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Protective effects of *Nigella sativa* on synaptic plasticity impairment induced by lipopolysaccharide

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Abstract

In the present study the protective effect of *Nigella sativa* (*N. sativa*) on synaptic plasticity impairment induced by lipopolysaccharide (LPS) in rats was investigated. Fifty-eight rats were grouped and treated as follows: 1) control (saline), 2) LPS, 3) LPS-*N. sativa*, and 4) *N. sativa.* In a Morris water maze test, the escape latency and traveled path to find the platform as well as time spent and the traveled distance in target quadrant (Q_1) were measured. Long term potentiation (LTP) from CA_1 area of hippocampus followed by high frequency stimulation to Schafer collateral was studied and slope, slope 10-90% and amplitude of field excitatory field potential (fEPSP) were calculated. The escape latency and traveled path in LPS group were significantly higher than those in the control group while, in LPS-*N. sativa* group these parameters were significantly lower than those in LPS group. The rats in LPS group spent less time and traveled shorter distance in Q_1 than the rats in the control group while, in LPS-*N. sativa* group the rats spent more time and traveled longer distance than the rats in LPS group. LPS significantly decreased slope, slope 10-90% and amplitude of fEPSP while, in LPS-*N. sativa* group these parameters increased compared to LPS group. The results indicated that the hydro-alcohol extract of *N. sativa* protected against synaptic plasticity and spatial learning and memory impairment induced by LPS in rats.

اثر محافظتی سیاهدانه بر اختلال در پلاستیسیتی سیناپسی القا شده بوسیله لیپوپلی ساکارید

چکیده

در مطالعه حاضر اثر محافظتی سیاهدانه بر اختلال در پلاستیسیتی سیناپسی القا شده بوسیله لیپوپلی ساکارید (LPS) در موش صحرایی بررسی شد. ۵۸ سر موش صحرایی نر به طور تصادفی بدین صورت گروه بندی و درمان شدند: ۱)کنترل (سالین)، ۲) LPS، ۳) لیپوپلی ساکارید–عصاره سیاهدانه (LPS-*N. saitva*) و ۴) سیاهدانه (*N. sativa*). در آزمون ماز آبی موریس تأخیر زمانی، مسیر طی شده برای رسیدن به سکو و همچنین زمان گذرانده شده و مسافت طی شده در ربع هدف (Q_1) ثبت شد. تقویت طولانی مدت در ناحیه CA1 هیپوکامپ به دنبال تحریک با فرکانس بالای مسیر شافرکولترال انجام و شیب، شیب ۱۰ تا ۹۰ درصد و دامنه پتانسیل پس سیناپسی تحریکی میدانی (fEPSP) محاسبه شد. تأخیر زمانی و مسیر طی شده برای رسیدن به سکو در موشهای گروه LPS به طور معنی داری از گروه کنترل بالاتر بود، در حالیکه این شاخصها در موش‌های گروه LPS-*N. sativa* پایینتر از موشهای گروه LPS بودند. زمان سپری شده و مسافت طی شده در Q_1 در موش‌های گروه LPS کمتر از گروه کنترل بود، در حالیکه این شاخصها در گروه LPS-*N. sativa* نسبت به گروه LPS بیشتر بودند. LPS شیب، شیب ۱۰ تا ۹۰ درصد و دامنه fEPSP را در مقایسه با گروه کنترل کاهش داد، در حالیکه در گروه LPS-*N. sativa* این شاخصها در مقایسه با گروه LPS افزایش یافت. نتایج نشان بیان می کند که عصاره هیدروالکلی سیاهدانه باعث حفاظت در برابر اختلال در پلاستیسیتی سیناپسی و بهبود اختلال یادگیری و حافظه فضایی القا شده با LPS در موش صحرایی می شود.

واژه های کلیدی: پلاستیسیتی سیناپسی، تقویت طولانی مدت، حافظه فضایی، سیاه دانه، لیپوپلی ساکارید

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Introduction

The immune system plays a crucial role in modulating brain functions such as learning, memory and synaptic plasticity.[1] The harmful effect of neuro-inflammation on learning and memory is mainly mediated via inflammatory cytokines including interleukin (IL)-1β,[2] IL-6 and tumor necrosis factor α (TNF-α).[3] Long term potentiation (LTP) is a well-known model of cellular synaptic plasticity which underlies memory storage in the hippocampus.[4] It has been reported that excessive production of pro-inflammatory cytokines attenuates synaptic plasticity and reduces LTP induction in hippocampus.[5] For example, in pathological levels, TNF-α has been shown to disturb LTP induction in the CA1 and dentate gyrus of the rat hippocampus.[6]

Lipopolysaccharide (LPS) is the potent particle of gram negative bacteria which is used experimentally to induce the inflammatory responses within the brain of rodents.[7] It has been reported that systemic and central administration of LPS results in a cascade of cytokines synthesis and release such as IL-1β, IL-6 and TNF-α from immune cells.[4] The results of scientific studies also show that LPS and LPS-triggered cytokines including IL-1β and IL-6 which disturb spatial learning and memory through suppressing of LTP in the hippocampus.[4,8] Inhibition of LTP in dentate gyrus by this bacterial endotoxin in *in-vitro* was also documented.[9]

Nigella sativa is an annual flowering plant which possesses several important active compounds including thymoquinone (TQ), thymohydroquinone, dithymoquinone and carvacrol.[10] In traditional medicine it is known as beneficial remedy against disorders occurred in respiratory system, digestive tract, kidney, liver and cardiovascular system as well as immune system.[11] Some of its ingredients such as TQ have been proposed to have anti-inflammatory effects via decreasing the level of pro-inflammatory cytokines and enhancing anti-inflammatory cytokines.[12] Furthermore, the beneficial effects of *N. sativa* on learning, memory and cognitive functions were well documented in both human and animal models.[13,14] For example, in animal models *N. sativa* oil has been found to enhance spatial memory in male diabetic rats.[15] Regarding the detrimental effects of overproduction of inflammatory cytokines on memory which may happen after LPS injection and based on the anti-inflammatory effects of *N. sativa,* the current study was designed to evaluate possible protective effects of *N. sativa* on synaptic plasticity impairment induced by LPS.

Materials and Methods

Animals and chemicals. Fifty-eight male Wistar rats (12 weeks old and 240 ± 10 g weight) were purchased from animal house of Mashhad University of Medical Sciences, Mashhad, Iran. The animals were housed in a standard (temperature 22 ± 2 ˚C and 12 hr light/dark cycle) condition with a freely access to the food and water. All experiments were conducted in accordance with procedures approved by the Committee on Animal Research of Mashhad University of Medical Sciences (proposal No. 931398). Twenty-eight rats were classified into four groups: (1) control (2) LPS, (3) LPS-NS, and (4) *N. sativa* (n = 7 in each group). The rest of the rats (30) were divided into three groups (1) control, (2) LPS and (3) LPS-NS (n = 10 in each group) and used for electrophysiological studies. LPS and extract of *N. sativa* were dissolved in saline. The animal of the control group received 2 mg kg^{-1} of saline. In LPS and LPS-NS groups, LPS (1 mg kg^{-1}, ip) was injected 2 hr before the behavioral and electro-physiological experiments. The extract of *N. sativa* (200 mg kg^{-1}, ip) was administered 30 min before LPS or saline in LPS-NS and *N. sativa* groups respectively. In LPS group, the rats received 2 mg kg^{-1} of saline instead of *N. sativa*. All drugs were prepared freshly. The LPS (*E. coli* 055:B5), was purchased from Sigma Aldrich (St. Louis, USA).

Preparation of the extract. *Nigella sativa* seeds were obtained from a local herbal shop at Mashhad, Khorasan Razavi Province, Iran. To prepare the hydro-alcoholic extract, 100 g of cleaned and dried *N. sativa* seeds were grounded and mixed with 70% ethanol in a Soxhlet apparatus.[16] The extract was then dried and stored at –20 ˚C.

Morris water maze (MWM) test. The MWM apparatus was made of a cylindrical tank (136 cm in diameter, 60 cm in high and 30 cm in deep) with boundaries of four quadrants. The tank was filled with water (23-25 ˚C) and a circular platform (10 cm in diameter and 28 cm in high) was hidden 2 cm beneath the surface of the water in the center of the northwest quadrant. Fixed visual cues at several locations around the maze and on walls of the room determined the navigation path. Before each experiment, for familiarizing with the apparatuses, the animals were placed in the maze filled with water without a platform for 30 sec. In acquisition test, the animals were placed randomly in the tank at one of four positions (north, east, south and west) and allowed to freely swim to find the hidden platform within 60 sec. Motion of the rats was recorded by a mounted camera above the center of the pool and the recorded data were transferred to a computer. The rats motions, time spent and traveled distance to reach the platform were recorded and analyzed by Radiab software (Borje Sanaat Co., Tehran, Iran). If the rat found the platform within 60 sec it was allowed to remain on the platform for 20 sec before the next trial otherwise, it was guided to the platform by the experimenter and permitted to stay on the platform for 20 sec. The experiments were repeated with four trials in each day for five consecutive days. The mean of the time spent and traveled distances were estimated to evaluate

the spatial learning ability in the examined rats. Twenty-four hours after acquisition test, the platform was removed and a probe test was performed. The time spent and the traveled path in the target quadrant (Q1) and non- target quadrants (Q2-Q4) were compared between groups.[17,18]

Electrophysiological study. In electrophysiological experiments, the rats were deeply anesthetized with urethane (1.6 g kg^{-1}), and then their head was fixed in a stereotaxic apparatus. After removing the skin and exposing the skull, the proper location of CA1 area of hippocampus and Schafer collateral pathway on the skull were drilled. A bipolar stimulating stainless steel electrode (A-M Systems, Carlsborg, USA) with 0.125 mm in diameter was fixed in Schafer collateral pathway of right hippocampus (anteroposterior = 3.0 mm; mediolateral = 3.5 mm; dorsoventral = 2.8 to 3.0 mm) and a unipolar recording electrode with the same characteristics of stimulating electrode was lowered into the CA1 area of the ipsilateral (anteroposterior = 4.1 mm; mediolateral = 3.0 mm; dorsoventral = 2.5 mm).[19] Proper location of the electrodes was determined using physiological and stereotaxic indicators. The stimulating electrode and recording electrode were connected to a stimulator and an amplifier respectively. Field excitatory post synaptic potential (fEPSP) was recorded from CA1 area of hippocampus in following high frequency stimulations of the Schafer collateral pathway. Signals were amplified (100×) and filtered (1 Hz to 3 kHz band pass) using differential amplifier. After surgery, the animals were allowed to rest for 30 min. Then for evaluating synaptic potency before induction of LTP, the input-output (I/O) protocol was performed. For this purpose, the intensity of stimuli was increased gradually with a constant current as input and fEPSP was recorded as output. Then, 50.0% of the maximum response was considered for recording baseline before and after high frequency stimuli. After indicating maximum response, the baseline recording was exerted at 30 min before induction of LTP. After ensuring a steady state baseline response, LTP induction was administered by the high frequency stimuli (HFS) protocol of 100 Hz. The fEPSP was recorded for 90 min after high frequency stimuli. Throughout excrement, the rats were alive. The values of the slope and amplitude of the fEPSP were averaged from the 10 consecutive traces. For computer-based stimulating and recording, Neurotrace software version 9 and Eletromodule 12 (Science Beam Institute, Tehran, Iran) was used. The analysis of responses was carried out using custom software from the same institute.[20]

Statistical Analysis. All data were expressed as mean ± SEM and were analyzed using ANOVA followed by Tukey's post hoc test. Differences were considered statistically significant when $p < 0.05$.

Results

The results of MWM showed that the escape latency and traveled path in LPS group were significantly higher than in control group ($p < 0.05$ and $p < 0.001$, respectively). The animals of the LPS-NS group had significantly lower time latency and traveled length to reach the platform in comparison to the LPS group ($p < 0.05$ and $p < 0.001$, respectively). There was no significant difference between the *N. sativa* and control groups when the time latency and traveled length to find the platform was compared (Figs. 1A and 1B).

In the probe day, the rats of the LPS group spent less time and traveled shorter distance in target quadrant (Q_1) than rats in control group ($p < 0.01$ and $p < 0.001$, respectively). The animals in LPS-NS group spent more time and traveled more distance in Q_1 than rats in LPS group ($p < 0.05$ and $p < 0.01$, respectively) (Figs. 1C and 1D).

There was no significant difference between *N. sativa* and control groups. There were no significant differences between the control, LPS and LPS-NS groups in the time spent and the traveled distance in non-target (Q2-Q4) quadrants. The results also showed that the time spent and the traveled distance in non-target (Q2-Q4) quadrants by the animals of *N. sativa* group was lower than that of animals of LPS group ($p < 0.05$ and $p < 0.01$, respectively).

The results also showed that applying of HFS, increased slope of fEPSP in all groups including control, LPS and LPS-NS compared to before applying stimulation ($p < 0.001$, Fig. 2). After applying of HFS, the slope of fEPSP in LPS group was lower than that of control group ($p < 0.001$, Fig. 2A). In LPS-NS group, the slope of fEPSP was higher than that of the LPS group ($p < 0.001$, Fig. 2) however, it was also lower than that of the control group ($p < 0.001$, Fig. 2A).

Applying of HFS also increased 10-90% slope of fEPSP in all groups including control, LPS and LPS-NS compared to before applying stimulation ($p < 0.001$, Fig. 3). After applying of HFS, the 10-90% slope of fEPSP in LPS group was lower than that of control group ($p < 0.001$, Fig. 2B). In LPS-NS group, the 10-90% slope of fEPSP was higher than that of the LPS group ($p < 0.001$, Fig. 2B) however; there was no significant difference between the LPS-NS and control groups (Fig. 2B).

High frequency stimulation also increased amplitude of fEPSP in all groups including control, LPS and LPS-NS compared to before applying stimulation ($p < 0.001$, Fig. 3). After applying of HFS, the amplitude of fEPSP in LPS group was lower than that of the control group ($p < 0.001$, Fig. 3). In LPS-NS group, the amplitude of fEPSP was higher than that of the LPS group ($p < 0.001$, Fig. 3) however, it was also lower than that of the control group ($p < 0.001$, Fig. 3).

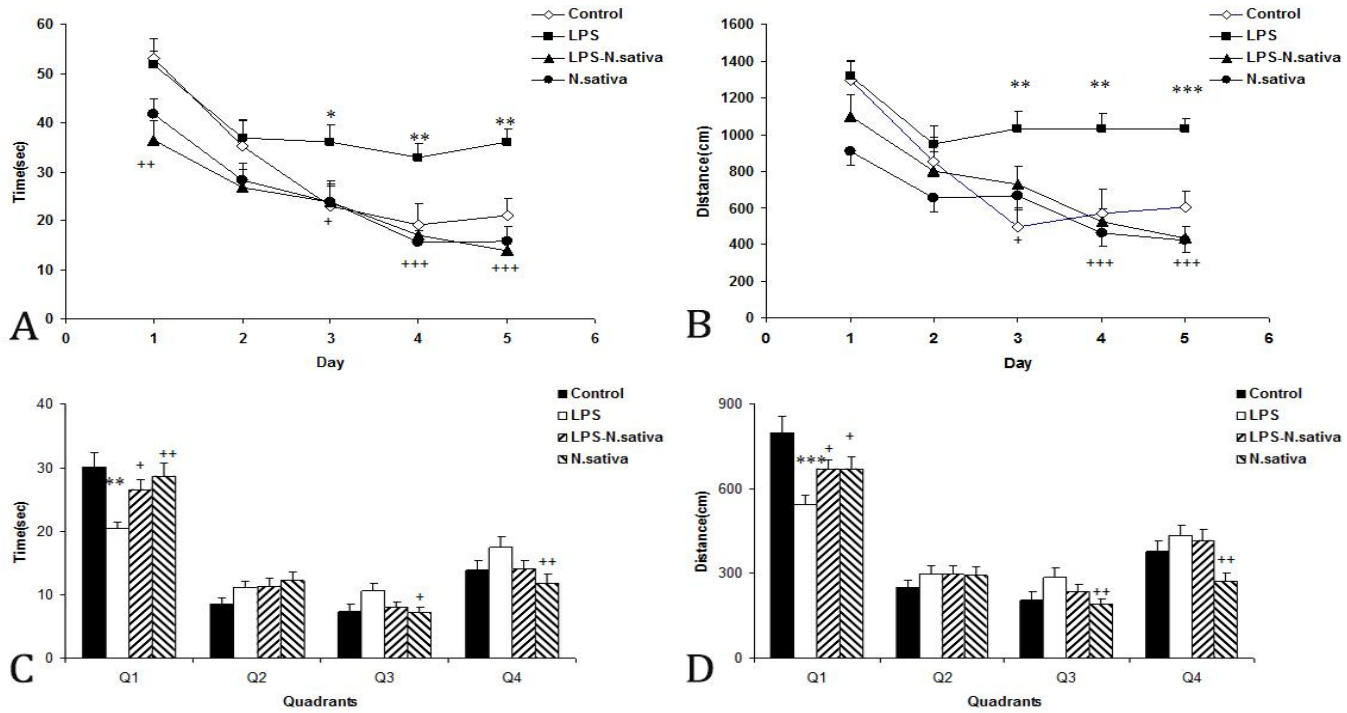

Fig. 1. Comparison of MWM test data between four groups. A) time latency, and B) the traveled distance to reach the platform during five days, C) time spent and D) the traveled distance in target quadrant (Q1) and non-target quadrants (Q2-Q4) in probe day, 24 hours after the last learning secession. Data are presented as mean ± SEM (7 in each group). The time latency of the LPS group was significantly higher than those of the control group whereas the animals of the LPS-NS group spent less time to reach the platform than the LPS ones. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with control group, $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ compared with LPS group.

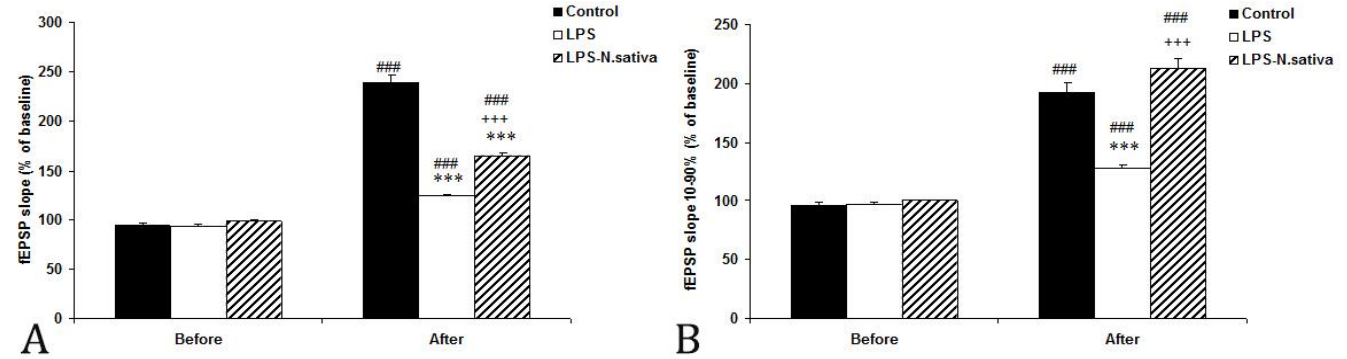

Fig. 2. A) Comparison of fEPSP slope recorded from CA1 area of the hippocampus before and after high frequency stimulation (HFS) in three groups; **B)** Comparison of fEPSP slope 10 - 90% recorded from CA1 area of the hippocampus before and after HFS in three groups. Data are presented as the average percentage change from baseline responses (n = 10 in each group). $^{###}p < 0.001$ compared to before high frequency stimulation in each group, $^{***}p < 0.001$ compared to control group, $^{+++}p < 0.001$ compared to LPS group.

Discussion

The results of the current study demonstrated that LPS impaired the spatial memory and synaptic plasticity in the examined rats which are consistent with our previous studies.[18,21] In our behavioral studies, the LPS-treated animals spent more time and traveled more distance to look for hidden platform than those of the control group.

In probe day, the rats of the LPS group also spent lesser time and traveled shorter distance than those in control group in the target quadrant. In electrophysiological studies, intraperitoneal administration of LPS also decreased the amplitude and slope of fEPSP in LPS group compared to the control group. This finding indicates that LPS attenuated synaptic plasticity and reduced LTP induction in the hippocampus of rat in current study. The destructive effects of LPS on learning and memory are attributed to synthesis and release of cytokines from macrophages and other cell types that are stimulated by LPS.[22] Injection of LPS into the fourth ventricle has been shown to accelerate inflammatory responses in the hippo-campus and entorhinal cortex which were accompanied with the death of pyramidal cells in CA3 region.[23]

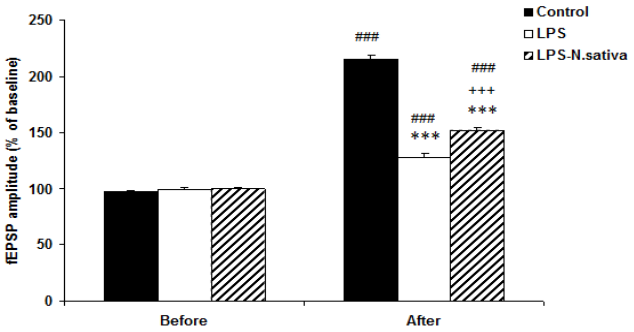

Fig. 3. Comparison of fEPSP amplitude recorded from CA1 area of the hippocampus before and after high frequency stimulation in three groups. Data are presented as the average percentage change from baseline responses (n = 10 in each group). ### $p < 0.001$ compared to before high frequency stimulation in each group, *** $p < 0.001$ compared to control group, +++ $p < 0.001$ compared to LPS group.

Pro-inflammatory cytokine, IL-1β, has been proposed to directly influence the nervous system and neurotransmitters such as serotonin, GABA, acetylcholine and noradrenaline in various brain areas.[24,25] The LTP, a useful model for studying molecular mechanisms involved in learning, memory and synaptic plasticity in the hippocampus[26] has been reported to be strongly influenced by neuro-inflammation.[23] LPS and IL-1β have been shown to debilitate the induction and maintenance of LTP in CA1 region and dentate gyrus of rats hippocampus.[4] Multiple studies have also indicated that increased levels of IL-1β in the hippocampus disturb synaptic plasticity resulting in LTP impairment that can attenuate learning and memory functions such as spatial learning task.[27] It has been found that LPS reduces paired-pulse facilitation in the CA1- subiculum pathway *in vitro* which may be due to higher levels of IL-1β followed by LPS injection.[9] In addition, influx of calcium via n-methyl-d-aspartate (NMDA) receptors has been shown to contribute in LTP induction in CA1 and CA3 regions of hippocampus.[28, 29] It has been documented that the IL-1β suppresses voltage-dependent calcium flow in the CA1 area[30] which in return, inhibits LTP induction in the hippocampus.[31] In an *in vitro* study, IL-1β has been also reported to reduce the amplitude of isolated field NMDA-EPSP which was fully antagonized by IL-1 receptor antagonist.[25] Regarding these data, it seems that overproduction of inflammatory cytokines followed by LPS-caused neuro-inflammation involves in deficit of synaptic plasticity and LTP induction impairment in the present study.

In our study the administration of *N. sativa* before LPS, restored LPS-induced spatial learning and synaptic plasticity impairments in the hippocampus of rats. Confirming this claim, in the behavioral experiments, the animals of LPS-NS group spent lesser time and traveled shorter distance for finding the scape platform than the rats of the LPS group. In the probe day, the animals of LPS-NS looked for the location of platform in target quadrant better than those of the LPS-treated group. In electrophysiological studies, injection of *N. sativa* before LPS also improved synaptic plasticity deficits caused by LPS. This data was confirmed by increasing the amplitude and slope of fEPSP in the animals of LPS-NS compared to the rats of LPS group.

To the best of our knowledge, the effects of *N. sativa* on LTP have not been previously reported to compare with the results of the present study. Additionally, responsible mechanism(s) for the effects of *N. sativa* extract on LTP was not investigated in the present study. It has been well evidenced that LTP is expressed by a persistent modification of postsynaptic AMPA (a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptors, a subclass of ionotropic glutamate receptors.[32,33] Researchers also showed that *N. sativa* can increase glutamate release and affects AMPA receptors[34] which may consider as possible mechanism for the effects of *N. sativa* on LTP.

Emerging studies have emphasized on immuno-modulatory properties and anti-inflammatory effects of *N. sativa* and its ingredients.[35,36] In an *in vitro* study, it has been shown that the aqueous extract of *N. sativa* can prevent the secretion of key inflammatory mediators such as IL-6, TNF-α and nitric oxide.[37] It has been proposed that TQ can reduce the level of pro-inflammatory cytokines including TNF-α[38] and enhance anti-inflammatory cytokines including IL-10.[12] In addition, TQ has been reported to improve the inflammatory responses through applying the inhibitory effects on cyclooxygenase (COX)-1 expression and generation of prostaglandin E2 (PGE2) in mice.[36] *Nigella sativa* oil was also considered as a natural modulatory factor of immune responses.[39] Considering these findings, it is suggested that improving the effect of *N. sativa* on LPS-induced synaptic plasticity and LTP induction impairment, at least in part, is due to its ability to serve as an anti-inflammatory agent in the current study.

The improvement effects of *N. sativa* on memory are most likely because of its ability to protect against cellular damage caused by oxidative stress.[40] It has been shown that *N. sativa* have a protective effect on lipid peroxidation process during ischemia reperfusion injury in the rat hippocampus.[41] Also, learning and memory improving effects of hydro-alcoholic extract of *N. sativa* in rats has been attributed to its protective effect against brain tissue oxidative damage.[42,43] Protection against acetylcholine esterase activity has also been considered as a possible mechanism for learning and memory improving effects of *N. sativa*.[34] However, all of these mechanisms require further investigation.

In conclusion, the results of the present study indicated that pre-treatment with *N. sativa* restored LPS-induced synaptic plasticity impairment in rat. Further studies are needed to be undertaken in order to determine more possible mechanism(s) and the responsible compounds for beneficial effects of *N. sativa* extract.

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Conflict of Interests

The authors have no conflict of interests to declare.

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