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ARTICLE

Effects of carvacrol and physical exercise on motor and memory impairments associated with Parkinson's disease

Efeitos do carvacrol e do exercício físico sobre o comprometimento motor e de memória associados à doença de Parkinson

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ABSTRACT

The present study was undertaken to investigate the effects of carvacrol and treadmill exercise on memory deficit, rotational behavior and oxidative stress biomarkers in a 6-OHDA-lesioned rat model of Parkinson's disease. Wistar rats were treated with carvacrol at a dose of 25 mg/kg and/or ran on a treadmill for a week. Then, 6-OHDA was microinjected into the medial forebrain bundle and treatments continued for six more weeks. Aversive memory, rotational behavior and oxidative stress biomarkers were assessed at the end of week six. The 6-OHDA-lesioned group showed a significant increase in rotational behavior and a decrease in step-through latency in the passive avoidance test compared with the sham group. These behaviors were accompanied by increased lipid peroxidation levels and decreased total thiol concentration in the striatum and/or hippocampus of the hemiparkinsonian rats. Moreover, treatment with carvacrol and exercise reduced rotational behavior and improved aversive memory deficit, which was accompanied by decreased lipid peroxidation levels and increased total thiol concentration in the striatum and/or hippocampus. In conclusion, treatment with carvacrol and treadmill exercise ameliorated motor and memory deficits by modulating oxidative stress in the striatum and hippocampus of hemiparkinsonian rats. Therefore, the combination of carvacrol and treadmill exercise could be an effective therapeutic tool for treatment of neurobehavioral deficits in Parkinson's disease patients.

Keywords: Exercise; memory; motor activity; oxidative stress, Parkinson disease.

RESUMO

O presente estudo foi realizado para investigar os efeitos do carvacrol e do exercício em esteira sobre o déficit de memória, comportamento rotacional e biomarcadores de estresse oxidativo em um modelo animal (ratos lesionados por 6-OHDA) da doença de Parkinson (DP). Ratos Wistar foram tratados com carvacrol na dose de 25 mg/kg e/ou correram em uma esteira por uma semana. Depois, 6-OHDA foi microinjetada no feixe do prosencéfalo medial e os tratamentos continuaram por mais seis semanas. A memória aversiva, o comportamento rotacional e biomarcadores de estresse oxidativo foram avaliados no final da semana 6. O grupo 6-OHDA mostrou um aumento significativo no comportamento rotacional e uma diminuição na latência no teste de esquiva passiva em comparação com o grupo "sham". Estes comportamentos foram acompanhados por aumento dos níveis de peroxidação lipídica e diminuição da concentração total de tiol no estriado e/ou hipocampo de ratos hemiparkinsonianos. Além disso, o tratamento com carvacrol e exercício reduziu o comportamento rotacional e melhorou o déficit de memória aversiva, que foi acompanhado pela diminuição dos níveis de peroxidação lipídica e aumento da concentração total de tiol no estriado e/ou hipocampo. Em conclusão, o tratamento com carvacrol e exercícios em esteira melhorou os déficits motor e de memória, modulando o estresse oxidativo no estriado e no hipocampo de ratos hemiparkinsonianos. Portanto, a combinação de carvacrol e exercício em esteira pode ser uma ferramenta terapêutica eficaz para o tratamento de déficits neurocomportamentais em pacientes com DP.

Palavras-chave: Exercício; memória; atividade motora; estresse oxidativo; doença de Parkinson.

Parkinson's disease (PD) is a heterogeneous neurodegenerative disorder characterized by loss of dopaminergic nigrostriatal neurons and clinical symptoms, such as resting tremor, rigidity, akinesia, and disturbances of postural

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reflex[1]. Cardinal motor symptoms of PD appear when as many as 58-64% of nigrostriatal dopaminergic neurons have been lost and striatal dopamine content has been reduced by 60-80%[2]. Parkinson's disease is also associated with a wide range of nonmotor symptoms, including cognitive decline, depression, anxiety and sleep disorders[3]. Cognitive impairments in PD patients resembles those seen in frontal lobe patients[4], and progression of these impairments can cause dementia. Dementia occurs in 83% of patients with PD after 20 years of diagnosis[5].

The histopathological hallmarks of PD are Lewy bodies, which are produced by the progressive accumulation of alpha-synuclein aggregates in the cytoplasm of selected neurons, leading to their death by necrosis and/or apoptosis[6]. Multiple other processes, including mitochondrial dysfunction, oxidative stress and neuroinflammation are thought to be involved in the onset and progression of PD. Alpha-synuclein may directly activate microglia, leading to increased production of pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-1beta, which affect the disease progression[7]. Alpha-synuclein can also interact with the mitochondrial membrane and accumulate inside the organelles. This leads to the damage of complex-I activity, resulting in mitochondrial dysfunction and increased oxidative stress[8]. Postmortem studies have shown that nigrostriatal neuronal death in PD is associated with increased lipid peroxidation[9] and reduced glutathione levels[10] in the substantia nigra.

At present, treatment with dopamine replacement medications such as L-DOPA or dopamine agonists are the most effective treatment in PD. However, these drugs merely control motor symptoms and do not meet the clinical challenges of the disease, such as dyskinesia, nonmotor symptoms, and neuroprotection. Thus, a therapeutic approach to PD treatment could include the modulation of oxidative stress.

Zataria multiflora Boiss (Avishan shirazi) is a perennial plant with small leaves, fibrous roots and several narrow branches[11]. This plant has a limited distribution in the world and grows only in Iran, Pakistan and Afghanistan[12]. *Zataria multiflora* has been used in Iranian traditional medicine for its antiseptic, analgesic and carminative properties[13]. Carvacrol (2-methyl-5-isopropylphenol) is one of the constituents of *Zataria multiflora*[11]. Carvacrol has been reported to have multiple biological and pharmacological actions, including antioxidant, anti-inflammatory, antibacterial, antifungal, antinociceptive, anti-apoptosis and anticancer activities[14]. Carvacrol also exerts several actions on the neuronal system, including acetylcholinesterase inhibition[15].

Several studies have demonstrated the beneficial effect of exercise on brain function, because it prevents oxidative stress in different brain regions of adolescent and aging rats[16]. A meta-analysis demonstrated that exercise might improve physical functions, life quality, balance, and gait speed in PD patients[17]. Exercise has also been shown to decrease the risk for the development and progression of PD[18]. While some studies suggest that exercise might exert neuroprotective effects on PD[19], other studies do not confirm its neuroprotective effects. For instance, it has been reported that treadmill exercise for six weeks in MPTP Parkinson's mice, does not prevent mitochondrial inhibition or nigrostriatal neurodegeneration[20]. This means that the neuroprotective effects of exercise, and the mechanism by which treadmill exercise exerts its effects in PD, are not completely understood. At present, few studies have been performed to show the effect of exercise on modulation of oxidative stress status in the experimental models of PD.

In the present study, the neuroprotective effects of carvacrol and/or treadmill exercise on motor and memory impairments were investigated in a unilateral 6-hydroxydopamine (6-OHDA) model of PD. For this purpose, apomorphine-induced rotations and aversive memory were evaluated. Daily exercise, for 30 minutes/day, five days/week for seven weeks, was used in this study to make the study comparable with clinical settings. Furthermore, the effects on lipid peroxidation levels and total thiol concentration in the striatum and hippocampus were investigated to understand the underlying mechanisms.

METHODS

Animals

Male Wistar rats (250-300g, procured from the Pasteur Institute of Iran, Tehran, Iran) were housed in an air-conditioned colony room at 22° ± 2°C with a 12-hour dark/light cycle and free access to water and food. The Ethics Committee for Animal Experiments at the 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, 6-OHDA, apomorphine hydrochloride and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Aldrich Co. 2,2´dinitro-5,5´-dithiodibenzoic acid (DTNB), trichloro acetic acid (TCA), thiobarbituric acid (TBA), tris, chloral hydrate, Tween 80 and hydrochloric acid were obtained from Merck.

Experimental design

The animals were randomly assigned into five groups (n = 8 in each group); 1) sham-operated group (injection of 0.2% ascorbate-saline into the left medial forebrain bundle (MFB), 1% Tween 80 intraperitoneal [ip] injection), 2) 6-OHDA-lesioned group (injection of 16 μg 6-OHDA into the left MFB, 1% Tween 80 ip), 3) carvacrol group (injection of 16 μg 6-OHDA into the left MFB, 25 mg/kg carvacrol, ip),

4) exercise group (injection of 16 µg 6-OHDA into the left MFB, treadmill exercise) and 5) carvacrol + exercise group (injection of 16 µg 6-OHDA into the left MFB, 25 mg/kg carvacrol and treadmill exercise).

Carvacrol was injected at a dose of 25 mg/kg/day according to previous studies, which have reported its antioxidant and anti-inflammatory effects[21]. Carvacrol was emulsified with 1% Tween 80 and dissolved in normal saline. The sham-operated group received 1% Tween 80 dissolved in normal saline at the same volume as the treated groups. It should be noted that the injection of carvacrol (25 mg/kg, ip) or 1% Tween 80 and treadmill exercise started one week before the 6-OHDA or ascorbate-saline injection and continued for six weeks after surgery.

Induction of Parkinson's disease

The animals were anesthetized with chloral hydrate (450 mg/kg, ip), then placed in a stereotaxic apparatus (Stoelting, USA). The scalp was cleaned with an iodine solution and lidocaine was injected (2% solution, subcutaneously). A midline skin incision was made with subsequent drilling of the skull, and 6-OHDA (16 µg/4 µl 0.2% ascorbate-saline) was injected into the left MFB by a Hamilton microsyringe according to the coordinates: AP: -3.6 mm; ML: -1.8 mm; DV: -8.2 mm[22]. The rats of the sham-operated group also received an identical volume of the ascorbate-saline as the vehicle. The injection rate was 1 µl/min and the needle was kept in place for an additional five minutes before being slowly retracted. After surgery, all rats were treated with penicillin (0.2 ml, ip), placed singly into a clean cage and kept warm until complete recovery occurred. After completion of the behavioral testing, the animals were euthanized and the brains were removed from the skulls on day 42. The striatum and hippocampus were dissected out and weighed. A 10% (w/v) tissue homogenate was prepared in NaCl 0.9% solution by a homogenizer (Heidolph, Germany).

Treadmill training

A five-lane motorized rat treadmill was used for exercise training. One week before essential protocol, the exercised groups of animals were trained on the treadmill, running for 5 days/week, 5 min/day with a speed of 10 m/min to reduce their stress to the new environment[23]. One week before surgery, the animals in the exercised group were trained on the treadmill running at 5 days/week, 40 min/day with a speed up to 17 m/min (5 min at 7 m/min, 30 min at 17 m/min, 5 min at 7 m/min). This protocol continued for six weeks after surgery.

Apomorphine-induced rotation test

The apomorphine-induced rotation test was performed on day 42. Apomorphine was dissolved in normal saline and injected intraperitoneally at a dose of 2 mg/kg. On the test day, the animals were allowed to habituate to a transparent plexiglass container (28 x 28 x 50 cm) for 10 minutes. One minute after the injection, ipsi- and contralateral rotations were counted for 30 minutes in a dimly-lit, quiet room. The data were expressed as the net (contralateral minus ipsilateral turns) average rotations per 30 minutes[24].

Passive avoidance memory

Passive avoidance memory was assessed by a shuttle box at the end of week six. The apparatus consisted of a light and a dark compartment, connected by a guillotine door. In the training session, animals were placed individually in the light compartment for one minute. After the opening of the door and the movement of the rat into the dark chamber, the door was closed and a 0.5 mA foot electric shock was delivered through the grid floor for three seconds. In the test session, each rat was again placed into the light compartment. The step-through latency of entering the dark compartment was measured as a positive index of memory performance, with a 300 second cut-off time[25].

Lipid peroxidation levels

The lipid peroxidation levels of the striatum and hippocampus were measured as malondialdehyde, which reacts with thiobarbituric acid as a thiobarbituric acid reactive substance (TBARS) to produce a red-colored complex that has a peak absorbance (A) at 535 nm. A mixture of TCA, TBA and HCl were added to 1 ml of homogenate, and the mixture was heated for 45 minutes in a boiling water bath. After cooling and centrifugation at 1000 g for 10 minutes, the absorbance was measured at 535 nm by a spectrophotometer (Secomam, France)[26]. The level of TBARS was calculated by: C (M) = absorbance/1.65 x 10^5.

Total thiol concentration

Total sulfhydryl groups were measured using DTNB as the reagent. This reagent reacts with the sulfhydryl groups to produce a yellow-colored complex that has a peak absorbance at 412 nm. Briefly, 1 ml tris-EDTA buffer was added to 50 µl homogenate and the sample absorbance was read at 412 nm against the tris-EDTA buffer alone (A1). Then, 20 µl of the DTNB reagent (10 mM in methanol) was added to the mixture and after 15 minutes, 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) x 1.07/0.05 x 13.6[26].

Statistical analysis

Data are presented as mean ± SEM. Data were analyzed using the Statistical Package for Social Sciences (SPSS), version 20 program. Comparisons between groups were performed by one-way analysis of variance followed by Tukey's *post hoc* test when appropriate. Results were considered significant at $p < 0.05$.

RESULTS

Apomorphine-induced rotations

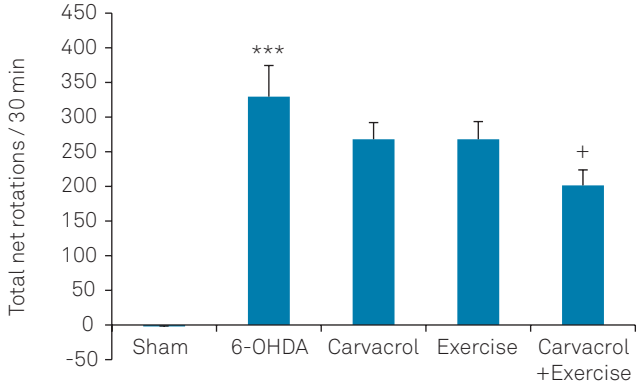
Apomorphine-induced rotational behavior was analyzed to assess the unilateral degeneration of the dopaminergic nigrostriatal neurons. Administration of apomorphine to 6-OHDA-lesioned rats produced contralateral rotations towards the lesion side at the end of the sixth week after surgery, indicating unilateral damage to the left striatum ($p < 0.001$, Figure 1). No such rotations were observed in the sham group rats. Moreover, pretreatment with carvacrol along with treadmill exercise reduced the contralateral rotations compared with the 6-OHDA-lesioned group ($p < 0.05$). However, pretreatment with carvacrol at a dose of 25 mg/kg and treadmill exercise alone did not change rotations compared with the 6-OHDA-lesioned group (Figure 1).

Passive avoidance memory

Learning and memory performance was assessed in the passive avoidance test, as it is a suitable model for evaluating hippocampal-dependent memory deficits in experimental animals. As shown in Figure 2, the step-through latency of 6-OHDA-lesioned rats was shorter than sham group rats at the end of week six ($p < 0.01$, Figure 2). Moreover, pretreatment with carvacrol at a dose of 25 mg/kg ($p < 0.01$) or treadmill exercise ($p < 0.05$) and in combination ($p < 0.01$) significantly increased the latencies compared with the 6-OHDA-lesioned group (Figure 2).

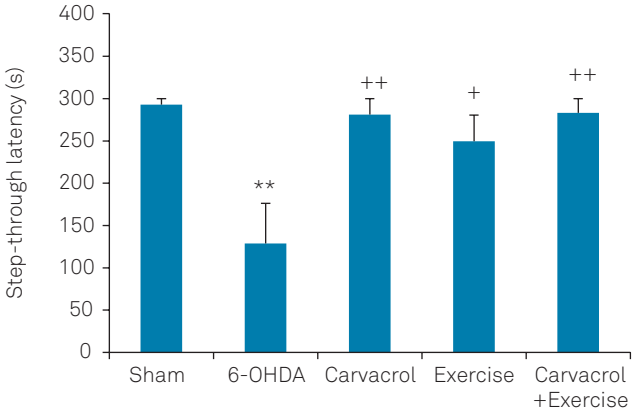
Lipid peroxidation levels

The degree of free radical damage following MFB lesion was assessed using lipid peroxidation, and measured as TBARS levels. A significant increase in the levels of TBARS was found in the striatum ($p < 0.05$) and hippocampus ($p < 0.01$) of 6-OHDA-lesioned rats compared with the sham group (Figure 3). Moreover, treadmill exercise alone and in combination with carvacrol decreased TBARS levels in the striatum ($p < 0.05$, $p < 0.05$) and hippocampus ($p < 0.001$, $p < 0.05$) at the end of week six (Figure 3).



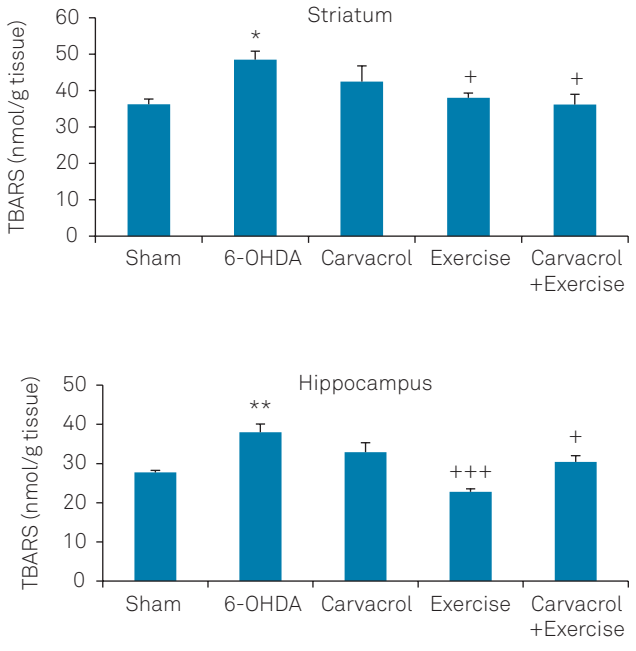
***p < 0.001 *vs* sham group, +p < 0.05 *vs* 6-OHDA-lesioned group.

Figure 1. Effects of carvacrol and treadmill exercise on apomorphine-induced rotations (mean ± SEM) among the experimental groups at the end of week six. The net number of rotations was measured 1 min after injection of apomorphine hydrochloride (2 mg/kg, ip) over a period of 30 min. Carvacrol was administered daily at a dose of 25 mg/kg for seven weeks.



**p < 0.01 vs sham group, +p < 0.05, ++p < 0.01 vs 6-OHDA-lesioned group.

Figure 2. Effects of carvacrol and treadmill exercise on step-through latency (mean ± SEM) among the experimental groups at the end of week six. The passive avoidance test was used to assess aversive memory. Carvacrol was administered daily at a dose of 25 mg/kg for seven weeks.



TBARS: thiobarbituric acid reactive substances. *p < 0.05, **p < 0.01 *vs* sham group, +p < 0.05, +++p < 0.001 *vs* 6-OHDA-lesioned group.

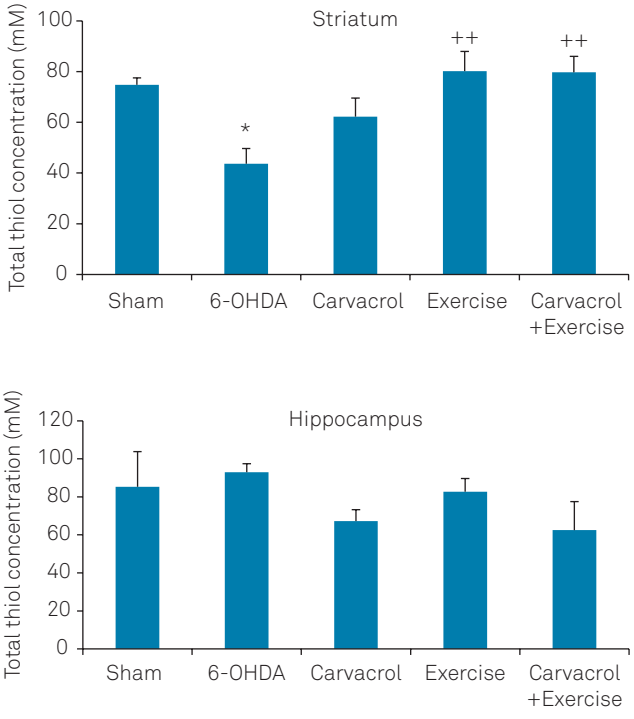
Figure 3. Effects of carvacrol and treadmill exercise on TBARS levels (mean ± SEM), an index of lipid peroxidation, in the striatum and hippocampus among the experimental groups at the end of week six. Carvacrol was administered daily at a dose of 25 mg/kg for seven weeks.

Total thiol concentration

The total thiol concentration was measured to evaluate the nonenzymatic defense potential of neurons against the oxidative stress. Statistical analysis showed that total thiol concentration in the striatum significantly decreased in the 6-OHDA-lesioned group compared with the sham group ($p < 0.05$, Figure 4). Moreover, treadmill exercise alone and in combination with carvacrol at a dose of 25 mg/kg significantly increased total thiol concentration in the striatum ($p < 0.01$, $p < 0.01$, Figure 4). There was no significant change in total thiol concentrations in the hippocampus of the sham and experimental groups at the end of week six (Figure 4).

DISCUSSION

The present study examined and compared memory and motor function in a 6-OHDA model of PD in rats. We observed the significant deficits in aversive memory and apomorphine-stimulated rotational behavior six weeks after 6-OHDA injection. These behavioral responses were accompanied by increased lipid peroxidation levels in the striatum and hippocampus and decreased total thiol concentration in the striatum. Our results also showed that long-term treatment with carvacrol and treadmill exercise ameliorated memory deficits and apomorphine-induced rotational behavior, by modifying oxidative stress biomarkers in the striatum and hippocampus.



*p < 0.05 *vs* sham group, ++p < 0.01 *vs* 6-OHDA-lesioned group.

Figure 4. Effects of carvacrol and treadmill exercise on total thiol concentration (mean ± SEM), an index of antioxidant potential, among the experimental groups at the end of week six. Carvacrol was administered daily at a dose of 25 mg/kg for seven weeks.

6-Hydroxydopamine is a hydroxylated analogue of dopamine found in the brains of PD patients[27]. The unilateral injection of 6-OHDA into the MFB of the rat is a commonly used animal model, because it causes increased oxidative stress and loss of the dopaminergic nigrostriatal neurons. Auto-oxidation of 6-OHDA leads to excess production of reactive oxygen species, such as superoxide radicals, hydrogen peroxide and hydroxyl radicals[28]. Overproduction of free radicals causes an imbalance in the redox system of neurons, and reacts with proteins and nucleic acids to alter their functions, or induce lipid peroxidation, leading to eventual neuronal death. In the present study, a microinjection of 6-OHDA into the MFB caused oxidative damage to membrane lipids, as evidenced by decreased total thiol concentrations and increased TBARS levels in the striatum at the end of week six. In line with this, studies have demonstrated that 6-OHDA leads to a reduction in glutathione content and superoxide dismutase and catalase activity, and an increase in lipid peroxidation in the striatum[24,29].

The unilateral damage to the nigrostriatal dopaminergic system by injection of 6-OHDA into the MFB is followed by a reduction in dopamine level in the striatum and an upregulation of dopaminergic postsynaptic receptors on the side of the lesion. These changes produce a prominent functional asymmetry that can be assessed by dopamine agonists such as apomorphine[30]. The rotations induced by apomorphine are considered to be reliable indicators of nigrostriatal dopamine depletion. In the present study, injection of apomorphine in the 6-OHDA group of rats caused significant contralateral rotations, which happens after degeneration of at least 75% of nigrostriatal dopaminergic neurons[31].

The present study also examined the effect of carvacrol, as an antioxidant agent, in a 6-OHDA model of PD. Our results showed that treatment with carvacrol at a dose of 25 mg/kg did not decrease the apomorphine-induced rotations and did not change the oxidative stress biomarkers in the striatum of PD rats. This is in contrast to a recent study that reported the neuroprotective effect of carvacrol at a single dose of 40 mg/kg upon the neurodegeneration induced by 6-OHDA intrastriatal injections in mice[32]. The reasons for this discrepancy could be related to the extent of the lesion, dosage of carvacrol and duration of treatment. We used a chronic 6-OHDA-induced rat model of PD with severe neurodegeneration. The initial oxidative stress caused by 6-OHDA may be attenuated by the antioxidant activity of carvacrol; however, when there is substantial ongoing oxidative stress and neurodegeneration, the antioxidant response wanes or is overwhelmed over time and, at that point, carvacrol cannot act as an antioxidant.

Our results also showed that exercise, one week before and six weeks after 6-OHDA injection, reduced lipid peroxidation levels and increased total thiol concentration in the

striatum. In confirmation of this result, it has been reported that treadmill exercise reduced the level of striatal carbonylated proteins and increased the superoxide dismutase levels in the striatum of mice with PD[33]. It has also been shown that aerobic exercise for eight weeks increased the level of antioxidant enzymes, such as superoxide dismutase and catalase, and reduced oxidative damage to lipids and proteins in the striatum of hemiparkinsonian rats[23].

Although behavioral analysis showed that treadmill training for seven weeks did not significantly attenuate the apomorphine-induced rotations in hemiparkinsonian rats, there was a tendency toward a decrease in rotations in the exercise group (Figure 1), suggesting that exercise could have a modest neuroprotective effect against this neurotoxin insult. In this regard, several studies have shown that treadmill exercise improved motor deficits in 6-OHDA-lesioned rats[23], while others have reported that treadmill training does not ameliorate locomotor deficits in the 6-OHDA model of PD[34]. This discrepancy in experimental results could be due to differences in experimental method, including severity of the nigrostriatal lesion, duration and intensity of the applied exercise regimen.

However, our findings also showed that treatment with carvacrol plus treadmill exercise significantly decreased the apomorphine-induced rotations, which was accompanied by decreased TBARS levels and increased total thiol concentration in the striatum. The positive effects of carvacrol and exercise on improving motor behavior could partly be due to their synergism effects on the redox system in the striatum. As shown in the results, carvacrol at a dose of 25 mg/kg also insignificantly increased total thiol concentration in the striatum. However, we cannot exclude a possible anti-inflammatory action of carvacrol[35] and exercise[36] causing the effects shown here. In addition, the mechanism of the effect of exercise in 6-OHDA injected rats may also involve improvement of mitochondrial functions[33] and increase of the levels of brain-derived neurotrophic factor (BDNF)[19] in the striatum, resulting in a decrease in 6-OHDA-induced toxicity.

Increasing number of studies have demonstrated that PD appears to be a multidimensional disease, and it is also associated with a number of cognitive impairments that result in a loss of quality of life in individuals with PD[5]. Experimental studies have also demonstrated cognitive deficits in animal models of PD and oxidative stress has been shown to play an important role in memory impairment[37]. Reactive oxygen species induced by 6-OHDA can react with biological target molecules and contribute to increased neuronal damage and death through protein oxidation, DNA damage, and peroxidation of membrane lipids. In the present study, 6-OHDA injections into the MFB also produced a memory deficit that was accompanied by increased lipid peroxidation levels in the hippocampus. In our study, the passive avoidance test was used to examine whether carvacrol and treadmill exercise could improve aversive memory impairments of parkinsonian rats. The task is based on the motivation of passive avoidance from the fear of foot shock. Our results showed that treatment with carvacrol and/or exercise training improved the aversive memory deficit induced by 6-OHDA. The memory-enhancing effect of treadmill training could partly be due to its impact on the redox system in the hippocampus. As shown in the results, treadmill exercise for seven weeks significantly decreased lipid peroxidation levels in the hippocampus. In agreement with this, it has been reported that treadmill exercise for four weeks attenuated 3,4-methylenedioxymethamphetamine-induced memory impairment and reduced lipid peroxidation in the rat hippocampus[38]. In addition, Macêdo et al.[16] reported that exercise improved memory and reduced malondialdehyde and carbonyl levels and increased superoxide dismutase and catalase activity in the hippocampus of young rats. Cho et al.[39] also reported that treadmill exercise (four weeks after intrastriatal 6-OHDA injection, 30 min/day for two weeks) alleviated short-term memory impairment by increasing cell proliferation in the hippocampus. In line with this, the present study found that the preventive effect of treadmill exercise on memory function in PD was mediated by modulating the redox system in the hippocampus, which has not been reported previously. Another mechanism, by which exercise exerts its beneficial effects on memory, could be increased levels of BDNF in the hippocampus. In support of this, it has been reported that treadmill exercise improved spatial learning ability via enhancement of the expression of BDNF in the hippocampus of lipopolysaccharide-injected rats[40].

As seen in the results, treatment with carvacrol significantly improved memory impairment in parkinsonian rats. In line with this, it has been reported that carvacrol (25, 50, 100 mg/kg) attenuated diabetes-associated cognitive deficits by decreasing malondialdehyde levels and increasing superoxide dismutase activity and reduced glutathione levels in the hippocampus and cortex of diabetic rats[21]. In a recent study, it was reported that chronic treatment with carvacrol improved passive avoidance memory in a rat model of PD; however, the mechanism of action was not examined[25]. Biochemical data in the present study did not confirm antioxidant activity of carvacrol in the hippocampus. The mechanism by which carvacrol improves memory deficits in PD could be due to anticholinesterase[15] and anti-inflammatory[35] activities of carvacrol.

In conclusion, long-term treatment with carvacrol and treadmill exercise ameliorated motor and memory deficits by modulating oxidative stress in the striatum and hippocampus of hemiparkinsonian rats. Moreover, our findings also indicated that the neuroprotective effects of exercise were associated with its antioxidative potential. Therefore, a combination of carvacrol and treadmill exercise could be an effective therapeutic tool for the treatment of neurobehavioral deficits in PD patients. Further research is needed to clarify the precise molecular mechanisms involved in the protective effects induced by carvacrol and exercise in the 6-OHDA model of PD.

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