

Melissa officinalis aqueous extract ameliorates 6-hydroxydopamine-induced neurotoxicity in hemi-parkinsonian rat

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A B S T R A C T

Background and Objective: Parkinson's disease (PD) is an age-related neurodegenerative disorder with massive loss of dopaminergic neurons in the substantia nigra pars compacta. L-Dihydroxyphenylalanine (L-DOPA) substitution is still the gold standard therapy for PD. However, there has been little information available on neuroprotective and regenerative therapies for PD. Due to the neuroprotective and anti-oxidant property of *Melissa officinalis* (MO), this research study was done to evaluate whether MO could improve behavioral and structural changes in an experimental model of early PD in rat.

Materials and Methods: In this study, rats (n = 48) were divided into 4 groups, i.e. sham-operated, SO-treated sham-operated, 6-OHDA-lesioned and MO-treated lesioned groups. The experimental model of PD was induced by unilateral intrastriatal injection of 6-hydroxydopamine (6-OHDA, 12.5 mg/5ml of saline-ascorbate; left side). The treated sham and lesioned groups received MO at a dose of 100 mg/kg once a day before surgery for three times at an interval of 24 h. One week post-surgery, the animals were tested for rotational behavior by apomorphine for an hour and the number of dopaminergic neurons in the substantia nigra pars compacta (SNc) was counted.

Results: MO pretreatment significantly improved apomorphine-induced turning behavior and partially prevented loss of SNc neurons with no significant effect on the Sham group.

Conclusion: These results suggest that MO pretreatment could exert neuroprotection against 6-OHDA neurotoxicity, as observed by preservation of dopaminergic neurons and attenuation of motor asymmetry and this may have potential benefit in neurodegenerative and movement disorders like PD.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder and the most common movement disorder hallmarked with degeneration of nigrostriatal dopaminergic neurons within basal ganglia leading to movement abnormalities like tremor, bradykinesia, rigidity, and postural imbalance (1). The main neuropathological feature of PD is the progressive and marked degeneration of the nigrostriatal dopaminergic neurons, whose cell bodies reside in the substantia nigra pars compacta (SNc) and nerve terminals project to the neostriatum (2, 3). The 6-hydroxydopamine

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(6-OHDA) neurotoxin has generally been used to induce the degeneration of dopaminergic neurons and in this way could produce experimental parkinsonism in rodents like rats (4). After 6-OHDA injection, some behavioral, biochemical, and pathological hallmarks of PD are observed (5). The toxic and deteriorating effect of 6-OHDA are due to enhanced oxidative stress, inflammatory processes and induction of apoptosis (6). Mitochondrial dysfunction and increased oxidative stress burden are also responsible for neuronal loss in patients with PD (7). Although great achievements have been attained in the development and innovation of novel agents for treatment of PD, until now, no pharmacological agent has convincingly had the capability to prevent and/or at least slow the progression of PD (8). Also, some patients with PD receiving dopamine replacement therapies like levodopa may develop some signs of dyskinesia that becomes a major complication in long term (9). Neuroprotective strategies have been accepted as an alternative options for slowing progression of PD (10). *Melissa officinalis* L. with the general name lemon balm belongs to the family Lamiaceae and grows widely in central and southern Europe and in Asia minor (11). This plant is an aromatic (lemony) perennial herb, up to about 1 m in height. Parts mostly used for medicinal purposes are dried leaves, often having flowering tops (11). Green lemon aromatized leaves of this plant are used as fresh leaves, as well as in their dried form in salads, sauces, soups, with vegetables and meat, and in desserts (11). This plant is also used for preparation of some herbal teas. In addition, *M. officinalis* has been used in a variety of practical applications in medical sciences. In this respect, its leaves contain some volatile oils (11). The leaf also contains polyphenolic compounds including caffeic acid derivatives like rosmarinic acid, trimeric compounds, and some flavonoids (11). Neuroprotective and neurological properties of *Melissa officinalis* has been proven in on hydrogen peroxide induced toxicity in PC12 cells (12). Meanwhile, *M. officinalis* can modulate a number of behavioral measures, with indications including administration as a mild sedative, in disturbed sleep, and in the attenuation of the symptoms of nervous disorders, including the reduction of excitability, anxiety, and stress (13). *M. officinalis* extract can attenuate the subjective effects of laboratory-induced stress. It can be a useful herbal medicine for the treatment of gastrointestinal spasms (13). The beneficial

effect of a *Melissa officinalis* plant extract infusion on the severity of physiological chronic stress induced by movement restriction has been proved (13). Considering these impressive array of beneficial effects of this plant, the present study tried to investigate the neuroprotective effect of *M. officinalis* in 6-OHDA rat model of hemi-parkinsonism.

2. Materials and Methods

2.1. Extraction

MO aerial part was dried under shade at room temperature. Thereafter, 100 g of its powder was mixed with 1000 ml of distilled boiling water for a period of 10 min under continuous stirring. The obtained mixture was filtered twice through a mesh and then one time through a filtered funnel, and the obtained liquid was dried on a magnet stirrer until a concentrated residue was obtained. This stock extract was maintained at -20 °C until being used. Lower concentrations of the extract were prepared by its dilution.

2.2. Animals

Adult male Wistar rats (250-300 g; n = 48) were procured from Pasteur's Institute of Tehran and housed in a temperature-controlled colony room under light/dark cycle with food and water available ad libitum. The used protocols were according to NIH guidelines for the care and use of laboratory animals. The animals were held in the colony room for at least one week before being tested. Only rats not showing any biased rotational behavior (net rotations less than 30/hour) following intraperitoneal injection of apomorphine hydrochloride (2 mg/kg) (Sigma Chemical, USA) were selected for the present study. The animals were randomly divided into four groups: sham-operated group, MO-treated sham-operated group (Sham + MO), lesion group (6-OHDA) and MO-treated lesion group (6-OHDA + MO). Unilateral intrastriatal 6-OHDA (SigmaAldrich, USA) injection (left side) was performed through a 5 ml Hamilton syringe on anesthetized rats (ketamine 80 mg/kg and xylazine 10 mg/kg, i.p.) using stereotaxic apparatus (Stoelting, USA) at the coordinates: L -3 mm, AP +9.2 mm, V + 4.5 mm from the center of the interaural line, according to the atlas of Paxinos and Watson. At the end of injection, the needle was left in place for an additional 5 min and then withdrawn at a rate of 1 mm/min.

The lesion group received a single injection of 5 ml of 0.9% saline containing 2.5 mg/ml of 6-hydroxydopamine-HCL (6-OHDA) and 0.2% ascorbic acid (W/V) at a rate of 1 ml/min. The sham group received an identical volume of ascorbate-saline solution. The 6-OHDA + MO group received the neurotoxin in addition to MO aqueous extract using rodent gavage dissolved in water at a dose of 100 mg/kg. MO extract was administered three times with the last administration 1 h before surgery.

2.3. Behavioral testing

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

2.4. Histological study

At the end of behavioral experiments, half of the rats in each group were deeply anesthetized with a high dose of ketamine (150 mg/kg) and perfused through the ascending aorta with 50-100 ml of 0.9% saline followed by 100-200 ml of fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) followed by 100 ml of 0.1 M PB containing 10% sucrose. Following perfusion, the brains were removed from the skull, blocks of forebrain and brainstem were prepared, and after final steps of preparation (immersion in 30% sucrose solution for 2-3 days), sections were cut at a thickness of 40 μ m on a freezing microtome (Leica, Germany) and collected in PB (0.1 M). Every second section was Nissl-stained with 0.1% cresyl violet (Sigma).

2.5. Neuronal counting

For each animal, mesencephalic sections (Interaural 2.9-4.2 mm) were examined by a method. Briefly, Nissl-stained neurons of the SNC were counted manually (Light microscopy; X400) using a superimposed grid to facilitate the procedure. At least two sections representative of

each of four Paxinos-Watson planes (4.2, 3.7, 3.2, 2.97; Interaural) were examined by scanning the entire extent on each side.

2.6. Statistical analysis

All data were expressed as mean \pm S.E.M. For the drug-induced rotational behavior, the non-parametric Kruskal-Wallis test was used. Inter-group differences for values of Nissl-stained neurons for the injected side and biochemical assays were found out using one-way ANOVA followed by Tukey's *post-hoc* test. In all analyses, the null hypothesis was rejected at a level of 0.05.

3. Results

The beneficial effect of MO extract was evaluated on apomorphine-induced rotations for a period of 1 hour (Fig. 1). There were no significant differences among the groups at baseline (before surgery). Statistical analysis of the total net number of rotations 1 week after the surgery showed that apomorphine caused a very significant contralateral turning in the rats of 6-OHDA group ($p < 0.0005$) and induced less significant rotations in 6-OHDA+ MO group ($p < 0.01$) in comparison with 6-OHDA group.

The results of histochemical studies (Figures 2 and 3) showed that there is no significant difference between sham and sham+ MO regarding number of Nissl-stained neurons on the left side of SNC. Meanwhile, a significant reduction was observed in 6-OHDA group ($p < 0.01$). In addition, number of Nissl-stained neurons on the left side of SNS was significantly higher in 6-OHDA+ MO versus 6-OHDA group ($p < 0.05$).

4. Discussion

In this study, we demonstrated that MO aqueous extract at a dose of 100 mg/kg significantly decreases apomorphine-induced rotations and attenuates loss of SNC neurons.

The selective degeneration of SNC dopaminergic neurons is likely to be due to direct toxicity effect in PD patients (14, 15). In addition, the neurotoxin 6-OHDA is commonly used for the induction of PD in experimental animals and could cause degeneration of dopaminergic neurons (16). The unilateral damage of the

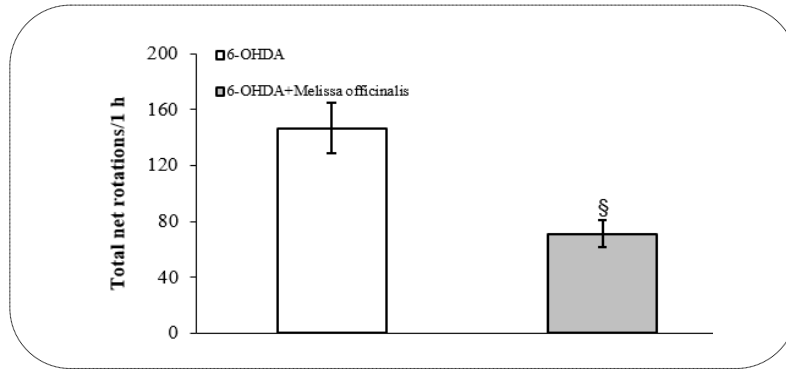


Figure 1. Total net number of rotations (mean \pm S.E.M.) induced by apomorphine (2 mg/Kg, i.p.) after 1 week over a period of 60 min in 6-OHDA-lesioned group. Note that the positive values indicate contralateral rotations. 6-OHDA stands for the neurotoxin 6-hydroxydopamine. \$ $p < 0.05$ (versus 6-OHDA)

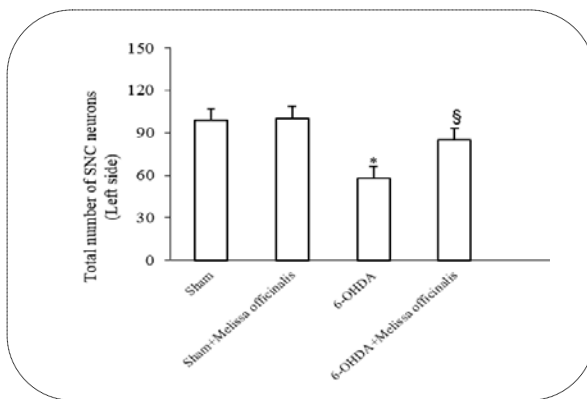


Figure 2. Total number of Nissl-stained neurons on the left side of substantia nigra pars compacta (SNC) in different groups after 1 week post-surgery. 6-OHDA stands for the neurotoxin 6-hydroxydopamine.

* $p < 0.01$ (in comparison with Sham), \$ $p < 0.05$ (versus 6-OHDA)

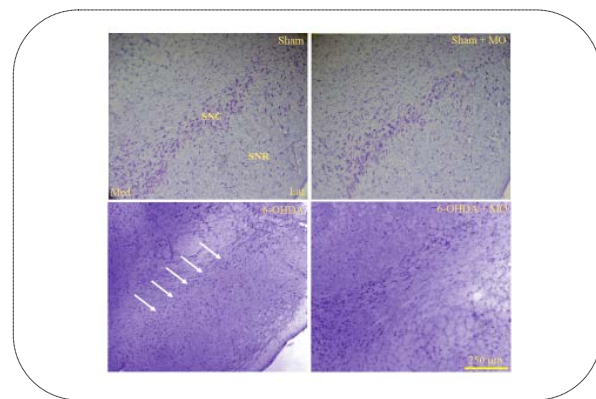


Figure 3. Photomicrograph of coronal sections through the midbrain showing Nissl-stained neurons in experimental groups. A severe reduction in the number of neurons in SNC was observed in the 6-OHDA lesioned group, but no such marked reduction was noted in the MO-treated lesioned groups in comparison with Sham group. Scale bar = 250 μ m (SNC and SNR = Substantia nigra pars compacta and pars reticulata, respectively)

nigrostriatal dopaminergic system through intrastriatal injection of 6-OHDA is followed by a reduction in the striatal dopamine level and an upregulation of dopaminergic postsynaptic receptors at the same side. These changes produce a prominent functional and motor asymmetry that can be evaluated by dopaminergic agonists like apomorphine (17). The observed attenuation of rotational behavior in MO-pretreated 6-OHDA group could be due to the possible neuroprotective effect of MO aqueous extract against SNC neurodegeneration and maintenance of striatal dopamine at a level that is not accompanied with a marked rotational behavior. In other words, nigrostriatal neurons within SNC were mainly preserved in the

presence of this extract against neurodegenerative effects induced by the neurotoxin 6-OHDA.

Oxidative stress is strongly involved in the toxicity of 6-OHDA-induced nigrostriatal lesions (18). Oxidative stress is an important factor that could affect the survival of dopaminergic neurons in PD. Neurons mostly depend on energy produced by mitochondria and are simultaneously faced with high levels of reactive oxygen species (ROS) as well as increased levels of free iron, which can promote OH generation (19). Overload of the free radical formation may lead to cell death. In addition, auto-oxidation of dopamine may produce dopamine quinone (20). Formation of species such as semiquinones and

other free radicals could especially damage nucleic acids, proteins, and membrane lipid components (21). Therefore, the therapeutic approaches are aimed at attenuation of oxidative stress. In addition, free radical scavengers may also be helpful in prolonging survival time of dopaminergic neurons (22). In this respect, MO extract could attenuate neuronal damage and loss through counteracting oxidative stress, possibly via regulating antioxidant defense system as well as inhibition of free radical generation (12).

Overall, the results of our study clearly suggest that MO aqueous extract could have neuroprotective effect in 6-OHDA model of parkinsonism. However, further studies are required to understand its exact mechanism of action.

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