

# Dose-dependent effect of *Hypericum perforatum* extract on motor imbalance following intrastriatal injection of 6-hydroxydopamine in the rat

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## ABSTRACT

**Background and Objective:** Parkinson's disease (PD) is a neurodegenerative disease with selective damage of mesencephalic dopaminergic neurons. Due to the protective, anti-inflammatory, and antioxidant effect of *Hypericum perforatum* (HP), this study was undertaken to assess dose-dependent effect of HP hydroalcoholic extract on motor imbalance following intrastriatal injection of 6-hydroxydopamine in the rat.

**Materials and Methods:** In this experimental research, male Wistar rats (n=35) were equally divided into sham, 6-hydroxydopamine (OHDA)-lesioned, and HP extract-treated lesion groups. Model of PD was induced by microinjecting 12.5 microgram of 6-OHDA dissolved in saline-ascorbate solution into the left striatum. Treated lesion groups received HP extract at doses of 50, 100, and 200 mg/kg/day p.o. started one week before the surgery for 1 week post-surgery. After 1 week, ipsilateral and contralateral rotations induced by apomorphine were counted and net scores were obtained.

**Results:** In the 6-OHDA-lesioned group, the dopaminergic agonist apomorphine induced contralateral rotational behavior ( $P<0.001$ ) as compared to sham. In addition, administration of HP extract at doses of 100 and 200 mg/kg significantly reduced the number of contralateral rotations ( $P<0.05$ ) versus 6-OHDA group.

**Conclusion:** Oral administration of HP extract at doses of 100 and 200 mg/kg could reduce motor imbalance and attenuate forced biased rotational behavior in 6-OHDA-induced model of PD.

## 1. Introduction

**P**arkinson's disease (PD) is a rather common neurodegenerative disorder with progressive nature and the most common movement disorder characterized with degeneration of nigrostriatal dopaminergic neurons within basal ganglia leading to movement abnormalities like tremor, bradykinesia, rigidity, and postural imbalance (1). The main neuropathological hallmark of this disease is the selective degeneration of the

nigrostriatal dopaminergic neurons within substantia nigra pars compacta (SNc) (2, 3). The neurotoxin 6-hydroxydopamine (6-OHDA) is generally used for induction of the degeneration of dopaminergic neurons and modeling of PD in rodents like rat (4). Following 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).

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Mitochondrial dysfunction and increased oxidative stress burden are also responsible for neuronal loss in patients with PD (7). Although great achievements have been made in the development and innovation of novel agents for treatment of PD, until now, no pharmacological agent has satisfactorily had the ability to stop or slow the progression of PD pathogenic mechanism (8).

*Hypericum perforatum* (*H. Perforatum*, St. John's wort) extract has traditionally been used for a wide range of disorders (9). The most common routine use of St. John's wort is for depression management (10, 11). *H. perforatum* could lower lipid peroxidation (12), protect neurons against oxidative stress induced by hydrogen peroxide in the PC12 cells (13-15), exhibit neuroprotective effect in rotenone-induced model of PD (16, 17), and improve bodily response to chronic stress (9). Considering these impressive array of beneficial effects of HP, the present study tried to investigate dose-dependent effect of HP hydroalcoholic extract on motor imbalance induced by intrastriatal injection of 6-hydroxydopamine in the rat.

## 2. Materials and Methods

### 2.1. Extraction

Aerial parts of the plant were dried for 1 week at room temperature under shade and were grinded. The extract was prepared with addition of 10 g of HP powder to 100 ml of 70% ethanol. The extraction was done using maceration method for 72 h at room temperature in darkness. Then, the solution was filtered three times, dried on a rotary evaporator at 40°C, yielding 2.14 g (21.4%) of the extract.

### 2.2. Animals

Adult male Wistar rats (205-265 g; n = 35) were obtained from Pasteur's Institute of Tehran and kept in a temperature-controlled room with food and water freely available. The used protocols were according to NIH guidelines for the care and use of laboratory animals. Only rats not showing any rotational behavior less than 30/hour following intraperitoneal injection of apomorphine (2 mg/kg) were chosen for the present study. The animals were randomly allocated to 5 groups, i.e. sham-operated group (sham), 6-OHDA-lesioned group (6-OHDA) and HP-treated lesioned groups. Unilateral

intrastratial 6-OHDA (Sigma Chemical, USA) injection (left side) was done via a 5 µl Hamilton syringe on anesthetized rats (a combination of 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 +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. The sham group received an identical volume of ascorbate-saline solution. The treated 6-OHDA groups received the neurotoxin in addition to HP hydroalcoholic extract using rodent gavage dissolved in water at doses of 50, 100, and 200 mg/kg started one week before the surgery for 1 week post-surgery.

### 2.3. Behavioral evaluation

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

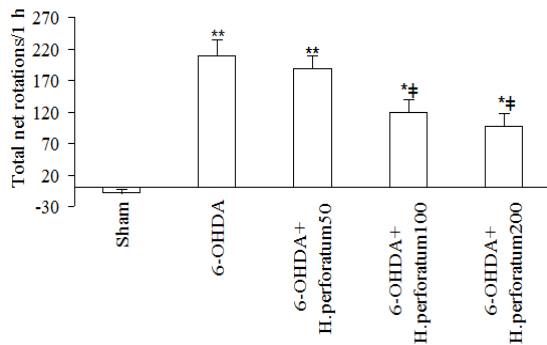
### 2.4. Statistical analysis

All data were expressed as mean ± S.E.M. For the drug-induced rotational behavior, one-way ANOVA followed by Tukey's post-hoc test was used. In all analyses, the null hypothesis was rejected at a level of 0.05.

## 3. Results

The beneficial effect of HP hydroalcoholic extract at different doses was studied on apomorphine-induced rotational bias for 1 hour (Fig. 1). There were no significant differences amongst the groups at baseline (before surgery). Statistical analysis of the total net number of rotations one week post-surgery showed that apomorphine caused a very significant contralateral turning in the rats of 6-OHDA group (p<0.001) and induced less significant rotations

in 6-OHDA+HP100 and 6-OHDA+HP200 groups ( $p < 0.005$ ) in comparison with sham group and the observed response for 6-OHDA+HP100 and 6-OHDA+HP200 groups were significantly attenuated versus 6-OHDA group ( $p < 0.05$ ).



**Fig. 1.** Total net number of rotations (mean  $\pm$  S.E.M.) induced by apomorphine (2 mg/kg, i.p.) 1 week after surgery over a period of 1 h in 6-OHDA-lesioned group. Note that the positive values indicate contralateral rotations. 6-OHDA stands for the neurotoxin 6-hydroxydopamine. The HP extract was used at doses of 50, 100, and 200 mg/kg. \*  $p < 0.005$ , \*\*  $p < 0.001$  (versus sham), #  $p < 0.05$  (versus 6-OHDA)

#### 4. Discussion

In this study, we demonstrated that HP hydroalcoholic extract could significantly attenuate motor imbalance at doses of 100 and 200 mg/kg. The selective degeneration of SNC dopaminergic neurons is likely to be due to direct toxicity effect in PD patients (18, 19). In addition, the neurotoxin 6-OHDA is commonly used for the induction of PD in experimental animals and could cause degeneration of dopaminergic neurons (20). 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 imbalance that can be evaluated by dopaminergic agonists like apomorphine (21). The observed attenuation of rotational behavior in HP-treated 6-OHDA group could be due to the possible neuroprotective effect of HP 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 may have been 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 (22). 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 (23). Overload of the free radical formation may lead to cell death. In addition, auto-oxidation of dopamine may produce dopamine quinone (24). Formation of species such as semiquinones and other free radicals could especially damage nucleic acids, proteins, and membrane lipid components (25). 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 (26). HP as a medicinal plant has reported to attenuate neuronal damage and loss through counteracting oxidative stress, possibly via regulating antioxidant defense system as well as inhibition of free radical generation (16, 17, 27).

Overall, the results of our study indicate that oral administration of HP extract at doses of 100 and 200 mg/kg could reduce motor imbalance and attenuate forced biased rotational behavior in 6-OHDA-induced model of PD. However, further research studies are warranted to understand its exact mechanism of action.

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