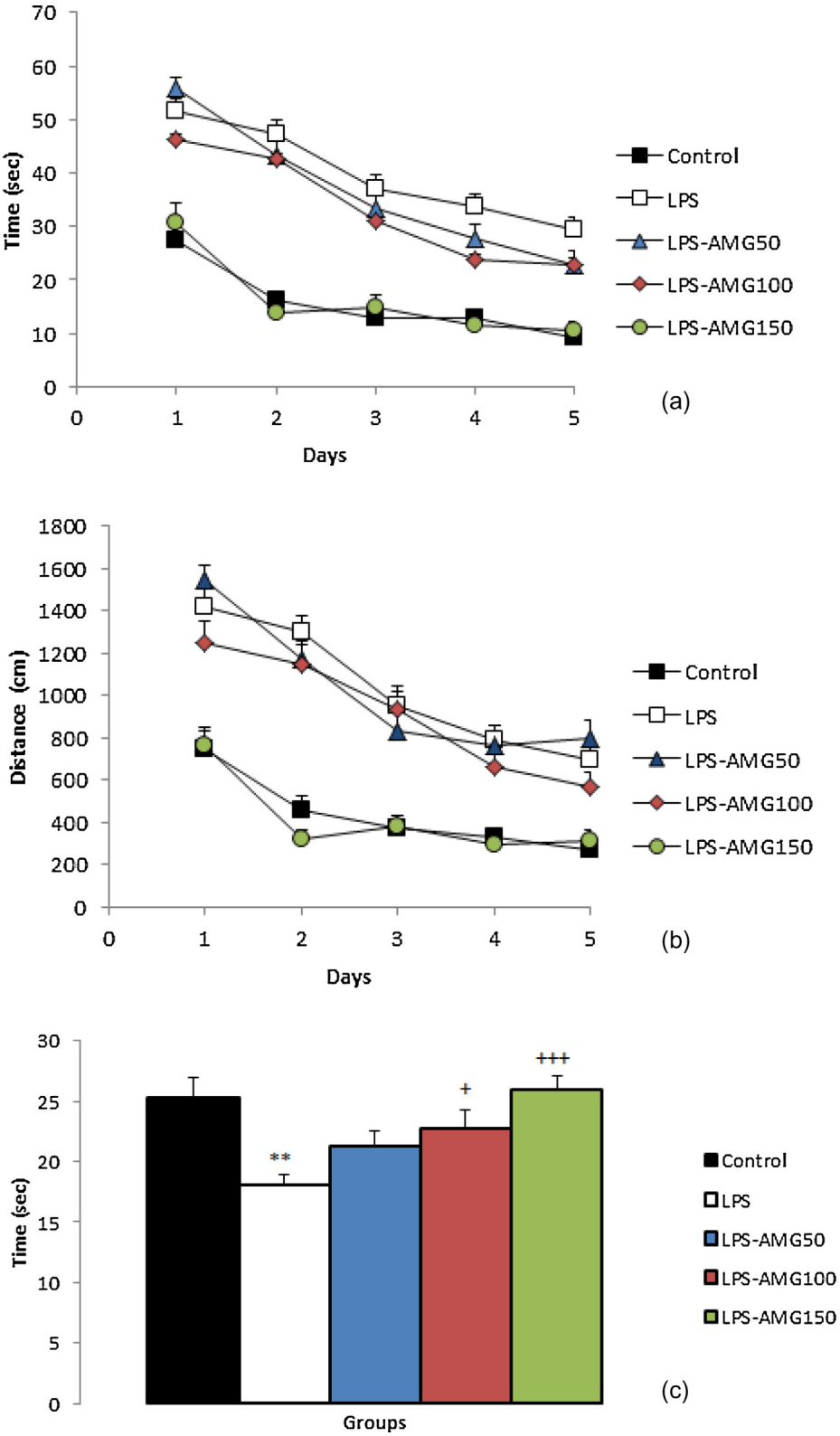

Fig. 1. A comparison of time latency (A), path length (B) to reach the platform and time spent in target quadrant (C) in MWM test among Control, LPS, LPS-AMG50, LPS-AMG100 and LPS-AMG150 groups. The data are presented as mean ± SEM (n = 8–10 per group). $^{**}P < 0.01$ comparison of LPS with Control group, $^{+}P < 0.05$ and $^{+++}P < 0.001$ comparison of LPS-AMG100 and LPS-AMG150 with LPS group.

and the concentrations were measured.

2.4.2. MDA assessment

MDA was measured as a biomarker of lipid peroxidation. MDA measurement method was described previously [21]. Briefly, one ml of the sample solution was mixed with 2 ml of thiobarbituric acid (TBA) + trichloroacetic acid (TCA + hydrochloric acid (HCl) (Merck) solution and was put in boiling water bath for 45 min. Finally, after

cooling, the whole solutions were centrifuged within 1000*g* for 10 min, and its absorbance was measured at 535 nm. MDA concentration was assayed based on the formula which was previously reported [21,22].

$C(M) = Absorbance/(1.56 \times 10^5)$.

2.4.3. The determination of total thiol concentration

The total thiol contents were measured in the tissue homogenates applying a method described by Ellman [23] and based on the previously described reports [21]. In summary, 50 μL of the supernatant of each sample and one mL of tris – ethylenediaminetetraacetic acid (EDTA) buffer was mixed, and the absorbance was read at 412 nm against tris-EDTA buffer alone labeled A1. Further, 20 μL of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) solution was added to A1, and the sample absorbance was read for the second time after 15 min labeled A2. The absorbance of DTNB was applied as blank (B). An equation was employed to calculate the total thiol concentration:

The total thiol concentration: (mM) = (A2−A1−B) × 1.07/0.05 × 13.6

2.4.4. Determination of SOD

SOD activity was measured based on Madesh and Balasurbamanian. The method is based on the generation of SOD through auto-oxidation of pyrogallol and dependent inhibition of 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) to formazan. The reaction stopped by dimethyl sulfoxide (DMSO). In sum, the supernatant of the sample was poured into the wells of the plate (96 wells). After 5 min, the DMSO was added, and the plate was observed with a micro plate reader at a wavelength of 570 nm. One unit of SOD was described as the amount of protein required to inhibit 50% reduction of MTT [24].

2.4.5. Determination of CAT

For CAT activity measurement, 100 μL H2O2 was mixed with phosphate buffer (pH = 7) and used for preparation of the solution that was applied for measurement (C buffer) 0.650 μL phosphate buffer (pH = 7) was utilized as solution blank. The cuvette for measurements was filled by the C buffer and sample homogenates. The reduction of absorption was determined by spectrophotometer at the wavelength of 240 nm for 5 min [25].

2.5. Statistical analysis

All data were expressed as mean ± SEM. SPSS software (11.5) was employed to evaluate the data. MWA and PA test data were analyzed during 5 days by the aid of repeated measures analyses of variance (ANOVA) followed by Tukey's post hoc comparisons test. The data of probe day of MWM test and biochemical data were compared by using one way ANOVA followed by Tukey's post hoc comparisons test. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Behavioral results

3.1.1. The results of MWM

The results of repeated measures ANOVA indicated that the day affected on the escape latency and traveled distance to reach the platform ($f(4, 195) = 131.679$; $P < 0.001$ for escape latency and $f(4, 195) = 87.620$; $P < 0.001$ for traveled distance)). Statistical analysis also demonstrated a significant group effect ($f(5, 1170) = 49.629$, $P < 0.001$ for escape latency and ($f(4, 195) = 46.985$, $P < 0.001$ for traveled distance)) and a significant interaction between factors (group × day) ($f(4, 195) = 2.516$, $P < 0.001$ for escape latency and ($f(4, 195) = 2.811$, $P < 0.001$ for traveled distance)) in the acquisition phase of learning. In addition, the results of the post hoc test indicated that training was accompanied by a reduction in the escape latency in all animals. Fig. 1A illustrates a significant difference in the elapsed time during the 5-day training period between LPS and control groups ($P < 0.001$). Also, traveling distance to find the platform in LPS group was higher than the control group ($P < 0.001$; Fig. 1B). Additionally, MWM results indicated that the animals of LPS group didn't recall the location of the platform when they were examined in the probe trial. The LPS group spent less time in the target quadrant in comparison to the control group ($P < 0.01$; Fig. 1C).

Moreover, the results demonstrated that the animals which were pre-treated by two higher doses of AMG (100 and 150 mg/kg) spent less time to get to the platform ($P < 0.05$-$P < 0.001$). However, the lowest dose was not efficient to change the traveling time in order to reach the platform during five training days (Fig. 1A). The animals in LPS- AMG150 groups also had a lower traveling distance compared to LPS group ($P < 0.001$; Fig. 1B). The highest dose of AMG was more potent than the two lower doses to decrease the spent time and traveling distance in order to find the location of the platform ($P < 0.001$; Fig. 1A and B). Furthermore, 100 and 150 of AMG enhanced the time spent in the target quadrant compared to the LPS group ($P = 0.05$ and $P < 0.001$ respectively; Fig. 1C).

3.1.2. The results of PA test

The animals of LPS group entered the dark chamber faster than the control group at 1, 24 and 48 h after receiving the electric shock ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; Fig. 2). Administrating of 150 mg/kg AMG before LPS injection resulted in an increased latency to enter the dark chamber at 1, 24 and 48 h after receiving the shock compared to LPS group ($P < 0.01$, $P < 0.05$ and $P < 0.05$,

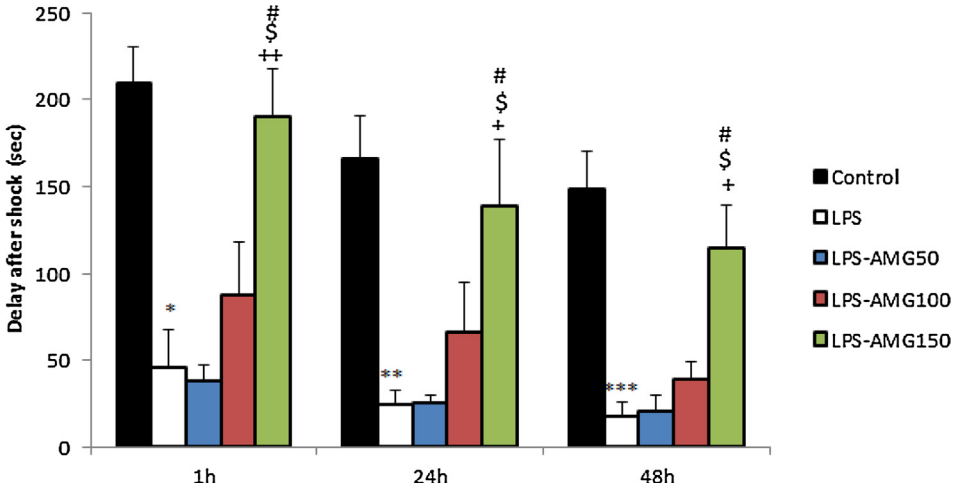

Fig. 2. Comparison latency time in PA test among Control, LPS, LPS-AMG50, LPS-AMG100 and LPS-AMG150 groups. Data are presented as mean ± SEM (n = 8–10 per group). $^{*}P < 0.05$ and $^{**}P < 0.01$ in comparison with Control group, $^{+}P < 0.05$ and $^{++}P < 0.01$ in comparison with LPS group. $^{\$}P < 0.05$ comparison of LPS-AMG150 with LPS-AMG50 group and $^{\#}P < 0.05$ comparison of LPS-AMG150 with LPS-AMG100 group.

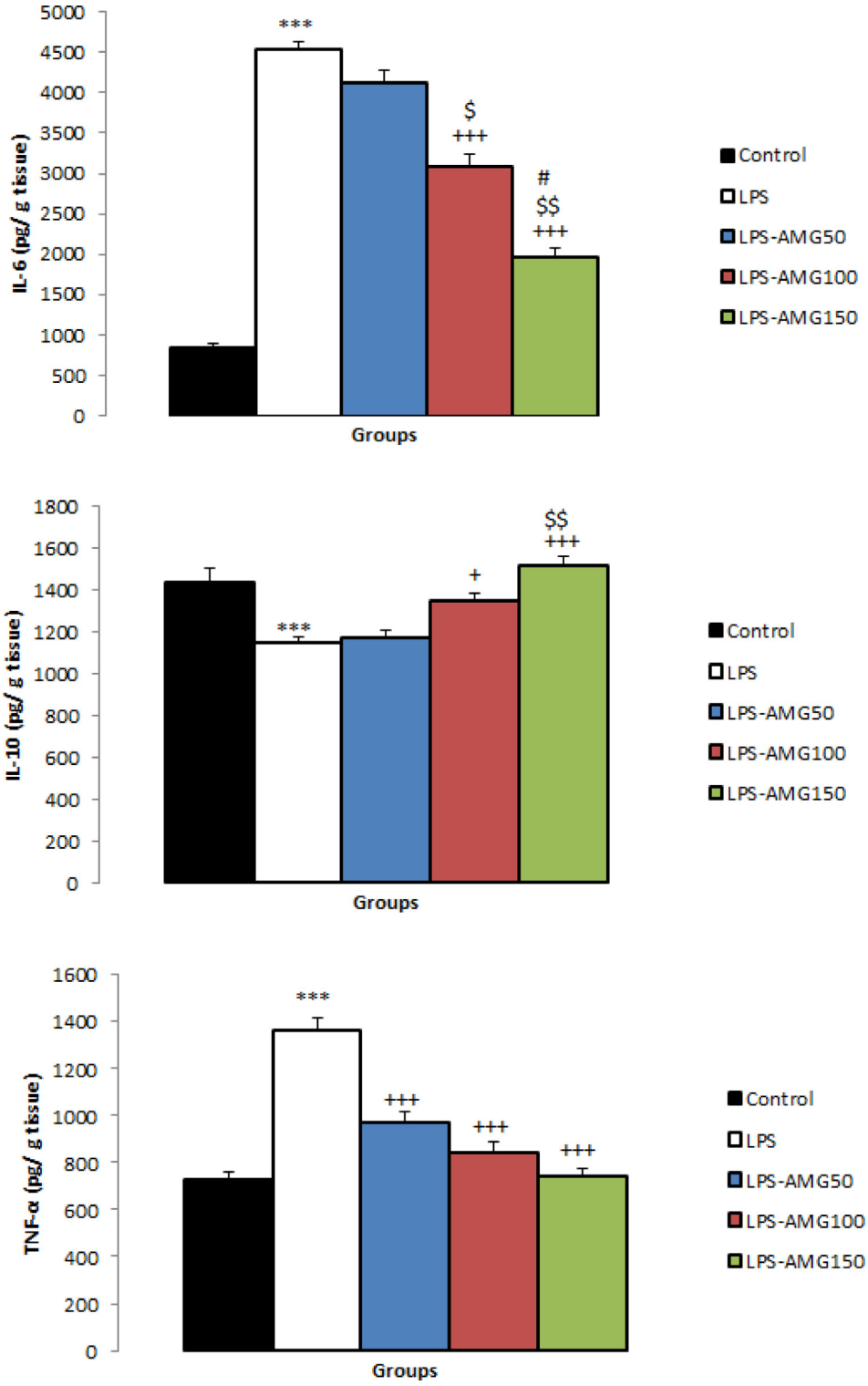

Fig. 3. Comparison of IL-6 (A), IL-10 (B) and TNFα (C) among Control, LPS, LPS-AMG50, LPS-AMG100 and LPS-AMG150 groups. Data are presented as mean ± SEM (n = 8–10 per group). $^{***}P < 0.001$ in comparison with Control group, $^{+}P < 0.05$ and $^{+++}P < 0.001$ in comparison with LPS group. $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ comparison of LPS-AMG100 and 150 with LPS-AMG50 group and $^{\#}P < 0.05$ comparison of LPS-AMG150 with LPS-AMG100 group.

respectively; Fig. 2). The latency for entering to the dark compartment in LPS- AMG 150 was higher than both groups treated by the two lower doses ($P < 0.05$; Fig. 2).

3.2. Biochemical measurements in the hippocampal tissues

3.2.1. Hippocampal IL-6, IL-10, and TNF-α levels

The hippocampal amount of IL-6 in LPS group was significantly elevated compared to the control group ($P < 0.001$). Pre-treatment by two higher doses of AMG (100 and 150 mg/kg) reduced the hippocampal IL-6 ($P < 0.001$ for both). However, the lowest dosage was not

effective (Fig. 3A). Moreover, the highest dose of AMG was more implicit than the lowest (P = 0.004) and the medium (P = 0.041) doses. Also, hippocampal IL-6 in LPS- AMG 100 group was lower than LPS-AMG 50 (P = 0.037; Fig. 3A).

The hippocampal content of IL-10 in LPS group was remarkably lower compared to the control group (p < 0.001). Pre-treatment by two higher doses of AMG (100 and 150 mg/kg) increased the hippocampal IL-10 (P = 0.033 and P < 0.001 respectively; Fig. 3B). Additionally, the highest dose of AMG was more potent than the lowest (P = 0.035; Fig. 3B).

The hippocampal tissues of LPS group had significantly elevated levels of TNF-α compared to the control group (P < 0.001). Pre-treatment by all doses of AMG (AMG 50, 100 and 150) decreased the hippocampal TNF-α (P < 0.001; Fig. 3C) significantly.

3.2.2. NO metabolites and Aβ concentration

NO metabolites in the hippocampal tissues of the LPS-treated group was higher than the control group (P < 0.001), while pre-treatment by two higher doses including 100 and 150 mg/kg AMG reduced the hippocampal tissues NO metabolites (P < 0.001). In addition, the highest dosage of AMG was more efficacious than the lowest (P < 0.01) and the medium (P < 0.05) doses. Also, hippocampal NO metabolites in LPS- AMG 100 group were lower than LPS-AMG 50 (P < 0.05; Fig. 4A).

The hippocampal content of Aβ in LPS group was meaningfully higher compared to the control group (p < 0.001). Pre-treatment by all doses of AMG (50, 100 and 150 mg/kg) decreased the hippocampal Aβ (P < 0.05-P < 0.001) (Fig. 4B). Additionally, the highest dosage of AMG was more advantageous than the lowest (P < 0.001) and the medium (P < 0.01) doses. Also, hippocampal Aβ in LPS- AMG 100 group was lower than LPS-AMG 50 (P < 0.01; Fig. 4B).

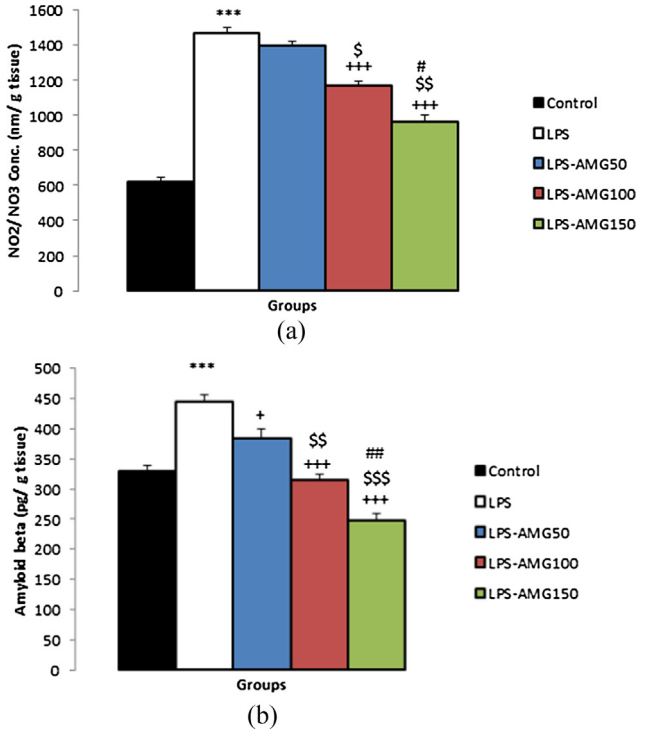

Fig. 4. Comparison of NO metabolites (A) and Aβ (B) among Control, LPS, LPS-AMG50, LPS-AMG100 and LPS-AMG150 groups. Data are presented as mean ± SEM (n = 8–10 per group). ***P < 0.001 in comparison with Control group, $^{+}$P < 0.05, $^{++}$P < 0.01 and $^{+++}$P < 0.001 in comparison with LPS group. $^{\$}$P < 0.05, $^{\$\$}$P < 0.01 and $^{\$\$\$}$P < 0.001 comparison of LPS-AMG100 and 150 with LPS-AMG50 group and $^{\#}$P < 0.05 and $^{\#\#}$P < 0.01 comparison of LPS-AMG150 with LPS-AMG100 group.

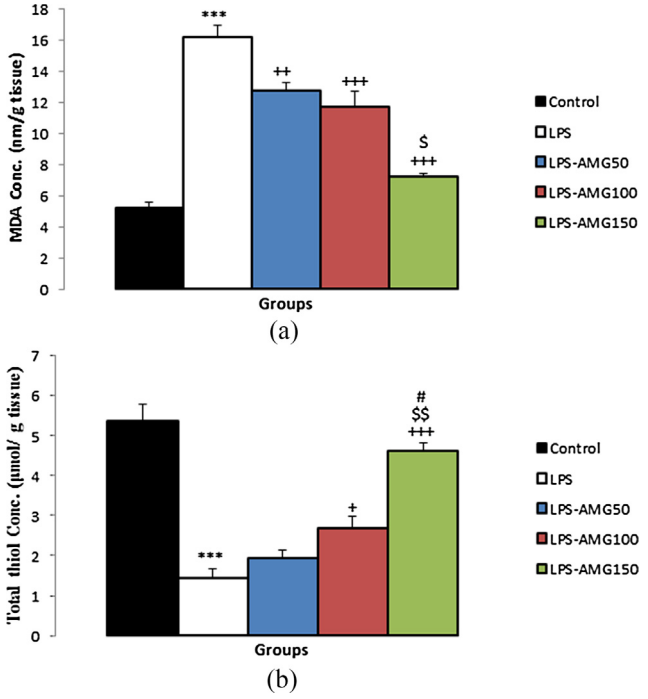

Fig. 5. The MDA concentrations (A) and total thiol concentrations (B) in hippocampal tissues of Control, LPS, LPS-AMG50, LPS-AMG100 and LPS-AMG150 groups. Data are presented as mean ± SEM (n = 8–10 per group). ***P < 0.001 in comparison with Control group, $^{+}$P < 0.05 and $^{+++}$P < 0.001in comparison with LPS group. $^{\$}$P < 0.05 and $^{\$\$}$P < 0.01 comparison of LPS-AMG150 with LPS-AMG50 group and $^{\#}$P < 0.05 comparison of LPS-AMG150 with LPS-AMG100 group.

3.2.3. MDA concentrations and thiol content

In comparison to the control group, the animals of LPS group had higher MDA concentrations (Fig. 5A) and lower thiol contents (Fig. 5B) (P < 0.001). Administration of all doses including 50, 100 and 150 mg/kg AMG declined hippocampal MDA compared to the LPS group (P < 0.01-P < 0.001; Fig. 5A). Remarkably, in the animals treated with the highest dose of AMG, MDA level was lower than the groups treated with the lowest dose (P = 0.04; Fig. 5A). Furthermore, the two higher doses of AMG augmented the hippocampal total thiol contents compared to LPS group (P < 0.05-P < 0.001; Fig. 5B), However, the lowest dose was not effective. Additionally, the highest dose was more effective than the lowest dose (P < 0.05) and the medium dose (P < 0.05), to improve the hippocampal thiol contents (Fig. 5B).

3.2.4. SOD and CAT activity

The findings also demonstrated that the LPS administration decreased hippocampal SOD (P < 0.001). The mentioned effect of LPS was weakened by the highest dose of AMG (P < 0.001); however neither 50 nor 100 mg/kg of AMG were significantly effective on LPS-induced deviations on SOD levels. Moreover, hippocampal SOD in the animals treated with 150 mg/kg AMG was higher than the lowest and medium doses of AMG (P = 0.006; Fig. 6A).

Administration of LPS also attenuated hippocampal CAT in comparison to the control group (P < 0.001). Pre-treatment by 100 and 150 mg/kg of AMG improved CAT in the hippocampus (P = 0.006-P < 0.001), but 50 mg/kg of AMG did not reveal any alteration in the hippocampal CAT content (Fig. 6B). In addition, hippocampal CAT in the animals treated with 100 and 150 mg/kg AMG was higher than the ones treated by 50 mg/kg (P = 0.029-P = 0.007).

3.2.5. BDNF content

The hippocampal content of BDNF in LPS group was significantly

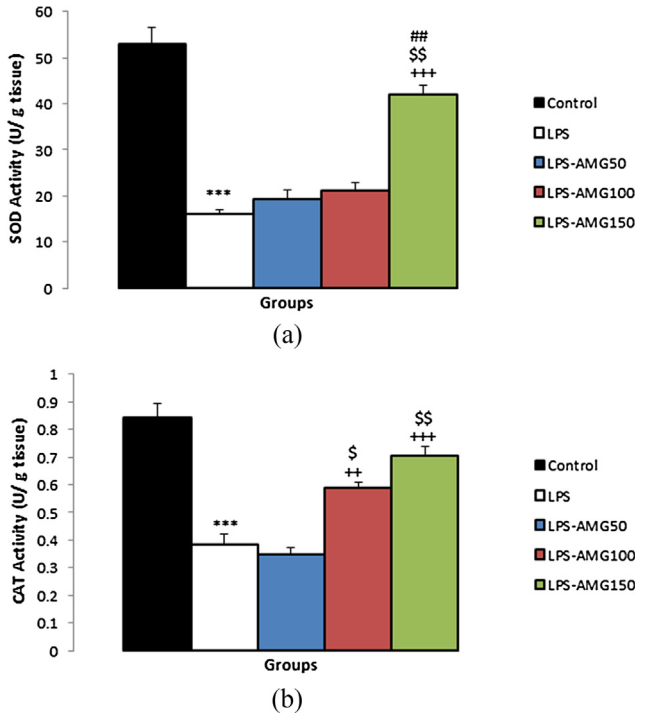

Fig. 6. The SOD (A) and CAT (B) activities in hippocampal tissues of Control, LPS, LPS-AMG50, LPS-AMG100 and LPS-AMG150 groups. Data are presented as mean ± SEM (n = 8–10 per group). $^{***}P < 0.001$ in comparison with Control group, $^{++}P < 0.01$ and $^{+++}P < 0.001$in comparison with LPS group. $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ comparison of LPS-AMG100 and 150 with LPS-AMG50 group and $^{\#\#}P < 0.01$ comparison of LPS-AMG150 with LPS-AMG100 group.

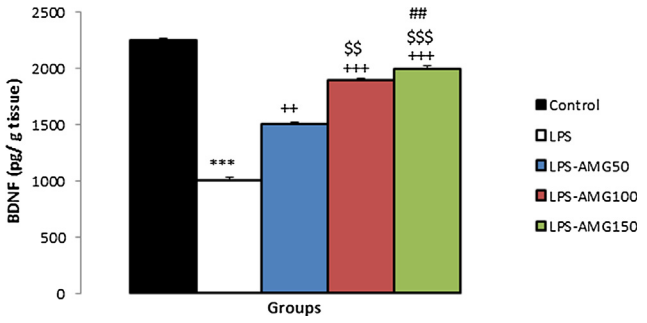

Fig. 7. BDNF in hippocampal tissues of among Control, LPS, LPS-AMG50, LPS-AMG100 and LPS-AMG150 groups. Data are presented as mean ± SEM (n = 8–10 per group). $^{***}P < 0.001$ in comparison with Control group, $^{++}P < 0.01$ and $^{+++}P < 0.001$in comparison with LPS group. $^{\$\$}P < 0.01$ and $^{\$\$\$}P < 0.001$ comparison of LPS-AMG100 and 150 with LPS-AMG50 group and $^{\#\#}P < 0.01$ comparison of LPS-AMG150 with LPS-AMG100 group.

lower compared to the control group ($P < 0.001$). All AMG treated groups had higher BDNF content compared to LPS group ($P = 0.007$-$P < 0.001$; Fig. 7). Additionally, the highest dose of AMG was more potent than the lowest dose ($P < 0.001$) and the medium dose of AMG ($P = 0.004$). Also, pre-treatment by a medium dose of AMG increased hippocampal BDNF content compared to lowest dose ($P = 0.007$; Fig. 7).

4. Discussion

The current examination aimed to elucidate the possible mechanisms for the protective effects of AMG against LPS-induced spatial and non-spatial memory impairments in rats. In the present study, chronic i.p. injection of LPS induced learning and memory impairment indicated by the results of MWM and PA test. In MWM test, the time spent and traveled distance to find the platform in LPS group was significantly longer than the control group. Interestingly, after removing the platform, the time spent in target quadrant in LPS group was lower than the control group which implies that the animals of LPS group did not recall the location of the platform. Furthermore, in PA test the animals of LPS group had lower latency to enter the dark compartment than the rats of the control group after-shock. In confirmation with the results of current study, researchers expressed that LPS administration induces learning and memory impairment in MWM and the Y-maze tests [26].

LPS is an endotoxin of gram-negative bacteria and performs to increase the inflammatory cytokine generation and cell apoptosis [27]. In recent years, it has been proved that inflammation plays an essential role in inducing learning and memory impairment [8]. Brain inflammation can induce tissue injury by increasing the microglia and astrocytes activity in the brain of LPS injected animals and leads to spatial memory dysfunction [8,28].

In the current study, impaired learning and memory that induced by LPS was associated with an elevated level of IL-6 and TNF-α and a decrease in IL-10 in the hippocampal tissues. It was quite perceived that neuroinflammatory processes are followed by activation of glial cells and production of a great variety of inflammatory markers, such as IL-1, IL-6 and, TNF-α which potentially contribute to neuronal dysfunction predominantly in the hippocampus region and subsequently in impairment of memory and learning [29]. IL-6 is a cytokine that has a proinflammatory property. Basically, IL-6 affects leukocytes in numerous ways including stimulating B-cell proliferation and antibody secretion as well as inducing proliferation of cytotoxic T cells [30]. Interleukin-10 (IL-10) is a powerful anti-inflammatory cytokine that affects macrophage function in various ways. IL-10 significantly decreases the amount of TNF-a, IL-1, IL-6, IL-8, and interferon-y (IFN-y) produced by stimulated macrophages [31]. IL-10 obstructs the ability of macrophages to secrete reactive oxygen species (ROS) and prevents the production of NO [32].

Additionally, a well-known relationship was elucidated between oxidative stress and the learning and memory deficits [33]. The results of the present study confirmed that LPS injection was followed by an oxidative stress status in the brain tissue which was presented by an increased level of MDA and NO metabolites while a decreased level of total thiol concentration and SOD and catalase activities. Consistently, it has been previously indicated that neuroinflammation induced by LPS is accompanied by an increased level of MDA and a decrease in glutathione, thiols, SOD and CAT [34–36].

In addition, it was demonstrated that the chronic injection of LPS was followed by an increased level of Aβ in the hippocampus which may also consider as a mechanism for learning and memory-impairing effects of neuroinflammation. The mentioned findings may also elucidate a relationship between neuroinflammation and AD [37,38]. Amyloid beta was measured utilizing an ELISA kit and it requires an evaluation by using more precise methods including PCR and immunohistochemistry. Previously, it was also suggested that systemic inflammation induced by LPS could lead to memory impairment by means of enhancing amyloid genesis [39].

In the present study, LPS also decreased BDNF in the hippocampus to consider as another possible mechanism(s) for learning and memory-impairing effects of neuroinflammation. It has been well known that many cellular populations of the immune system can affect the production of neurotrophins, a family of proteins that indorse survival and differentiation of specific neuronal populations [40]. In particular, the immune cells have some interactions with BDNF [41]. Moreover, the production of BDNF appears selectively controlled by LPS [42]. In AD patients, the results described above are contradictory, however, several studies described a BDNF modification at mRNA and protein level, in both the brain [43] and the periphery [44], suggesting an

involvement of this neurotrophin in AD. In confirmation with the presented results, in another study LPS decreased the level of BDNF, nerve growth factor (NGF) and NT-3 [45]. In the present study BDNF level was evaluated by ELISA method however, it was better to measure BDNF expression using qPCR or by immunohistochemistry in the hippocampus.

The former studies mentioned that the excessive generation of NO is accompanied by several biochemical events such as lipid peroxidation, oxidation of protein and thiols oxidation to induce and oxidative stress status [46]. An overproduced level of NO was considered to be accompanied by learning and memory impairment [47,48] which was attributed to an increased level of iNOS activity [47,49]. There is also a lot of evidence indicating that iNOS inhibition attenuates inflammatory responses and oxidative damage while improving learning and memory [50]. AMG as a selective iNOS inhibitor has been able to suppress inflammation and inhibit Aβ deposition to demonstrate neuroprotective, learning and memory-improving effects [17]. In the current study, AMG enhanced spatial and non- spatial memory in MWM and PA test. In MWM test AMG-treated rat spent less time to find the platform and increased the time spent in target quadrant compared to animals in LPS group. Also, in PA test latency for entering to the dark compartment in AMG treated groups was higher than LPS group. In a previous study, it was revealed that AMG had protective effects against learning and memory deficit induced by LPS. It was also mentioned that AMG had a protective effect against oxidative injury in the brain [50]. In a previous study, the effects of a single dose of AMG were examined and it was indicated that AMG improved learning and memory of the rats. Later in the present study the effects of three doses of AMG were investigated. They were chronically administered. In current study also, all doses of AMG decreased NO metabolites in the hippocampus which confirms that iNOS has a mediatory effect in learning and memory-impairing effects of LPS in rats which was mentioned in the present study. Similar to the previous work, all doses of AMG had protective effects against the hippocampal tissue oxidative damage which was presented by a decrease in hippocampal MDA while total thiol concentration and CAT and SOD activities were increased in the hippocampal tissues of LPS treated animals.

Previously, it was also indicated that the injection of 100 mg/kg of AMG for one week decreased TNF-α concentration in the serum of LPS-treated rats [50]. Recent reports indicate that, in the inflammatory process, glia activation causes an expression of iNOS and pro-inflammatory cytokines, such as IL-1β and TNF-α. IL-1β and TNF-α trigger the production of other cytokines, such as IL-6. Besides, IL-1β and TNF-α regulate iNOS expression in astrocytes and microglia to produce high concentrations of NO [51–53]. In order to have a better perception of the possible mechanism(s) the effects of three doses including 50, 100 and 150 mg/kg of AMG were examined in the current study and the levels of the cytokines in the hippocampus were measured. Findings of the presents study indicated that AMG increased IL-10 as an anti-inflammatory biomarker while, decreased IL-6 and TNF-α as inflammatory cytokines. Considering these results, it is suggested that AMG treatment is able to inhibit activation of glia and release of cytokines to improve the memory [14].

It was suggested that there is a synergy between Aβ and cytokines such as IL- 6 and TNF-α to induce microglial NO release [54,55], whereas others argued that an increased level of NO discharge was the result of astrocytic activation [56,57]. Moreover, it remains controversial whether Aβ is able to induce neuronal NO release or not [58]. The results of the present study illustrated that AMG prevented an overproduction of NO to improve learning and memory of LPS treated rats.

It was well documented that Aβ peptides may engender a dysfunctional encoding state in neurons, leading to neurodegeneration [59]. On the other hand, BDNF was perceived as a supporting factor of neurons and their connections that are vulnerable to AD [60,61]. It was demonstrated that BDNF promotes the neural cells survival [21] and has a protective effect against Aβ- induced neurotoxicity [62]. On the other hand, Aβ has been able to decrease BDNF expression [63,64]. In the present study, a decreased level in Aβ concentration in the hippocampus by AMG was accompanied by an increased level of BDNF. Considering these facts, improving BDNF as a possible mechanism(s) for learning and memory-improving effects of AMG might be suggested. However, it needs to be investigated more in the future studies. The mechanism of action of AMG is still unknown for reestablishing BDNF level in the hippocampus although the following prediction of the relationship between BDNF and AMG was presumed. First, BDNF can shelter hippocampal neurons against neuroinflammation induced by different inflammatory mediators [65]. Second, BDNF prevents NO-mediated neurotoxicity to protect neurons from damages [66]. Third, the metabolism of free radicals can be adjusted by BDNF to increase the SOD enzyme content in the neurons to decrease the accumulation of free radicals, thus, protecting neurons from free radical attack [67].

5. Conclusion

The results indicated that protective effects of AMG against LPS-induced memory were accompanied by decreasing of inflammatory cytokines, Aβ, oxidative stress and increasing of anti-inflammatory mediators and BDNF.

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Conflict of interest

The authors declared no conflict of interest.

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