The effects of thymoquinone on hippocampal cytokine level, brain oxidative stress status and memory deficits induced by lipopolysaccharide in rats

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ABSTRACT

Objective: The study objective was to determine the protective effects of thymoquinone (TQ) on brain tissues oxidative stress status, hippocampal cytokine level, and learning and memory deficits induced by lipopolysaccharide (LPS) in rats.

Methods: Animals were randomly divided into the following groups and treated: (1) Control (saline), (2) LPS (1 mg/kg i.p.), (3–5) 2, 5 or 10 mg/kg TQ extract 30 min before LPS injection. The treatment was started since two weeks before the behavioral experiments and continued during the behavioral tests (LPS injected 2 h before each behavioral experiment). Finally, the brains were removed for biochemical assessments.

Results: Morris water maze (MWM) test results showed that LPS increased escape latency compared to control group whereas TQ decreased them vs. LPS group. In passive avoidance (PA) test, LPS reduced the latency to enter the dark compartment vs. control group, while TQ treatment attenuated this effect of LPS. Additionally, LPS increased interleukin-6 (IL-6) and tumor necrosis alpha (TNF-α) in the hippocampal tissues. It also elevated malondialdehyde (MDA) and nitric oxide (NO) metabolites and decreased thiol content, superoxide dismutase (SOD) and catalase (CAT) in both hippocampus and cortex vs. control group, while TQ decreased IL-6, TNF-α, MDA and NO metabolites and increased thiol content, SOD and CAT compared to LPS group.

Conclusion: Findings of current study indicated that TQ improved LPS-induced learning and memory impairments induced by LPS in rats by attenuating the hippocampal cytokine levels and brain tissues oxidative damage.

1. Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder, which is associated with memory and cognitive impairments especially in the elderly population. A relationship between neuroinflammation and neurodegenerative diseases such as AD has been well documented. There are many evidence showing the significant influence of immune systems on mental functions such as learning, memory and neuronal plasticity [1]. Additionally, inflammation has been evidenced as a major reason of disturbed learning and memory [2,3]. On the other hand, systemic inflammation has been reported to be accompanied with oxidative stress status, which the later also has a critical role in memory and learning impairments [4]. Previous findings showed that inflammation may cause dysfunction in central cholinergic system in both hippocampal tissues and cerebral cortex in rats [5]. On the other hand researcher found that acetylcholine receptor antagonists have a detrimental effect on memory and this fact was emphasized by studying on Alzheimer’s patient [6].

Tissue damages and infection through activating of microglia can increase proinflammatory cytokines and mediators specially in hippocampus and eventually by neuronal dysfunction and defects in cell proliferation and differentiation of the neuronal progenitor cells can lead to suppressing of neurogenesis, memory, learning and long term potentiation (LTP) [7]. In addition, many studies have shown that inflammation induced by lipopolysaccharide (LPS) resulted in learning and memory impairments through releasing of pro-inflammatory...
cytokines and inducing of reactive oxygen species (ROS) production [8,9]. There is also a good confirmation that oxidative stress contribute in the central nervous system (CNS) disorder pathogenesis and also in learning and memory impairments [10].

Thus it is suggested that protecting against the oxidative injury and use of agents with antioxidant, anti-inflammatory and acetyl choline-terase (AchE) inhibitory properties can reverse the memory impairment. So researcher found that thymoquinone (TQ), the most active component of the volatile oil of Nigella sativa seed, is one of this agent and exert anti-inflammatory and neuroprotective effects [11,12]. Traditionally and experimentally, Nigella sativa and TQ have been used for their therapeutic effects on many degenerative diseases like Parkinson, AD and schizophrenia [13-15]. Therefore, in the current study we aimed the effects of TQ on LPS induced learning and memory impairments, hippocampal cytokine level and brain tissues oxidative damage in rats.

2. Materials and methods

2.1. Animals

Twelve weeks-old male Wistar rats (240 ± 10 g) were purchased from local animal house located in Mashhad University of Medical Sciences, then accommodated in groups of 5 in individually ventilated cages and kept under standard conditions (temperature 22 ± 2 °C, humidity of 54 ± 2% and 12 h light/dark cycle). Food and water were freely available. The Ethical Committee of Animal Research approved the experimental procedures.

2.2. Chemicals and groups

LPS (Sigma-Aldrich Chemical Co) freshly dissolved in sterile saline prior to injection. TQ, also purchased from sigma, dissolved in sterile warm saline supplement with ethanol prior to injection. Animals were randomly divided into five groups as follows: (1) Control, (2) LPS, (3) LPS-TQ 2 mg/kg (LPS-TQ 2), (4) LPS-TQ 5 mg/kg (LPS-TQ 5) and (5) LPS-TQ 10 mg/kg (LPS-TQ 10).

The animals in the LPS, LPS-TQ 2, LPS-TQ 5 and LPS-TQ 10 groups were treated with a daily injection of LPS (1 mg/kg/day; i.p.) during two weeks and also 120 min before behavioral tests. The groups 3-5 received 2, 5 and 10 mg/kg of TQ dissolved in 0.1 ml ethanol 96% and diluted by 2 ml saline (i.p.) 30 min before LPS administration. Both control and LPS groups received 2 ml/kg saline supplemented by ethanol instead of TQ supplements. In addition, control group received saline instead of LPS.

2.3. Behavioral procedures

2.3.1. Morris water maze apparatus and procedures

A circle shaped black pool was filled with 23–24 °C water (pool dimensions: 60 cm deep × 136 cm diameter). A circular platform (10 cm diameter, 28 cm high) was submerged about 2 cm below water surface. The apparatus had of two compartments (dark and lighted sections) with a grid floor. The two parts were connected to each other by a small gate. This test is based on native preference of animals to stay in the dark environment. Prior starting the training sessions, animals were introduced to the apparatus for 5 min per day in two consecutive days. In following day, they were placed in the light compartment and the time latency for entering the dark compartment was measured, as well as the time spent in dark and light compartments. During the training phase, the animals were placed in the lighted compartment, away from the gate and facing toward the walls. When they entered the dark part, they received an electric shock (1.5 mA for 2 s). The retention or test phase conducted at one hour, and then 24 h after the training sessions, by placing animals in the light compartment, and measuring the time latency to enter the dark compartment, as well as the time spent in the dark and light compartments.

2.4. Biochemical assessments

After completing the behavioral tests, animals were euthanized. Brain tissues were dissected, and hippocampal and cortical tissues were separated on an ice-cold surface. The tissues were homogenized with phosphate buffer solution (pH 7.4). The homogenates were centrifuged at 1500 rpm for 10 min and content of malondialdehyde (MDA), thiol, superoxide dismutase (SOD), Catalase (CAT) and nitric oxide (NO) metabolites were determined in hippocampal as well as cortical tissues. Additionally, IL-6 and TNF-α were measured in hippocampal tissues.

2.4.1. Measurement of IL-6 and TNF-α

Specific ELISA kits (ebioscience Co, San Diego, CA, USA) and the instructions provided by the manufacturer were used to determine TNFα and IL-6 concentrations in the hippocampal and IL-6 in the cortical tissues. The measured absorbance of the samples in a micro-plate reader (Biotek, USA) was compared with an established standard curve in the same measurement and the concentrations were calculated.

2.4.2. MDA assessment

MDA, is a biomarker of lipid peroxidation, was measured based on MDA reaction with thiobarbituric acid (TBA), which produces a pink complex with a peak absorbance at 535 nm [16]. 375 mg of TBA was dissolved in 2 ml of hydrochloric acid (HCl), then 15 g trichloroacetic acid (TCA) was added, and the total volume was reached to 100 ml with distilled water. To dissolve TBA, the solution was incubated in a water bath at 50 °C. One ml of 10 percent homogenates solution was mixed with 2 ml of TBA + TCA + HCl solution and the solution was incubated in a boiling water bath for 45 min. After a few minutes, it was centrifuged and its absorbance was measured at 535 nm. MDA level was calculated based on the following formula (C (M) = A/1.65 × 10⁵) [16].

2.4.3. Determination of thiol content

The thiol contents was measured in the tissue homogenates using a method described by Ellman [17]. The supernatants were incubated with 5,5′-dithiobis(2-nitrobenzoic acid) (DTN) in a 1 ml tris-ethylene-diaminetetraacetic acid (EDTA) buffer (pH = 8.6). Reaction of DTNB with SH groups creates a yellow complex which has a peak absorbance at 412 nm. In summary, 50 μl of homogenate and 1 ml of tris-EDTA buffer were mixed and the absorbance was read at 412 nm against tris-EDTA buffer alone (A1). Then, 20 μl DTNB reagent (10 mM in methanol) was added and the sample absorbance was read again after 15 min (A2). DTNB reagent was used as blank (B). The following equation was used for calculation of total thiol concentration [18].
Total thiol concentration (mM) = \((A_2 - A_1 - B) \times 1.07/0.05 \times 13.6\)

2.4.4. Determination of superoxide dismutase

Superoxide dismutase activity was measured based on a colorimetric assay described by Madesh and Balasubramanian, and by using 96 wells micro plates. The method is based on the production of superoxide dismutase through auto-oxidation of pyrogallol and dependent revived inhibition of 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) to formazan. The reaction stops by adding dimethyl sulfoxide (DMSO), which stabilizes the color [19]. Briefly, the homogenized tissue was poured into the wells and incubated at room temperature for 5 min. The reaction was stopped by adding DMSO and then read with a micro plate reader at a wavelength of 570 nm, and 630 nm as a reference wavelength. One unit of SOD is defined to amount of protein needed to inhibit 50% reduction of MTT. The results were expressed as units per gram tissues.

2.4.5. Determination of catalase

Catalase activity was measured based on its ability to decompose hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), which is reflected in reduction of absorption at 240 nm [20]. For this purpose, 30 mM H\textsubscript{2}O\textsubscript{2} was used as substrate and 50 mM phosphate buffer (pH = 7) used as an alternative substrate in a solution blank. Test solutions contained the appropriate volume of hydrogen peroxide and tissue homogenates. The reaction started by adding H\textsubscript{2}O\textsubscript{2} and reduction of absorption was measured by spectrophotometer at 240 nm wavelength for 3 min.

2.4.6. Measurement of nitric oxide (NO) metabolites

NO metabolites were measured using Griess reagent. Briefly, a standard curve was prepared and tissue samples (50 \textmu l of tissue suspension) were added to the Griess reagents, sulphanilamide and NED (Naphthylethylenediamine) solutions. Absorbance was measured at 520 nm using a microplate reader, and the level of NO metabolites were calculated from standard curve [21].

2.5. Statistical analysis

The data were provided as means ± SEM. A repeated measures analysis of variance (ANOVA) test and was used to compare the elapsed time to reach the platform during 5 days MWM between the groups. One-way ANOVA and LSD post hoc tests were used to analyze the time spent in the target quadrant in probe trial of MWM test, the data of PA test, and the biochemical data. Differences were considered statistically significant when P < 0.05.

3. Results

3.1. Behavioral results

3.1.1. The results of Morris water maze

According to the results of MWM test, training was associated with a reduction in the escape latency in all animals. Fig. 1A demonstrates a significant difference in the elapsed time across the 5-day training period between LPS and control groups (P < 0.05–P < 0.01). Additionally, MWM results demonstrated that the animals of LPS group didn’t remember location of the platform when they were examined in the probe trial. The LPS group spent longer time in the target quadrant compared to control group (P < 0.001; Fig. 1B). Furthermore, the results demonstrated that the animals pre-treated by two higher doses of TQ (5 and 10 mg/kg) spent less time to reach the platform (P < 0.05–P < 0.01) however, the lowest dose was not effective to change the traveling time to reach the platform during five training days (Fig. 1A). Additionally, all doses including 2, 5 and 10 mg/kg of TQ improved the time spent in the target quadrant compared to the LPS group (P < 0.01–P < 0.001; Fig. 1B). There was no significant difference between three doses of TQ.

3.1.2. The results of passive avoidance test

Animals of LPS group entered the dark chamber faster than control group at both 3 and 24 h after receiving the electric shock (P < 0.01–P < 0.001). Administrating of TQ before LPS injection resulted in an increased latency to enter the dark chamber in treatment groups at both 3 and 24 h after receiving the shock compared to LPS group (P < 0.05–P < 0.001). There was no significant difference between the three doses of TQ (Fig. 2A). Results of PA also revealed that the animals of LPS-treated group spent a longer time in the dark room than the control group at both 3 and 24 h after the shock (P < 0.01–P < 0.001). Pre-treatment by all doses of TQ decreased the time spent in the dark compartment where they had previously received a shock (P < 0.05–P < 0.001). There was no significant difference between the three doses of TQ in the time spent in the dark compartment (Fig. 2B). Additionally, injection of LPS decreased the time spent in the light compartment at both 3 and 24 h after the shock (P < 0.01–P < 0.001). The animals of all TQ-treated groups including LPS-TQ 2, LPS-TQ 5 and LPS-TQ 10 groups spent longer times in the light compartment compared to the LPS group (P < 0.01–P < 0.001). There was no significant difference between the three groups in the time spent in the light compartment (Fig. 2C).

3.2. Biochemical measurements in hippocampal tissues

3.2.1. Hippocampal IL-6 and TNF-α levels

The hippocampal content of IL-6 in LPS group was significantly higher compared to the control group (p < 0.001). Pre-treatment by two higher doses of TQ (5 and 10 mg/kg) decreased the hippocampal IL-6 (P < 0.05 and P < 0.001 respectively), however the lowest dose was not effective (Fig. 3A). Additionally, the highest dose of TQ was more effective than the lowest (P < 0.001) and the medium (P < 0.01) doses.

Additionally, the hippocampal tissues of LPS group had significantly higher TNF-α levels compared to the control group (p < 0.05). Pre-treatment by higher doses of TQ (TQ10) significantly decreased the hippocampal TNF-α (P < 0.05). Additionally, the highest dose of TQ was more effective than the lowest (P < 0.01) (Fig. 3B).

3.2.2. NO metabolites

NO metabolites in the hippocampal tissues of LPS-treated group was higher than control group (P < 0.05), while pre-treatment by all doses including 2, 5 and 10 mg/kg TQ decreased the hippocampal tissues NO metabolites (P < 0.05, P < 0.01 and P < 0.001 respectively). However, no significant difference was observed between different doses of TQ (Fig. 4).

3.2.3. MDA concentrations and thiol content

In comparison to control group, the animals of LPS group had a higher MDA concentration (Fig. 5A) and lower thiol contents (Fig. 5B), however the lowest dose was not effective. Interestingly, in the animals treated with the highest dose of TQ, MDA level was lower than that in the groups treated with the medium and the lowest doses (P < 0.05–P < 0.001) (Fig. 5A). Additionally, the two higher doses of TQ increased the hippocampal 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 to improve the hippocampal thiol contents (P < 0.01).

3.2.4. SOD and CAT

The results also showed that the LPS administration decreased hippocampal SOD (P < 0.001). This effect of LPS was attenuated by...
10 mg/kg of TQ (P < 0.001), however neither 2 nor 5 mg/kg TQ were significantly effective on LPS-induced changes on SOD levels. Additionally, hippocampal SOD in the animals treated with 10 mg/kg TQ was higher than ones treated by 2 mg/kg (P < 0.001) (Fig. 6A).

Administration of LPS also decreased hippocampal CAT compared to control group (P < 0.001). Pre-treatment by all three doses of TQ, including 2, 5 and 10 mg/kg, decreased the hippocampal tissues CAT content (P < 0.001). However, no significant difference was observed between different doses of TQ (Fig. 8).

3.3. Biochemical results in cortical tissues

3.3.1. Cortical IL-6 level

The cortical content of IL-6 in LPS group was significantly higher compared to the control group (P < 0.001). Pre-treatment by all three doses of TQ decreased the cortical IL-6 (P < 0.001) (Fig. 7). Additionally, the highest dose of TQ was more effective than the medium and the lowest doses (P < 0.001). The results also showed that the highest dose was more effective than medium dose (P < 0.01).

3.3.2. NO metabolites

Additionally, LPS administration increased the NO metabolites concentrations in the cortical tissues of LPS group compared to the control group (P < 0.001). Pre-treatment by all doses of TQ, including 2, 5 and 10 mg/kg, decreased the hippocampal tissues NO metabolites (P < 0.001). However, no significant difference was observed between different doses of TQ (Fig. 8).

3.3.3. MDA concentrations and thiol content

The biochemical assessment also showed that LPS administration was associated with increased levels of MDA (Fig. 9A), and reduced concentrations of thiol in cortex samples (Fig. 8B) (both P < 0.001). Administration of all three doses of TQ, including 2, 5 and 10 mg/kg, resulted in reduction of cortical MDA compared to LPS group (P < 0.01–P < 0.001) (Fig. 9A). Furthermore, treatment by the highest dose of TQ was more effective to decrease cortical MDA vs. other doses of TQ (P < 0.001) (Fig. 9A). Additionally, the highest dose was able to increase cortical thiol contents compared to LPS (P < 0.001), while the lowest and the medium doses did not change the cortical total thiol content compared to LPS group. Also, treatment by the highest dose of TQ was more effective to increase cortical thiol content when was compared to the effect of medium and the lowest TQ doses (P < 0.001) (Fig. 9B).

3.3.4. SOD and CAT

The level of SOD in cortex of LPS-treated group was lower compared to control ones (P < 0.001). TQ, in three different doses, was able to elevate cortical SOD vs. LPS group (P < 0.01–P < 0.001). Furthermore, cortical SOD in the animals treated with 5 and 10 mg/kg of TQ was higher than that of 2 mg/kg (P < 0.001). The effects of 5 mg/kg and 10 mg/kg TQ on cortical SOD were not significantly different (Fig. 10A).
LPS administration also decreased cortical CAT (\(P < 0.001\)), which was prevented by all three doses of TQ (\(P < 0.001\)). Additionally, cortical CAT in the animals treated with both 5 and 10 mg/kg TQ was higher than those treated by 2 mg/kg TQ (\(P < 0.001\)). Interestingly, the highest dose was more effective than the medium dose (\(P < 0.001\)) (Fig. 10B).

4. Discussion

The present investigation aimed at elucidating mechanistically the possible protective effects of TQ against LPS-induced spatial and non-spatial memory impairments in rats. Although different mechanisms may contribute to create memory impairment, it has been clarified in recent years that inflammation plays a key role in this process [22]. Inflammation of the brain has been suggested to induce tissue damages through increasing of activity of microglia and astrocytes in the brain.
Fig. 3. The hippocampal IL-6 (A) and TNF-α (B) levels. Data are shown as Mean ± SEM (n = 10 per group). * P < 0.05 and ** P < 0.001 compared with control group, + P < 0.05 and +++ P < 0.001 compared with LPS group, $$$ P < 0.001 compared to LPS-TQ 2 group, & & P < 0.01 compared to LPS-TQ 5 group.

Fig. 4. The hippocampal NO metabolites. Data are shown as Mean ± SEM (n = 10 per group). * P < 0.05 compared with control group, + P < 0.05, ++ P < 0.01 and +++ P < 0.001 compared with LPS group.
LPS, the most potent agent to induce inflammation, is an endotoxin that mimics infection by gram-negative bacteria and prompts inflammatory cytokine production, mitochondrial dysfunction, and cell death [24], thereby leading to LTP suppression in the hippocampus and impaired spatial memory function [25]. In the present study, we showed that LPS administration for a mild term period was associated with impaired learning and memory in rats, when they were examined in both MWM and PA tests. The results also showed that the animals of LPS-treated group spent more time to find the platform during 5 days MWM learning. Interestingly, after removing the platform, the LPS group had impaired ability to remember its location. Additionally in PA test, the animals of LPS-treated group entered the dark compartment, where they had previously received an electronic shock, with less latency than control group. The results also showed that LPS administration increased the time spent in the dark, while decreased the time spent in the light compartment. It has been well known that neuro-inflammatory processes is followed by activation of glial cells and release of wide variety of inflammatory markers, such as IL-1, IL-6 and TNF-α which potentially contribute to neuronal dysfunction particularly in hippocampus area and eventually in impairment of memory and learning [26]. In the present study, learning and memory impairment induced by LPS was accompanied with an increased level of IL-6 and TNF-α in the hippocampal and IL-6 in the cortical tissues however, the TNF-α concentration was not determined in the cortical tissues. Many studies demonstrated that an increased level of immune system activity lead to a decreased level of spatial learning and memory performance in the rodents [27]. It has been indicated that the different parts of brain have different level of sensitivity to inflammation. Among them, hippocampus is more sensitive to inflammation, and its response to inflammatory status reflects in impaired spatial and contextual memory processes [28,29]. The possible mechanism might be the high concentration of inflammatory cytokine receptors in hippocampus, which results in cytokines inhibit LTP, and induces a deficit in hippocampus performance, reflected in learning and memory impairment [30,31]. Several studies demonstrated that TNF-α level elevates in a various neuropathological situations that are relevant with learning and memory shortages [32]. In this regard, it has been clarified that there is correlation between TNF-α signaling pathway with inducible nitric oxide synthase (iNOS) expression in a mouse model of AD [33,34]. Also, it is been reported that activation of microglia leads to production of reactive nitrogen species (RNS) and reactive oxygen species (ROS) which triggers neuronal damage in neurodegenerative disorders [35]. An increased level of ROS production has been suggested to be involved in LPS-induced hippocampal synaptic plasti-
It is well documented that oxidative damage and inflammation lead to learning and memory impairments [36]. A close relationship between the degree of oxidative damage and the learning and memory deficits was described [37,38]. Increased generation of ROS/RNS causes oxidative/nitrosative stress, leading to cellular macromolecules (lipids, proteins, and DNA) damages and impairments in normal neuronal functions. Oxidative/nitrosative stress has been shown to impair synaptic plasticity and cognitive functions, as observed in some neurodegenerative disorders and aging process [37–40]. In addition, ROS may cause neuroinflammation via increased production of proinflammatory cytokines and chemokines, such as TNF-α, the leads to the loss of synapses, and consequently cognitive deficits [41]. In the present study, LPS administration increased MDA in both hippocampal and cortical tissues, while decreased thiol content, and SOD and CAT activities. On the other hand, phytochemicals with antioxidant and anti-inflammatory properties have been shown to improve learning and memory functions [42–45]. According to this fact and the evidence that has repeatedly confirmed antioxidant effects of *Nigella sativa* and its main component TQ [10,46–48], it was assumed that administration of TQ might be beneficial for treatment of memory impairment induced by LPS. Neuroprotective and improving effects of *Nigella sativa* and TQ on learning and memory have been documented [10,46–50]. This study confirmed beneficial effects of TQ on learning and memory, since TQ-treated rats spent less time to find the platform compared to LPS group. The animals of TQ-treated groups also better remembered location of the platform, which was presented by a longer time spent in the target quadrant. Learning and memory improving effects of TQ was also confirmed in PA test, which was presented by a longer delay for entering to the dark room after the shock. TQ also increased the time spent in the light, while decreased the time spent in the dark
Fig. 8. Cortical NO metabolites. Data are shown as Mean ± SEM (n = 10 per group). *** P < 0.001 compared with control group, +++ P < 0.001 compared with LPS group.

Fig. 9. Cortical MDA concentrations (A) and thiol contents (B). Data are shown as Mean ± SEM (n = 10 per group). *** P < 0.001 compared with control group, ++ P < 0.01 and + + P < 0.001 compared with LPS group, $$$ P < 0.001 compared to LPS-TQ 2 group, & & & P < 0.001 compared to LPS-TQ 5 group.
compartment where they have previously received a shock.

Our results also revealed that administration of TQ was accompanied with reduction in hippocampal IL-6 and TNF-α content. Other researchers have also suggested that both *Nigella sativa* and TQ exerted anti-inflammatory properties by suppressing NF-kB and inhibition of cytokine production [51]. We also previously showed that a hydro-alcoholic extract *Nigella sativa* and its main component TQ improved depression, anxiety and sickness behaviors induced by LPS [52,53]. Additionally, it has been previously reported that *Nigella sativa* supplementation in patients with rheumatoid arthritis for a period of eight weeks significantly increased anti-inflammatory cytokine (IL-10) while, reduced pro-inflammatory cytokine, TNF-α [54]. The anti-inflammatory effects of *Nigella sativa* has been repeatedly attributed to its lipid-soluble component including TQ [55–58]. Considering the results of present study, protection against neuro-inflammation might be considered as an explanation for the benefits of TQ on nervous system functions especially on learning and memory.

In this study, TQ also restored SOD and CAT activities, while reduced MDA and NO metabolites levels in the brains of LPS-treated rats, again suggesting the antioxidant and anti-inflammatory properties of TQ as a possible mechanism in prevention of LPS-induced learning and memory impairments. Accordingly, the anti-oxidant effects of TQ has been frequently demonstrated in many studies and using a varied range of animals models [59–61]. Similar to our work, another study showed that TQ was able to reduce MDA levels in hippocampal tissues of rats [15]. We have previously considered the anti-oxidative effects of *Nigella sativa* as a responsible mechanism for its learning and memory improving effects in several animal models, such as models using scopolamine, hypothyroidism and repeated seizures as inducers of learning and memory impairment [10,46,47]. In general, considering the results of present study it seems that both antioxidative and anti-inflammatory effects of TQ should be involved in its beneficial effects on LPS-induced learning and memory impairments. Additionally recent in vitro studies showed that TQ is able to show antioxidant and anti-inflammatory effects by suppressing LPS-induced microglial cells activation [62–64]. Considering these reports and the fact that microglial activation has an important role in pathology of neurodegenerative diseases, such as AD, protection against microglial activation might be considered as a possible mechanism for the beneficial effects of TQ on learning and memory observed in the present study, however it needs to be more investigated in future studies.

In addition, inhibition of iNOS activity and prevention from NO overproduction has been suggested as a possible mechanism for memory improving effects of TQ in PTZ-induced kindling cognitive impairments [65] which may consider as possible mechanism for learning and memory effects of TQ which was seen in the present study.
study. Using animals models, a correlation between TNF-α signaling pathway with iNOS in AD has been suggested (33, 34). In an our previous study, aminoguanidine as an iNOS inhibitor, ameliorated deleterious effects of LPS on memory and long term potentiation in rats[66]. Additionally, it is well documented that acetylcholine has a key role in learning and memory process, therefore its decreased release in AD is involved in memory impairment [67]. Pharmacological studies have demonstrated that TQ is able to inhibit acetylcholinesterase (AChE) activity, and prevent degeneration of nervous cells and further decline in cognitive ability [68]. Considering these reports, it is possible that AChE inhibitory effects of TQ are also involved in its improving effects on learning and memory observed in the present study, but more investigations are required in future.

5. Conclusion

In conclusion, the results of current study indicated that TQ improves learning and memory impairments induced by LPS in rats. Moreover, the effects of TQ are accompanied with attenuating hippocampal cytokine levels and improving oxidative damage biomarkers in the brain tissues.

Conflict of interest

The authors declare no conflict of interest.

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