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Malva sylvestris aqueous extract could ameliorate 6-hydroxydopamine-induced motor asymmetry with no protective effect on dopaminergic nigrostriatal neurons in the rat

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ABSTRACT

Background and Objective: Parkinson's disease (PD) is a common neurological disorder due to degeneration of dopaminergic neurons within pars compacta of substantia nigra (SNC). With regard to protective effect of *Malva sylvestris* (MS), this study was conducted to evaluate the effect of aquaeous extract of this plant in an experimental model of PD induced by 6-hydroxydopamine (6-OHDA).

Materials and Methods: Rats were divided into sham-operated, MS-treated sham-operated, lesioned and MS-treated lesioned groups. The hemi-PD early model was induced by unilateral intrastriatal injection of 6-OHDA (12.5 µg/5µl of saline-ascorbate, left side). The treated groups received aquaeous extract of MS (i.p.) at a dose of 100 mg/kg once a day for one week at an interval of 24 h till 1 h pre-surgery. One week after surgery, the animals were tested for rotational behavior by apomorphine for an hour and the number of dopaminergic neurons in the SNC was counted.

Results: After one week, apomorphine caused a significant contralateral turning ($P<0.001$) in 6-OHDA-lesioned group and a reduction in the number of neurons on the left side of the SNC in the lesioned group ($P<0.05$) in comparison with sham group. In addition, pretreatment with MS extract at a dose of 100 mg/kg significantly decreased the rotational behavior in lesioned rats ($p<0.01$) but could not significantly prevent the reduction of SNC neurons versus lesioned group.

Conclusion: Intraperitoneal administration of aquaeous extract of *Malva sylvestris* could reduce motor asymmetry and has not neuroprotective effect against 6-OHDA toxicity in an experimental model of PD.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder with progressive nature highlighted by degeneration of nigrostriatal dopaminergic neurons and debilitating motor symptoms like tremor, bradykinesia, rigidity, and postural imbalance (1). The 6-hydroxydopamine (6-OHDA) neurotoxin is generally used to induce the degeneration of dopaminergic neurons in rodents like rat (2). After 6-OHDA injection, some behavioral, biochemical, and pathological hallmarks of PD are observed (3).

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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 halt or postpone the progression of PD (4). Neuroprotective treatments have been useful alternative options for slowing PD progression (5).

Among the numerous species that has been used in folk medicine, *Malva sylvestris L.* (Malvaceae; MS) stands out due to its various uses. Currently, the consumption of MS is worldwide and new research has revealed its important therapeutic potential (6-11). Leaves of *Malva sylvestris* possess very strong antioxidant properties including radical-scavenging activity, reducing power and lipid peroxidation inhibition in lipossomes and brain cells homogenates. This plant is also the richest in nutraceuticals such as powerful antioxidants, unsaturated fatty acids (e.g. alpha-linolenic acid), and minerals (8). Since no previous work on this plant regarding models of CNS disorders has been conducted before and considering its impressive array of beneficial effects, the present study tried to investigate the neuroprotective potential of MS in 6-OHDA rat model of hemi-parkinsonian rat.

2. Materials and Methods

2.1. Extraction

MS leaves were 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 (200-240 g; n = 32) were obtained 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 (2mg/kg) (Sigma, USA) were selected for the present study. The animals were randomly divided into four groups: sham-operated group, MS-pretreated sham-operated group (Sham + MS), lesion group (6-OHDA) and MS-treated lesion group (6-OHDA + MS). Unilateral intrastriatal 6-OHDA (Sigma Chemical, USA) injection (left side) was performed through a 5 µl 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 µl of 0.9% saline containing 2.5 µg/µl of 6-hydroxydopamine-HCL (6-OHDA, Sigma Chemical, USA) 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 6-OHDA + MS group received the neurotoxin in addition to MS aqueous leaf extract using rodent gavage dissolved in water at a dose of 100 mg/kg. MS extract was daily administered one week 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) 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 30 µm 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, USA).

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 ± S.E.M. For the drug-induced rotational behavior, the parametric one-way ANOVA was used. Inter-group differences for values of Nissl-stained neurons for the injected side 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 MS 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 ($p<0.001$) versus sham and induced less significant rotations in 6-OHDA+ MS group ($p<0.01$) in comparison with 6-OHDA group.

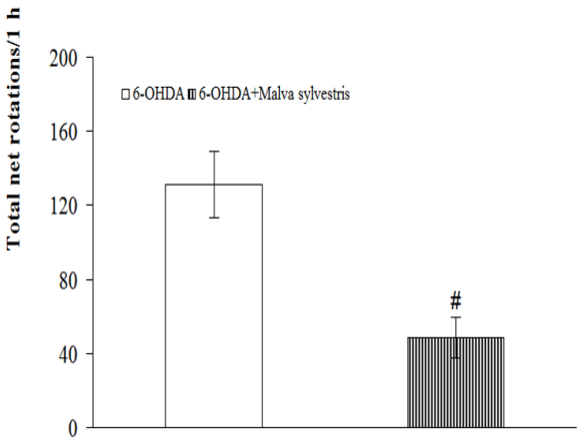

Fig. 1. Total net number of rotations (mean ± S.E.M.) induced by apomorphine (2 mg/Kg, i.p.) after 1 week over a period of 60 min. Note that the positive values indicate contralateral rotations. 6-OHDA stands for the neurotoxin 6-hydroxydopamine.
# $p<0.01$ (versus 6-OHDA)

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 ($p<0.05$).

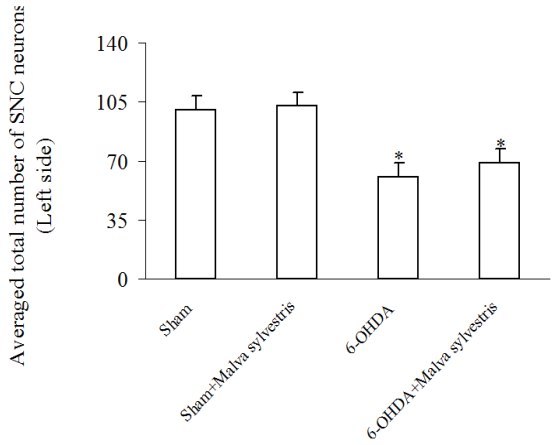

Fig. 2. Total number of Nissl-stained neurons on the left side of substantia nigra pars compacta (SNC) in different groups after 1 week post-surgery. 6-OHDA stands for the neurotoxin 6-hydroxydopamine.

* $p<0.05$ (in comparison with Sham)

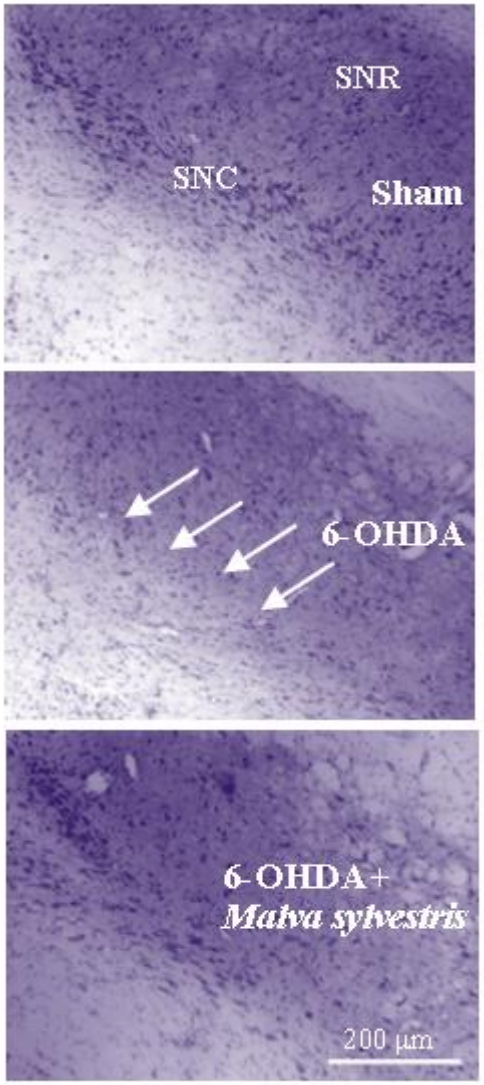

Fig. 3. Photomicrograph of coronal sections through the midbrain showing Nissl-stained neurons in experimental groups. A severe reduction in the number of neurons in SNC was observed in the 6-OHDA lesioned group and such marked reduction was also noted to a lesser degree in the MS-pretreated lesioned groups in comparison with Sham group. Scale bar= 200 µm (SNC and SNR = Substantia nigra pars compacta and pars reticulate, respectively)

4. Discussion

In this study, we demonstrated that MS aqueous extract at a dose of 100 mg/kg significantly decreases apomorphine-induced rotations with no significant protective effect on dopaminergic SNC neurons.

The selective degeneration of SNC dopaminergic neurons is likely to be due to direct toxicity effect in PD patients (12, 13). In addition, the specific neurotoxin 6-OHDA is generally used for the induction of PD in experimental animals and could cause degeneration of dopaminergic neurons (14). The unilateral damage of the 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 (15). Since MS extract in our study did not protect SNC neurons against 6-OHDA neurotoxicity, therefore, the observed attenuation of rotational behavior in MS-pretreated 6-OHDA group could be due to the enhanced release of dopamine from the remaining dopaminergic terminals and/or the occurrence of sprouting within the neostriatum, in this way maintaining striatal dopamine at a level that is not accompanied with a marked rotational behavior. In this respect, dopaminergic sprouting is known to contribute to compensatory mechanisms in 6-OHDA lesioned rats and monkeys (16). It has been known that reduced storage capacity leads to pulsatile delivery of dopamine in the striatum and this could explain the emergence of wearing off and dyskinaesia in PD. Lee et al in 2008 have shown that surviving DA neurons in 6-OHDA lesioned rats sprout to re-innervate the striatum and to maintain terminal density until approximately 60% of neurons that have been lost (16).

Taken together, the results of this study indicated that MS aqueous leaf extract could attenuate motor asymmetry with no neuroprotective effect in 6-OHDA model of PD. However, further studies are required to explore its dose-dependent effect regarding its possible neuroprotective potential.

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