Physiology and Pharmacology

Physiol Pharmacol 23 (2019) 286-295 **www.phypha.ir/ppj**

Original Article

Effects of carvacrol on spatial learning performances, hippocampal interleukin-1ß level and oxidative stress markers in lipopolysaccharide-treated rats

Manizheh Gholami, Ziba Rajaei*, Maryam Malek

Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Introduction: Peripheral administration of lipopolysaccharide (LPS), can cause production of cytokines in the brain and subsequently impair learning and memory function. Carvacrol is a phenolic monoterpene that is found in the essential oils of the Lamiaceae family. Anti-inflammatory and antioxidant activities of carvacrol have been demonstrated in previous studies. The aim of the current study was to evaluate the effects of carvacrol on spatial learning performances in LPS-treated rats.

Methods: Male Wistar rats were pretreated with carvacrol at doses of 10, 25 and 50mg/kg for a week. Then, the animals received LPS injection (1mg/kg, ip) and treatments continued for 3 more weeks. Spatial learning performances were assessed in rats by the Morris water maze from post-injection days 18 to 21. Biochemical assays (interleukine-1β, lipid peroxidation and total thiol levels) were performed in the hippocampus and cerebral cortex at the end of the experiment.

Results: LPS-treated rats displayed higher escape latency and longer traveled distance as compared to control rats. In addition, chronic treatment of LPS-treated rats with carvacrol at a dose of 25mg/kg significantly decreased escape latency and traveled distance as compared to untreated-LPS rats. Biochemical assessments showed no significant difference in inflammatory and oxidative stress markers levels among the groups.

Conclusion: Our findings demonstrated that chronic treatment with carvacrol improves spatial learning performances in LPS-treated rats. This might be due to antioxidant, anti-inflammatory and anticholinesterase activities of carvacrol in early LPS challenge.

Keywords:
Carvacrol;
Lipopolysaccharide;
Spatial learning;
Cytokines;
Oxidative stress;
Inflammation

Received: 24 Apr 2019
Accepted: 20 Jul 2019

***Correspondence to:**
Z. Rajaei

Tel: +98-3137929181
Fax: +98-3136688597

Email:
rajaeiz@med.mui.ac.ir

Introduction

Neuroinflammation is implicated in the pathogenesis of many neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease and multiple schlerosis (Frank-Cannon et al., 2009; Block and Hong, 2005). AD is a progressive neurodegenerative disorder which associated with memory loss, spatial disorientation and deterioration of intellectual capacity (Carrero et al., 2012) and the fourth leading cause of death in developed countries (Ray and Lahiri, 2009). Neuroinflammation stimulates glial cells (astrocytes and microglia) to produce

cytotoxic mediators, such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), reactive oxygen species (ROS) and nitric oxide (Olajide et al., 2013). It is known that pro-inflammatory mediators disrupt neurochemical systems such as acetylcholine, and cellular functions in the hippocampus and basal forebrain which are involved in learning and memory processes (Tyagi et al., 2007).

The most effective stimulators to induce neuroinflammation in experimental animals are peripheral or central administration of lipopolysaccharide (LPS) (Spulber et al., 2012). LPS is an endotoxin which is released from the surface of gram-negative bacteria when they multiply or die and lyse (Wyns et al., 2015). LPS increases production of cytokines and free radicals from activated microglia, which mediate detrimental effects of neuroinflammation (Qin et al., 2004). It has been reported that intraperitoneal injection of LPS induces hippocampal apoptosis, cognitive impairment, learning deficits and beta-amyloid generation in the hippocampus (Lee et al., 2008; Shaw et al., 2001).

It has been demonstrated that herbal remedies are a potential source of antioxidant and anti-inflammatory compounds (Lima et al., 2013). Attention has been focused on herbal remedies owing to their safety and cost-effectiveness (Purushoth et al., 2012). Therefore, usage of natural products may be a therapeutic strategy for treating or preventing neuroinflammation in neurodegenerative diseases.

Carvacrol is a phenolic monoterpene that is found in the essential oils of the Lamiaceae family, formerly known as Labiatae, which includes the genera Origanum and Thymus (Oliveira et al., 2012; Guimarães et al., 2010). Several studies have shown a number of pharmacological activities of carvacrol, such as antioxidant and antinociceptive (Guimarães et al., 2010; Yu et al., 2012), anti-inflammatory (Landa et al., 2009), antibacterial (García-García et al., 2011), antifugal (Ahmad et al., 2011), anti-carcinogenic (Karkabounas et al., 2006) and antispasmodic (Oliveira et al., 2012) properties. The antioxidant activity and radical scavenging properties of carvacrol has been demonstrated in acute gastric lesions (Oliveira et al., 2012).

Based on anti-inflammatory and antioxidant activities of carvacrol, the present study was designed to evaluate the neuroprotective effects of carvacrol at doses of 10, 25 and 50mg/kg against LPS-induced neuroinflammation and spatial learning impairment in rats.

Materials and methods

Animals

Adult male Wistar albino rats (200-230g) procured from Royan Institute (Isfahan, Iran) were kept under controlled condition (22±2°C), 12h dark/light cycle with free access to pellet diet and water. The Ethic Committee for Animal Experiments at Isfahan University of Medical Sciences approved the study and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication, 8th edition, 2011).

Drug and chemicals

Carvacrol (Cat. No. 282197), E.coli LPS (L2880) and EDTA (E5134) were purchased from Sigma-Aldrich Co. The 2,2´dinitro-5,5´-dithiodibenzoic acid (DTNB, D218200), trichloro acetic acid (TCA, 100810), 2-thiobarbituric acid (TBA, 108180), tris (108382), Tween 80 (822187) and hydrochloric acid (HCL) were obtained from Merck.

Experimental design

The animals were randomly classified into five groups (n=8), including: 1) control group; 2) LPS group and 3-5) LPS groups treated with carvacrol intraperitoneally at doses of 10, 25 and 50mg/kg.

Carvacrol was emulsified with 1% Tween 80 and dissolved in normal saline. The control and LPS groups received 1% Tween 80 dissolved-saline at the same volume as the treated groups. Injection of carvacrol or 1% Tween started one week before LPS injection and continued for 23 days after LPS injection.

LPS injection

Rats were injected intraperitoneally with LPS at a dose of 1mg/kg (Vasconcelos et al., 2014). LPS (Sigma-Aldrich Co., USA) were dissolved in normal saline.

Morris water maze test

The Morris water maze, which is a hippocampal-dependent task, was used to examine spatial learning performances in rats. The maze consisted of a black

circular pool (150cm in diameter and 50cm height) that was filled with tap water (25±1°C), so that water depth was 30cm. The pool contained an escape circular platform (10cm in diameter) in the South-eastern quadrant, which was submerged approximately 2cm below the surface of the water. The location of the hidden platform was fixed during the experiment. The pool placed in a large dark room with various cues for spatial orientation, which was stable throughout the period of experiment. The swimming of each rat in the pool was monitored with a video camera and analyzed with a computerized tracking system. The training period consisted of four 60-second trials per day for 4 consecutive days with the platform located in the same position. Each rat was released into the water facing the wall of the pool from one of four different quadrants. In all the trials, rats were allowed to swim until to find the platform to escape from the water. If a rat could not escape within 60s, it was directed to the platform and placed on the platform for 30s. The escape latency and traveled distance for the animals to reach the platform were calculated by a computer software (NeuroVision, Tajhiz Gostar Co.). The daily score per animal was an average of the 4 trials it received on that day (Ahmadi et al., 2017).

Dissection and homogenization

After completion of the behavioral testing, rats were euthanized and the brains were removed from the skulls on day 23. The hippocampus and total cortex (approximately 400mg) were dissected out and weighed. A 10% (w/v) tissue homogenate was prepared in NaCl solution.

Cytokine levels

After centrifugation of hippocampal homogenates at 3000rpm for 5min, the supernatants were assayed for IL-1β using commercially available ELISA kits (BMS 630, ebioscience) according to the manufacturer's instructions. Results are shown as pg/ml.

Lipid peroxidation levels

Lipid peroxidation levels of the hippocampus and cortex were estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS), which is the end product of lipid peroxidation. Briefly, a mixture of TCA, TBA acid and HCl were added to 1ml of homogenate and the mixture was heated for 45min in a boiling water bath. After cooling, the samples were centrifuged at 1000g for 10min and the absorbance was measured at 535nm. The level of TBARS was calculated by: C(M)= Absorbance/1.65×10^5 (Rajaei et al., 2013).

Total thiol concentration

Total sulfhydryl groups were measured using DTNB as the reagent. Briefly, 1ml tris-EDTA buffer was added to 50µl homogenate and the sample absorbance was read at 412nm against the tris-EDTA buffer alone (A1). Then, 20µl of the DTNB reagent (10mM in methanol) was added to the mixture and after 15min, the sample absorbance was read again (A2). The absorbance of the DTNB reagent was also read as a blank (B). The total thiol concentration (mM) was calculated by: (A2-A1-B)×1.07/0.05×13.6 (Rajaei et al., 2013).

Statistical analysis

Data were expressed as mean±SEM. Statistical analysis was performed using statistical package SPSS, version 20. The data distributions for normality was checked by the Kolmogorov-smirnov test. Statistical analysis was carried out using one-way ANOVA and two-way repeated measures ANOVA followed by Tukey's post hoc test. A statistical *P*-value <0.05 was considered significant.

Results

Effects of carvacrol on spatial learning performances

Statistical analysis with two-way repeated measures ANOVA revealed that the escape latency ($F_{(3,105)}$=67.9, *P*<0.001, Fig. 1A) and traveled distance ($F_{(3,105)}$=75.22, *P*<0.001, Fig. 2A) for the trained rats decreased over the course of the 4 learning days in all groups, indicating spatial learning acquisition (Figs. 1A and 2A). The results also showed that LPS-treated rats displayed significantly higher escape latency ($F_{(4,35)}$=3.01, *P*<0.05, Figs. 1A and B) and traveled distance ($F_{(4,35)}$=2.88, *P*<0.05, Figs. 2A and B) compared to control rats. This indicates that LPS treatment disturbed the spatial learning acquisition phase in Morris water maze. Moreover, chronic treatment of LPS-treated rats with carvacrol at a dose of 25mg/kg significantly decreased escape latency (*P*<0.05, Figs. 1A and 1B) and traveled distance

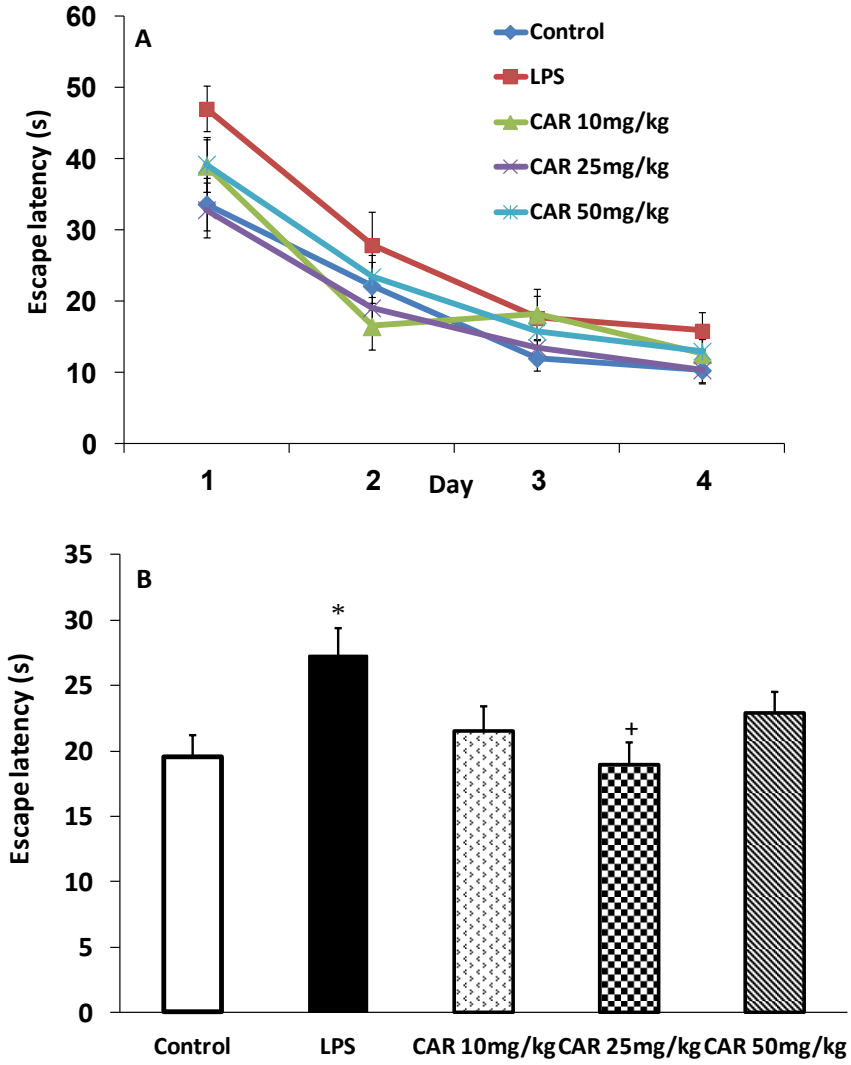

Fig.1. Effects of carvacrol at doses of 10, 25 and 50mg/kg on the performance of spatial memory acquisition phase in Morris water maze, (A) Escape latency during 4 days, (B) overall escape latency. Data are mean±SEM for eight animals in each group. $^{*}P<0.05$ vs control group, $^{+}P<0.05$ vs LPS group.

($P<0.05$, Figs. 2A and B) compared to un-treated LPS rats.

Effects of carvacrol on IL-1β levels

Statistical analysis showed that there was no significant change in IL-1β levels in the hippocampus of control and experimental groups ($F_{(4,35)}=1.07$, $P>0.05$, Fig. 3).

Effects of carvacrol on lipid peroxidation levels

TBARS levels, an index of lipid peroxidation, in the hippocampus and cortex of the control and experimental groups of rats are shown in Figure 4. There was no significant difference in lipid peroxidation levels in the hippocampus ($F_{(4,35)}=0.23$, $P>0.05$) and cortex ($F_{(4,35)}=1.23$, $P>0.05$) of control, untreated-LPS and LPS treated with carvacrol at doses of 10, 25 and 50mg/kg at the end of the experiment.

Effects of carvacrol on total thiol concentration

Figure 5 shows total thiol concentration in the hippocampus and cortex of the control and experimental groups. There was no significant difference in total thiol concentration in the hippocampus ($F_{(4,35)}=2.14$, $P>0.05$) and cortex ($F_{(4,35)}=2.45$, $P>0.05$) of control, untreated-LPS and LPS treated with carvacrol at doses of 10, 25 and 50mg/kg at the end of the experiment.

Discussion

The present study evaluated the effect of chronic administration of carvacrol on spatial learning impairment induced by LPS, as a rodent model of

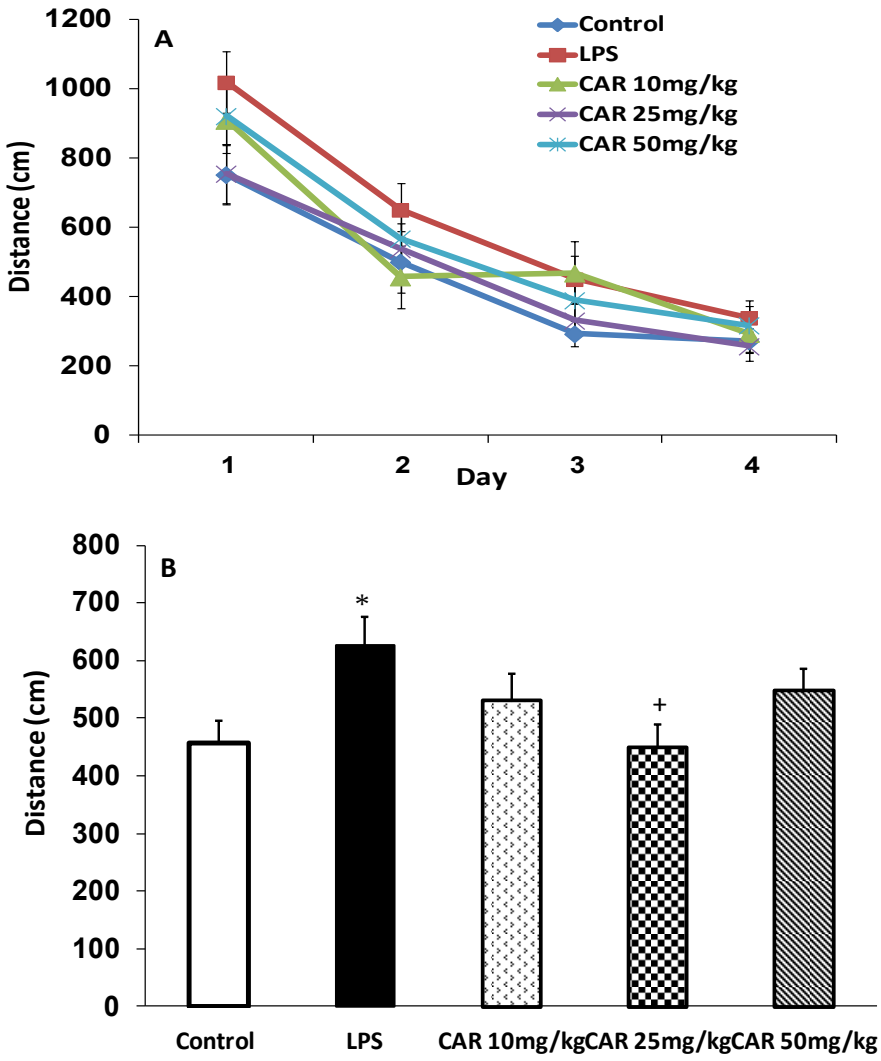

Fig.2. Effects of carvacrol at doses of 10, 25 and 50mg/kg on the performance of spatial memory acquisition phase in Morris water maze, (A) traveled distance during 4 days, (B) overall traveled distance. Data are mean±SEM for eight animals in each group. $^{*}P<0.05$ vs control group, $^{+}P<0.05$ vs LPS group.

inflammation and dementia. The current study confirmed that LPS significantly impaired spatial learning performances in the Morris water maze paradigm, since an increase in escape latency and traveled distance to reach the hidden platform was observed in LPS group during 4 days learning. This indicates that LPS treatment disturbed spatial memory acquisition phase in Morris water maze. Previous studies also confirmed that LPS is able to impair learning and memory (Hou et al., 2014; Arai et al., 2001; Ming et al., 2015). For instance, Huo et al. (2014) reported that single injection of LPS induced learning and memory impairment of mice in Morris water maze test. It has also been reported that LPS impaired passive avoidance learning and memory, and also performance in the novel object recognition test (Lee et al., 2008; Ming et al., 2015). The result of the present study also showed for the first time the beneficial effect of carvacrol on spatial learning performances in LPS-treated rats, since treatment with carvacrol at a dose of 25mg/kg shortened the time and distance to reach the platform during 4 days learning in LPS-injected rats. Interestingly, there was no significant difference in escape latency and traveled distance between control and CAR25 groups in our study. This beneficial effect of carvacrol on spatial learning is supported by previous studies which have reported the positive effect of carvacrol on learning and memory deficits in different models, such as diabetes (Deng et al., 2013) and Parkinson's disease (Haddadi et al., 2018).

Several mechanisms might be involved in impairment of spatial learning and memory ability in LPS-treated rats, including increased proinflammatory cytokines (Joshi et al., 2014; Tyagi et al., 2008), increased oxidative stress (Bai et al., 2016) and dysfunction of

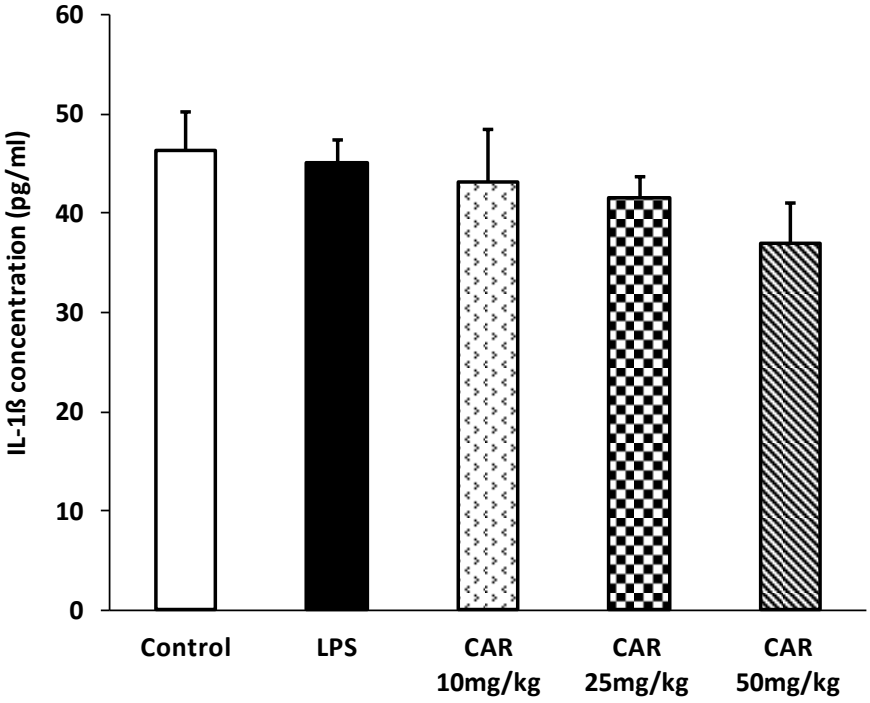

Fig.3. Effects of carvacrol at doses of 10, 25 and 50mg/kg on IL-1ß levels in the hippocampus of LPS-treated rats. Data are mean±SEM for eight animals in each group.

cholinergic system (Tyagi et al., 2008; Houdek et al., 2014).

Administration of LPS can cause cognitive impairment in animal models by mechanisms involving expression of pro-inflammatory mediators (Vasconcelos et al., 2014). Previous studies have demonstrated the potential for a single inflammatory event to initiate processes which leads to long-term neurodegeneration, even 5 months after a single intraperitoneal LPS injection (Ming et al., 2015). It has been well documented that LPS induced acute inflammation and loss of learning and memory function (Joshi et al., 2014; Tyagi et al., 2008). LPS activates glial cells to secret the proinflammatory cytokines, such as: TNFα and IL-1β which are involved in pathology of neuroinflammation (Tyagi et al., 2007). It has been reported that pro-inflammatory cytokines secretion occurs within 6h via the NF-κB pathway in the mouse hippocampus (Skelly et al., 2013; Rosi et al., 2006). It is known that spatial learning and memory require integrative control functions of the hippocampus (Bliss and Collingridge, 1993). Release of pro-inflammatory cytokines by microglia fuels a cycle of neuroinflammation that can cause damage to neurons (Saijo and Glass, 2011). Activated microglia also inhibit neurogenesis in the hippocampus following LPS injection, thereby exacerbating the extent of injury on learning and memory processes (Terrando et al., 2010). Carvacrol has been reported to exhibit anti-inflammatory activities (Lima et al., 2013; Alvarenga et al., 2016). For example, Lima et al. (2013) have reported that carvacrol exerts anti-inflammatory effect by reducing the production of inflammatory mediators, such as IL-1β and prostanoids. Therefore, the positive effect of carvacrol on learning performances observed in LPS-treated rats in the present study may be partly due to the ability of carvacrol to reduce neuroinflammation. However, in the current study, we observed no significant changes in IL-1β levels in the hippocampus after LPS injection. The reason might be such that we measured cytokine levels 23 days after LPS injection, when spatial learning impairment was confirmed. According to previous studies, injection of LPS induced increases in TNF-α and IL-1β levels in the mouse hippocampus 6h after the injection and they returned to the baseline levels at 24h postinjection (Zhang et al., 2015; Terrando et al., 2010). Therefore, carvacrol could have been exerted its anti-inflammatory activity early during LPS injection.

Oxidative stress is also considered as a causative factor in the progressive loss of cognition in clinical as well as animal studies (Joshi et al., 2014). LPS injection to rodents leads to increased production of ROS and development of oxidant/antioxidant

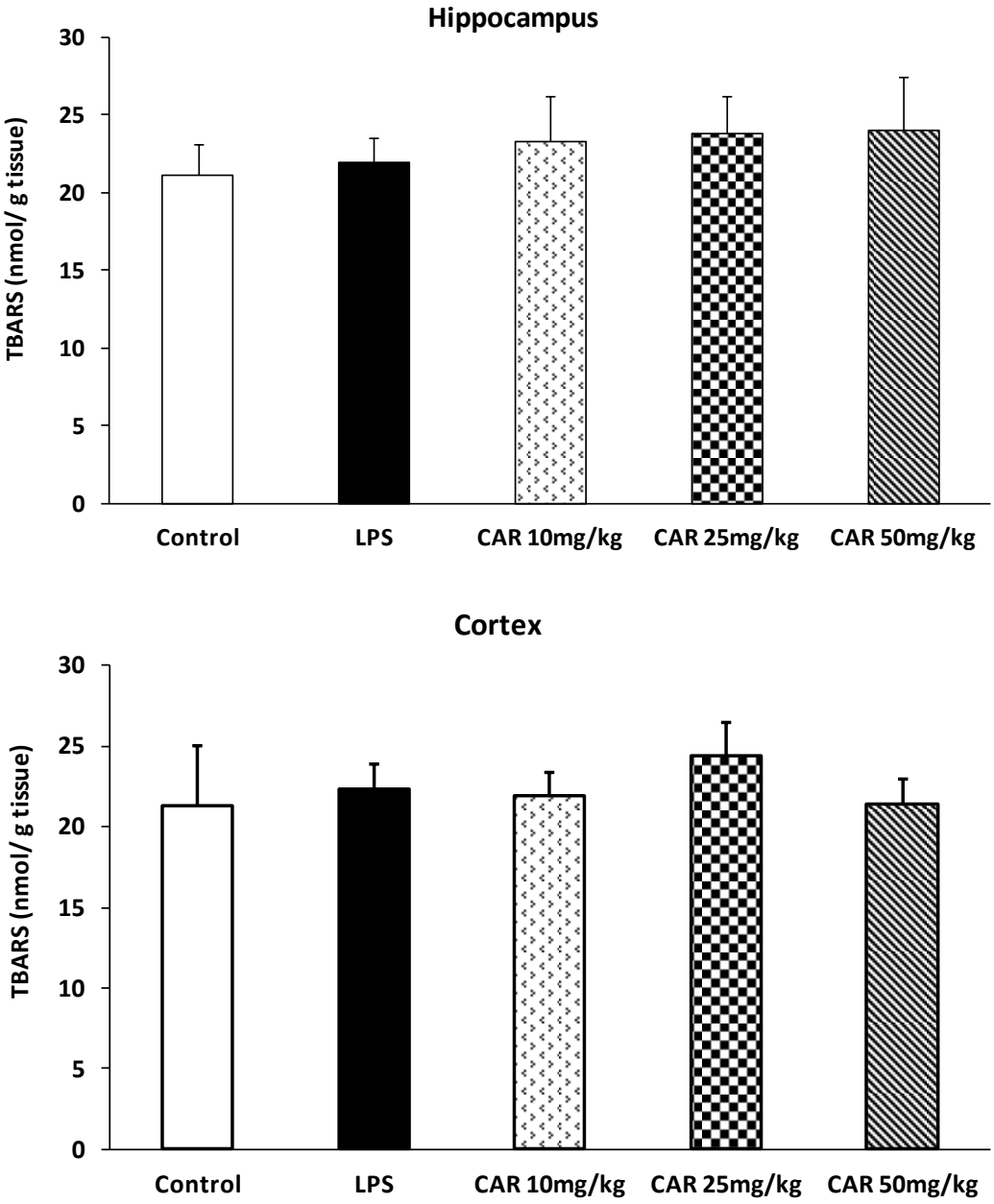

Fig.4. Effects of carvacrol at doses of 10, 25 and 50mg/kg on lipid peroxidation levels (TBARS) in the hippocampus and cerebral cortex of LPS-treated rats. Data are mean±SEM for eight animals in each group.

imbalance (Bai et al., 2016). Activated microglia following LPS injection produces potentially neurotoxic substances, such as: oxygen radicals and proteolytic enzymes, which causes neuronal death (Spulber et al., 2012; Correale, 2014; Zindler and Zipp, 2010). Oxidative stress is implicated in neuronal damage in different conditions, such as: ischemia, hypoxia and especially the chronic neurodegenerative diseases, including: Alzheimer's and Parkinson's disease (Tanaka et al., 2006). Oxidative damage to the rat synapse in the cerebral cortex and hippocampus has been previously reported to contribute to memory deficit (Ahmadi et al., 2017). Previous studies have reported that carvacrol is effective as an antioxidant compound in oxidative insults (Alvarenga et al., 2016; Wang et al., 2017; Yu et al., 2013). For instance, it has been reported that carvacrol attenuates the cognitive dysfunction, oxidative stress and apoptosis of the mice treated with ethanol (Wang et al., 2017). Also, carvacrol protects against acute myocardial infarction of rats via anti-oxidative and anti-apoptotic pathways (Yu et al., 2013). Therefore, carvacrol might protect learning deficit by reducing oxidative stress in rats. In the present study, we observed no significant changes in TBARS and total thiol levels in the brain after LPS injection. This might be due to measurement of oxidative stress biomarkers 23 days

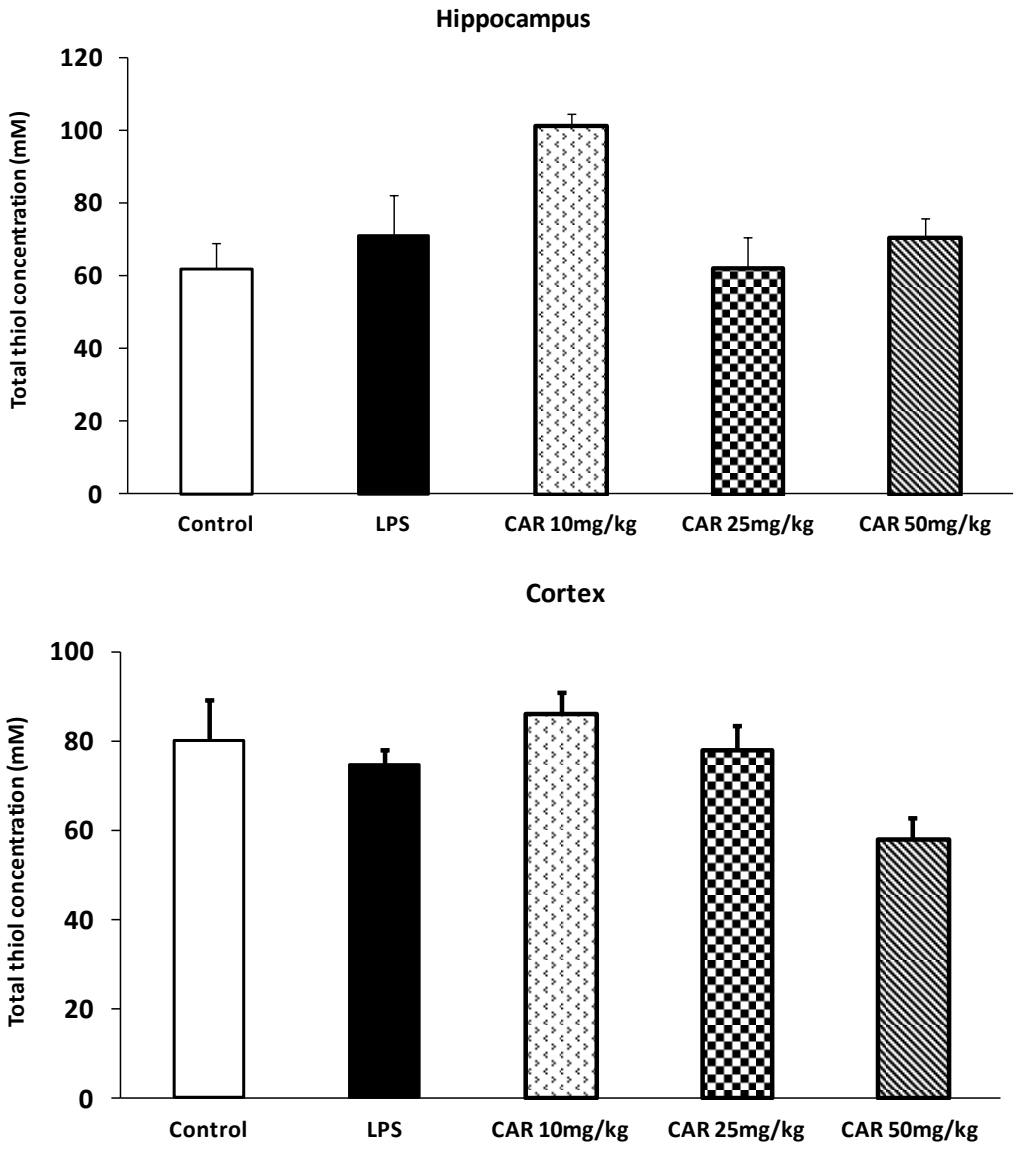

Fig.5. Effects of carvacrol at doses of 10, 25 and 50mg/kg on total thiol concentration in the hippocampus and cerebral cortex of LPS-treated rats. Data are mean±SEM for eight animals in each group.

after LPS injection. In this context, it has been shown that the levels of malondialdehyde highly increased 1.5h after LPS injection, while it approached baseline levels at 24h post-LPS (Theobaldo et al., 2012). Collectively, it could be concluded that the improvement of learning behavior by carvacrol could be partly due to its antioxidant activity against early LPS oxidative damage.

Cognitive deficits induced by LPS have also been linked to increase of acetylcholinesterase activity. Intraperitoneal injection of LPS has been reported to impair memory performance via reduction of acetylcholine which is involved in cognitive function (Houdek et al., 2014). Ming and colleagues (2015) have also shown that acute systemic LPS (1mg/kg) induces a lasting increase in whole-brain acetylcholinesterase activity that corresponded with decreased memory. Previous studies have shown that carvacrol improved spatial learning and memory impairments induced by scopolamine, a muscarinic receptor antagonist, in rats (Azizi et al., 2012). More importantly, the acetylcholinesterase inhibitory activity of carvacrol has been reported in several studies (Jukic et al., 2007; Kaufmann et al., 2011). Therefore, the effect of carvacrol on learning improvement of LPS-rats could also be related to enhancement of central cholinergic neurotransmission through inhibition of acetylcholinesterase activity.

Conclusion

In conclusion, our findings demonstrated that chronic

treatment with carvacrol improved spatial learning impairments induced by LPS. This might be due to antioxidant, anti-inflammatory and anticholinesterase activities of carvacrol. Our behavioural results in the present study provided evidence for the first time that carvacrol could improve spatial learning performances in LPS-treated rats, adding new information in support of its beneficial effect on the improvement of learning and memory deficits.

Acknowledgments

The results presented in this work have been taken from a student's thesis. This study was supported by a grant from the Council of Research, Isfahan University of Medical Sciences.

Conflict of interest

None.

References

Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, et al. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur J Clin Microbiol Infect Dis 2011; 30: 41-50.

Ahmadi M, Rajaei Z, Hadjzadeh MA, Nemati H, Hosseini M. Crocin improves spatial learning and memory deficits in the Morris water maze via attenuating cortical oxidative damage in diabetic rats. Neurosci Lett 2017; 642: 1-6.

Alvarenga EM, Souza LK, Araújo TS, Nogueira KM, Sousa FB, Araújo AR, et al. Carvacrol reduces irinotecan-induced intestinal mucositis through inhibition of inflammation and oxidative damage via TRPA1 receptor activation. Chem Biol Interact 2016; 260: 129-140.

Arai K, Matsuki N, Ikegaya Y, Nishiyama N. Deterioration of spatial learning performances in lipopolysaccharide-treated mice. Jpn J Pharmacol 2001; 87: 195-201.

Azizi Z, Ebrahimi S, Saadatfar E, Kamalinejad M, Majlessi N. Cognitive-enhancing activity of thymol and carvacrol in two rat models of dementia. Behav Pharmacol 2012; 23: 241-9.

Bai K, Xu W, Zhang J, Kou T, Niu Y, Wan X, et al. Assessment of free radical scavenging activity of dimethylglycine sodium salt and its role in providing protection against lipopolysaccharide-induced oxidative stress in mice. PLoS One 2016; 11: e0155393.

Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993; 361: 31-9.

Block ML, Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 2005; 76: 77-98.

Carrero I, Gonzalo MR, Martin B, Sanz-Anquela JM, Arévalo-Serrano J, Gonzalo-Ruiz A. Oligomers of β-amyloid protein (Aβ1-42) induce the activation of cyclooxygenase-2 in astrocytes via an interaction with interleukin-1β, tumour necrosis factor-α, and a nuclear factor κ-B mechanism in the rat brain. Exp Neurol 2012: 236: 215-27.

Correale J. The role of microglial activation in disease progression. Mult Scler 2014; 20: 1288-95.

Deng W, Lu H, Teng J. Carvacrol attenuates diabetes-associated cognitive deficits in rats. J Mol Neurosci 2013; 51: 813-9.

Frank-Cannon TC, Alto LT, McAlpine FE, Tansey MG. Does neuroinflammation fan the flame in neurodegenerative diseases? Mol Neurodegener 2009; 4: 47.

García-García R, López-Malo A, Palou E. Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against Listeria innocua. J Food Sci 2011; 6: M95-100.

Guimarães AG, Oliveira GF, Melo MS, Cavalcanti SC, Antoniolli AR, Bonjardim LR, et al. Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. Basic Clin Pharmacol Toxicol 2010; 107: 949-57.

Haddadi H, Rajaei Z, Alaei H, Shahidani S. Chronic treatment with carvacrol improves passive avoidance memory in a rat model of Parkinson's disease. Arq Neuropsiquiatr 2018; 76: 71-77.

Hou Y, Xie G, Miao F, Ding L, Mou Y, Wang L, et al. Pterostilbene attenuates lipopolysaccharide-induced learning and memory impairment possibly via inhibiting microglia activation and protecting neuronal injury in mice. Prog Neuropsychopharmacol Biol Psychiatry 2014; 54: 92-102.

Houdek HM, Larson J, Watt JA, Rosenberger TA. Bacterial lipopolysaccharide induces a dose-dependent activation of neuroglia and loss of basal forebrain cholinergic cells in the rat brain. Inflamm Cell Signal 2014; 1: e47.

Joshi R, Garabadu D, Teja GR, Krishnamurthy S. Silibinin ameliorates LPS-induced memory deficits in experimental animals. Neurobiol Learn Mem 2014; 116: 117-31.

Jukic M, Politeo O, Maksimovic M, Milos M, Milos M. In vitro acetylcholinesterase inhibitory properties of thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone. Phytother Res 2007; 21: 259-61.

Karkabounas S, Kostoula OK, Daskalou T, Veltsistas P, Karamouzis M, Zelovitis I, et al. Anticarcinogenic and antiplatelet effects of carvacrol. Exp Oncol 2006; 28: 121-5.

Kaufmann D, Dogra AK, Wink M. Myrtenal inhibits acetylcholinesterase, a known Alzheimer target. J Pharm Pharmacol 2011; 63: 1368-71.

Landa P, Kokoska L, Pribylova M, Vanek T, Marsik P. In vitro anti-inflammatory activity of carvacrol: Inhibitory effect on COX-2 catalyzed prostaglandin E(2) biosynthesis. Arch Pharm Res 2009; 32: 75-8.

Lee JW, Lee YK, Yuk DY, Choi DY, Ban SB, Oh KW, et al. Neuro-inflammation induced by lipopolysaccharide

causes cognitive impairment through enhancement of beta-amyloid generation. J Neuroinflammation 2008; 29: 5-37.

Lima Mda S, Quintans-Júnior LJ, de Santana WA, Martins Kaneto C, Pereira Soares MB, Villarreal CF. Anti-inflammatory effects of carvacrol: evidence for a key role of interleukin-10. Eur J Pharmacol 2013; 699: 112-7.

Ming Z, Sawicki G, Bekar LK. Acute systemic LPS-mediated inflammation induces lasting changes in mouse cortical neuromodulation and behavior. Neurosci Lett 2015; 590: 96-100.

Olajide OA, Bhatia HS, de Oliveira AC, Wright CW, Fiebich BL. Inhibition of neuroinflammation in LPS-activated microglia by cryptolepine. Evid Based Complement Alternat Med 2013; 2013: 459723.

Oliveira IS, da Silva FV, Viana AF, dos Santos MR, Quintans-Júnior LJ, Martins Mdo C, et al. Gastroprotective activity of carvacrol on experimentally induced gastric lesions in rodents. Naunyn Schmiedebergs Arch Pharmacol 2012: 385: 899-908.

Purushoth T, Panneerselvam P, Vijaykumar R, Clement Atlee W, Balasubramanian S. Antiinflammatory, anti arthritis and analgesic effect of ethanolic extract of whole plant of merremia emarginata burm. F. Cent Europ J Exp Biol 2012; 1: 94-9.

Qin L, Liu Y, Wang T, Wei SJ, Block ML, Wilson B, et al. NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. J Biol Chem 2004; 279: 1415-21.

Rajaei Z, Hadjzadeh MA, Nemati H, Hosseini M, Ahmadi M , Shafiee S. Antihyperglycemic and antioxidant activity of crocin in streptozotocin-induced diabetic rats. J Med Food 2013; 16: 206-10.

Ray B, Lahiri DK. Neuroinflammation in Alzheimer's disease: different molecular targets and potential therapeutic agents including curcumin. Curr Opin Pharmacol 2009; 9: 434-44.

Rosi S, Vazdarjanova A, Ramirez-Amaya V, Worley PF, Barnes CA, Wenk GL. Memantine protects against LPS-induced neuroinflammation, restores behaviorally-induced gene expression and spatial learning in the rat. Neuroscience 2006; 142: 1303-15.

Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. Nat Rev Immunol 2011; 11: 775-87.

Shaw KN, Commins S, O'Mara SM. Lipopolysaccharide causes deficits in spatial learning in the watermaze but not in BDNF expression in the rat dentate gyrus. Behav Brain Res 2001; 124: 47-54.

Skelly DT, Hennessy E, Dansereau MA, Cunningham C. A systematic analysis of the peripheral and CNS effects of systemic LPS, IL-1β, TNF-α and IL-6 challenges in C57BL/6 mice. PLoS One 2013; 8: e69123.

Spulber S, Edoff K, Hong L, Morisawa S, Shirahata S, Ceccatelli S. Molecular hydrogen reduces LPS-induced neuroinflammation and promotes recovery from sickness behaviour in mice. Plos One 2012; 7: e42078.

Tanaka S, Ide M, Shibutani T, Ohtaki H, Numazawa S, Shioda S, et al. Lipopolysaccharide-induced microglial activation induces learning and memory deficits without neuronal cell death in rats. J Neurosci Res 2006; 83: 557-66.

Terrando N, Rei Fidalgo A, Vizcaychipi M, Cibelli M, Ma D, Monaco C, et al. The impact of IL-1 modulation on the development of lipopolysaccharide-induced cognitive dysfunction. Crit Care 2010; 14: R88.

Theobaldo MC, Barbeiro HV, Barbeiro DF, Petroni R, Soriano FG. Hypertonic saline solution reduces the inflammatory response in endotoxemic rats. Clinics (Sao Paulo) 2012; 67: 1463-8.

Tyagi E, Agrawal R, Nath C, Shukla R. Effect of anti-dementia drugs on LPS induced neuroinflammation in mice. Life Sci 2007; 80: 1977-83.

Tyagi E, Agrawal R, Nath C, Shukla R. Influence of LPS-induced neuroinflammation on acetylcholinesterase activity in rat brain. J Neuroimmunol 2008; 205: 51-6.

Vasconcelos AR, Yshii LM, Viel TA, Buck HS, Mattson MP, Scavone C, et al. Intermittent fasting attenuates lipopolysaccharide-induced neuroinflammation and memory impairment. J Neuroinflammation 2014; 11: 85.

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

Wyns H, Plessers E, De Backer P, Meyer E, Croubels S. In vivo porcine lipopolysaccharide inflammation models to study immunomodulation of drugs. Vet Immunol Immunopathol 2015; 166: 58-69.

Yu H, Zhang ZL, Chen J, Pei A, Hua F, Qian X, et al. Carvacrol, a food-additive, provides neuroprotection on focal cerebral ischemia/reperfusion injury in mice. PLoS One 2012; 7: e33584.

Yu W, Liu Q, Zhu S. Carvacrol protects against acute myocardial infarction of rats via anti-oxidative and anti-apoptotic pathways. Biol Pharm Bull 2013; 36: 579-84.

Zhang XY, Cao JB, Zhang LM, Li YF, Mi WD. Deferoxamine attenuates lipopolysaccharide-induced neuroinflammation and memory impairment in mice. J Neuroinflammation 2015; 12: 20.

Zindler E, Zipp F. Neuronal injury in chronic CNS inflammation. Best Pract Res Clin Anaesthesiol 2010; 24: 551-62.