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Protective Effect of Carvacrol in 6-hydroxydopamine Hemi-parkinsonian Rat Model

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A B S T R A C T

Background and Objective: A huge amount of investigational evidence support a role for oxidative stress as an intermediary of nerve cell dysfunction in Parkinson's disease (PD). Polyphenols such as carvacrol have been indicated to prevent neuronal deterioration caused by increased oxidative load, thus, this study evaluated whether carvacrol administration would attenuate behavioral abnormalities in an animal model of PD.

Materials and Methods: In this study, unilateral intrastriatal 6-hydroxydopamine injected rats were daily pretreated with carvacrol (10 mg/kg) started three days before the surgery. Apomorphine-induced rotations and level of stress oxidative markers in the midbrain were assessed after 2 weeks.

Results: Carvacrol administration lessened the rotation number in lesioned rats. Also, carvacrol decreased the 6-OHDA-induced malondialdehyde and nitrite level and intensified the antioxidant enzyme catalase, indicative of a protective effect against lipid peroxidation and free radicals synthesis.

Conclusion: In summary, carvacrol shows a protective effect against 6-OHDA neurotoxicity, partly through attenuating oxidative stress.

1. Introduction

Parkinson's disease (PD) is a neurological disorder involving the dopaminergic neurons death in the substantia nigra, followed with the loss of their terminals in the striatum. The lessening of dopamine level leads to the motor disturbances related to PD (1). The major causes of dopaminergic neurons deterioration in PD is oxidative stress, DNA rupture and iron accumulation (2). Free radicals destroy the dopaminergic neurons and interfere with oxidation- phosphorylation in mitochondria that causing to depletion energy production and finally to cell death (3). A large body of investigation indicate that 6-OHDA with generation of cytotoxic free radicals exerts its neurotoxicity effect on nigrostriatal dopaminergic neurons (4). Albeit there is a massive progression in generation of agents to treat PD, none of these elucidate the reason of the progressive death of dopaminergic neurons (5). At present, current treatments control the symptoms of PD without suppression of disease development. Moreover, since dopamine metabolism intensifies oxidative stress, long-term use of some drugs such as levodopa that control mainly motor dysfunction may be injurious to dopaminergic neurons and may put in danger the safety of patient (6). Hence, recently plentiful considerations have been focused on protection-based approaches.

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Of experimental viewpoint, flavonoids are natural constituents of the human diet that due to antioxidant and ROS scavenging, show a diversity of biological and phamalogical activities like cardioprotection, neuroprotection and chemoprevention (7, 8).

The aromatic plants belonging to the family Lamiaceae, such as oregano and thyme comprise many types of polyphenols. One of them that is principally accountable for the useful effect of these plants is carvacrol [2-Methyl-5-(1-methylethyl) phenol].

Carvacrol have been broadly used in traditional medicine and some spices containing it are commercially available (9). Some studies revealed that carvacrol exhibits a large number of beneficiary effects including fungicidal (10,11), insecticidal (12) and antimicrobial activities (13). Moreover, carvacrol has anticarcinogenic, antitumor, antimutagenic and antioxidant activities (14, 15). Since carvacrol is capable to cross the blood-brain barrier (16), it can reach to different sites in central nervous system and interact with receptors (17, 18). Regarding valuable effects of carvacrol, especially its antioxidant properties, this study was conducted to assess the protective effect of carvacrol administration in an early model of Parkinson's disease in the rat.

2. Materials and Methods

Adult male Wistar rats (250–300 g; n = 32) (Pasteur's Institute, Tehran, Iran) were housed four per cage in a temperature-controlled colony room under a seasonal light/dark cycle with freely access to food and water. They were held in the colony room for at least 1 week before being tested. The rats selected for this study did not show any rotational behavior (net rotations less than 30/h) following intraperitoneal injection of apomorphine hydrochloride (2 mg/kg). The animals were randomly divided into four groups: sham-operated group (Sham), carvacrol-treated sham-operated group (Sham + Car), lesion group (Lesion) and carvacrol-treated lesion group (Lesion+Car). Unilateral intrastriatal 6-OHDA injection (left side) was performed through a 10 μL Hamilton syringe on anesthetized rats (ketamine 100 mg/kg and xylazine 5 mg/kg, i.p.) using a stereotaxic apparatus (Stoelting, USA) at the coordinates: L -3mm, AP+9.2 mm, V+4.5 mm from the center of the interaural line, according to the atlas of Paxinos and Watson (Paxinos and Watson, 1986). The 6-OHDA group received a single injection of 5 μL of 0.9% saline containing 2.5 μg/μL of 6- hydroxydopamine-HCl (6-OHDA, Sigma) and 0.2% ascorbic acid (w/v) at a rate of 1 μL/min. The Sham group received an identical volume of ascorbate-saline solution.

The Lesion+ Car group received the neurotoxin in addition to intraperitoneal pretreatment with carvacrol (10 mg/kg/day dissolved in propylene glycol; Sigma) started three days before surgery.

2.1. Behavioral testing

The animals were tested for apomorphine hydrochloride (2 mg/kg, i.p.)-induced rotational behavior, 1 week before (baseline) and 2 weeks after the surgery. The rotations were measured according to a method described previously (19). First, the animals were allowed to habituate for 10 min and then 1 min after the injection of the drug, full rotations were counted in a cylindrical container (diameter 33 cm and height 35 cm) at 10 min intervals for the first 60 min in a quiet isolated room. The net number of rotations was defined as the positive scores minus the negative scores.

2.2. Determination of midbrain MDA concentration

After removing of decapitated brains, anterior third left of left midbrain block was blotted dry, weighed, then made into 10% tissue homogenate in ice-cold 0.9% saline solution, centrifuged (1000×g, 4ºC, 10 min) to remove particulates, obtained supernatant was aliquotted, then stored at −80 ºC until assayed. The MDA concentration (thiobarbituric acid reactive substances, TBARS) in the supernatant was measured as described before (20). Briefly, trichloroacetic acid and TBARS reagent were added to supernatant, then mixed and incubated at 100 ◦C for 80 min. After cooling on ice, samples were centrifuged at 1000×g for 20 min and the absorbance of the supernatant was read at 532 nm. TBARS results were expressed as MDA equivalents using tetraethoxypropane as standard.

2.3. Assay of midbrain NO concentration

The Griess method was used for measuring of

NO content in the supernatant of midbrain homogenate according to the previous works (20). Because NO has a short half life and is rapidly converted to the stable end products nitrate (NO3−) and nitrite (NO2−), the principle of the assay is the conversion of nitrate into nitrite by cadmium and followed by color development with Griess reagent (sulfanylamide and N-naphthylethylenediamine) in acidic medium. The total nitrite was measured by Griess reaction. The absorbance was determined at 540 nm with a spectrophotometer.

2.4. Determination of catalase activity

For assessment of homogenate catalase activity, we used the Claiborne's method (21). For this purpose, H_2O_2 was added to a combination of 50 mM potassium phosphate buffer (pH 7.0) and by calculating the absorbance changes at 240 nm for 60 s, the rate of H_2O_2 decomposition was measured. The enzyme activity was expressed as unit/mg protein.

2.5. Protein assay

The Bradford method was used for assessing of protein content of the supernatant using bovine serum albumin (Sigma Chemical, St. Louis, MO) as the standard (22).

2.6. Statistical analysis

All data were expressed as mean ±SEM. For the apomorphine hydrochloride-induced rotational behavior, one-way ANOVA followed by the post-hoc test was used. The values of Nissl-stained cells for the injected and non-injected sides were compared using two-tailed Student's *t*-test for paired samples and the inter-group differences were analyzed using one-way ANOVA. In all analyses, a difference at $p < 0.05$ was regarded as significant.

3. Results

3.1. Rotational behavior

In this study, there was no mortality due to drug administration. Because, there was no significant behavioral and histological differences between the Sham and Sham+ Carvacrol groups, the results of these groups were considered together. The protective effect of carvacrol on the apomorphine hydrochloride - induced rotational behavior was recorded for a period of 1 h. As shown, before surgery, the induced rotational behavior exhibited no significant difference. But, two weeks after the surgery, the total net number of rotations made over 1 h period showed a very significant contralateral turning in the rats of the Lesion group ($p < 0.001$) and induced less significant rotations in the Lesion+Car group ($p < 0.01$) in comparison with the Sham and Lesion groups, respectively (Table 1).

3.2. Oxidative stress markers

Regarding midbrain lipid peroxidation and oxidative stress markers (Figures 1-3), 6-OHDA injection resulted in significant elevation of MDA and nitrite content ($P < 0.001$) and a significant reduction of catalase activity ($P < 0.01$). Treatment of lesioned rats with carvacrol significantly attenuated the increased MDA and nitrite content ($P<0.001$). While the level of catalase activity was slightly increased in Lesion+Car group in comparison with lesioned group, but this augmentation was not significant.

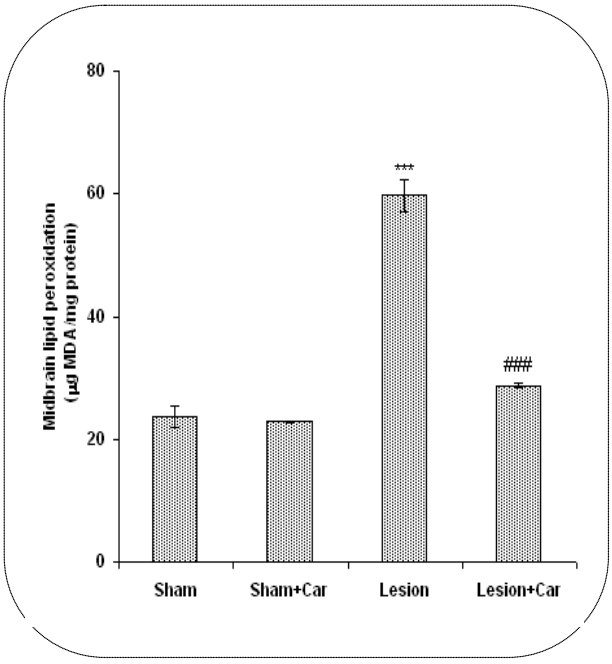

Figure 1. Malondialdehyde (MDA) concentration in midbrain homogenate from different groups. Animals were pretreated with carvacrol at a dose of 10 mg/kg before intrastriatal 6-OHDA injection (left side); ***p<0.001 (vs. sham); ###p<0.001 (vs. lesion). (means±SEM).

Table 1. Total net number of apomorphine (2 mg/kg, i.p.)-induced rotational behavior (mean±SEM) for a period of 60 min

	Sham	Sham+Car	Lesion	Lesion+Car
1 week pre-surgery	1.85±0.93	1.25±0.47	1.16±0.6	1.16±0.87
2 weeks post-surgery	1.14±0.13	1±0.91	214.6±11.19[a]	48±3.73[b]

[a]p < 0.00 1 (compared with the Sham group), [b] p < 0.01 (compared with the Lesion group).

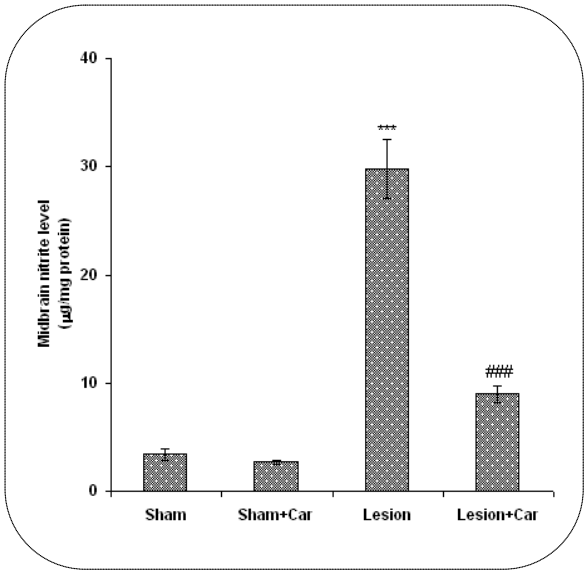

Figure 2. Nitrite content in midbrain homogenate from different groups. Animals were pretreated with carvacrol at a dose of 10 mg/kg before intrastriatal 6-OHDA injection (left side). ***p<0.001 (vs. sham); ###p<0.001 (vs. lesion). (means±SEM).

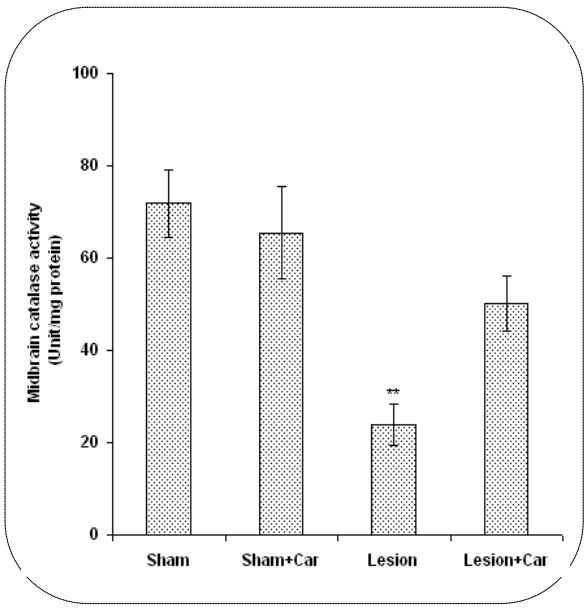

Figure 3. Catalase activity in midbrain homogenate from different groups. Animals were pretreated with carvacrol at a dose of 10 mg/kg before intrastriatal 6-OHDA injection (left side). **p<0.01 (vs. sham) (means±SEM).

4. Discussion

As a great number of studies have mentioned, dopamine acts as a modulator of cognitive, learning and memory processes and motor activity (23). Therefore, dysfunction of this neurotransmitter could robustly lead to mood, cognitive and emotional changes.

Intrastriatal administration of 6-OHDA that is used for development of an animal model of unilateral Parkinsonism (2), causes to lower dopamine level in striatum. This reduction leads to an upregulation of dopaminergic postsynaptic receptors on the same side. These variations generate an important motor irregularity that can be assessed by direct acting (apomorphine) and indirect-acting (amphetamine) dopaminergic agonists (24). Studies have revealed that the apomorphine-induced rotational behavior could be considered as trustworthy indicators of nigrostriatal dopamine exhaustion (25).

On the other hand, 6-OHDA toxification is primarily induced by free radicals production in nigrostriatal system. Lipid peroxidation with cell membrane destruction lead to synthesis of free radicals including reactive aldehyde metabolites (26). One of the major metabolites that is used for evaluating of oxidative destruction is malondialdehyde (MDA) (27). In the present study, we observed that the number of rotations induced by apomorphine decreased in the carvacrol-treated 6-OHDA-lesioned group. This potential protective effect of carvacrol in attenuation of rotational behavior could be associated to potency of carvacrol in prevention of nigral neurons degeneration and the maintenance of striatal dopamine amount at a level near normal. Some studies reveal that carvacrol is a regulator of dopaminergic transmissions in brain so that intracerebroventricular administration of this neurotransmitter exerts neuroprotective properties in a experimental model of cerebral artery occlusion (28).

The oregano extract has been shown to inhibit the reuptake and degradation of the monoamine neurotransmitters through reduction of monoamine oxidase activity and enzymatic destruction. These changes could lead to accumulation of monoamine transmitters in synaptic cleft (29). In addition, carvacrol may have reduced neuronal damage and death by neutralizing oxidative stress. In a study, it has been demonstrated that carvacrol could ameliorate amyloid β(Aβ) or scopolamine-induced cognitive weakening. It seems that the carvacrol anticholinesterase, antioxidant, and anti-inflammatory properties are involved in its useful effects (30). Moreover, carvacrol is effective as an analgesic compound in various nociceptive models, probably by inhibition of peripheral mediators that could be related with its strong antioxidant effect observed *in vitro* (31). It has been shown that carvacrol supplementation could increase antioxidant enzyme activities, impede lipid oxidation, enhance digestive enzyme activities, and improve immune response (32). Also, in the present study, the antioxidant effect of carvacrol was revealed. So that, it decreased MDA and nitrite levels in lesioned-carvacrol treated group.

Collectively, the results of our study indicate that intraperitoneal administration of carvacrol may exert benefitial effects on behavioral abnormalities induced by 6-OHDA through its anti-oxidant properties.

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