

# The Flavonoid Hesperetin Alleviates Behavioral Abnormality in 6-Hydroxydopamine Rat Model of Hemi-Parkinsonism

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## ABSTRACT

Parkinson's disease (PD) is a neuropathological and debilitating disorder involving the degeneration of mesencephalic dopaminergic neurons. Neuroprotective effect of hesperetin has already been reported, therefore, this study examined whether the administration of this flavonoid would attenuate behavioral abnormalities in an experimental model of PD in rat. For this purpose, unilateral intrastriatal 6-hydroxydopamine (6-OHDA, 12.5 µg/5µl of saline-ascorbate)-lesioned rats were pretreated i.p. with hesperetin (10 mg/kg). It was found out that hesperetin administration attenuates the rotational behavior in lesioned rats. In summary, hesperetin administration attenuates behavioral abnormality in hemiparkinsonian rats and this may be of benefit, along with other therapies, in neurodegenerative disorders including PD.

### Key Words:

Hesperetin,  
Parkinson's Disease,  
6-hydroxydopamine,  
Rotational Behavior.

## 1. Introduction

**P**arkinson's disease (PD) is a progressive and debilitating neurodegenerative disorder involving the degeneration of dopaminergic neurons in the substantia nigra pars compacta and is characterized by motor symptoms including hypokinesia, rigidity, tremor, and postural imbalance (Sauer, Oertel, 1994). Oxidative stress and increased lipid peroxidation, low glutathione levels, DNA damage and iron deposition has been reported as the main causes of dopaminergic neurons degeneration in PD. Oxidative stress not only destroys the dopaminergic neurons, but it also compromises mitochondrial oxidative phosphorylation, leading to decreased energy output by these organelles and eventually to secondary death of the cells (Schwartz, Huston, 1997). There is also an increasing amount of evidence that the neurotoxicity of 6-OHDA for model-

ing of PD is mainly due to its oxidation, resulting in generation of cytotoxic free radicals, which are believed to play a pivotal role in degeneration of the nigrostriatal dopaminergic system (Dauer, Przedborski, 2003).

Although great advances have been made in development of agents to treat PD, none yet address the underlying problem associated with it, i.e. the progressive loss of dopaminergic neurons. Presently, most treatments for PD are aimed to control the symptoms (Wu, Frucht, 2005). Although dopamine replacement therapy using agents like levodopa could effectively relieve symptoms, it does not prevent disease progression. In addition, there is a progressive increase in the prevalence of drug-related motor fluctuations and dyskinesia. Run-down of effectiveness, on-off effect, end of dose deterioration, and peak-dose dyskinesia are the most common side-effects and sometimes are quite difficult to deal with. Because dopamine metabolism

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increases oxidative stress and metabolites of levodopa are thought to be toxic, long-term use of levodopa may be harmful to dopaminergic neurons and may endanger the patient's health (Bezard, Brotchie, Cross, 2001). For this reason, search for neuroprotection-based strategies has received much attention in recent years. Of clinical interest, flavonoids are naturally occurring polyphenolic compounds as normal constituents of the human diet and are known for a variety of biological activities. Various bioactive flavonoids have been reported to exhibit important therapeutic activities involving cardioprotection, neuroprotection as well as chemoprevention in addition to possessing powerful antioxidant properties (Baluchnejadmojarad, et al, 2009). Recent studies have focused on the abilities of dietary polyphenols to protect against neuronal damage resulting from aging and neurodegenerative processes (Maher, 2006). Much attention has focused on the potential neuroprotective effects of flavonoids, which have been shown to protect against both age-related cognitive and motoric decline and against 6-hydroxydopamine neurotoxicity and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioning of the nigrostriatal tract (Yao, Vieira, 2007; Vauzour et al, 2008). This study was designed to investigate the possible beneficial effect of hesperetin on rotational behavior in 6-hydroxydopamine rat model of hemi-parkinsonism.

## 2. Methods

Adult male Wistar rats (210-260 g; n = 24) (Pasteur's Institute, Tehran, Iran) were housed three to four per cage in a temperature-controlled colony room under light/dark cycle with food and water available ad libitum. Procedures involving animals and their care were conducted in conformity with 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 (0.5 mg/kg) (Sigma Chemical, St. Louis, Mo., USA) were selected for the present study. The animals were randomly divided into 3 groups: sham-operated group, lesion group (6-OHDA) and hesperetin-treated lesion group. Unilateral intra-striatal 6-OHDA (Sigma Chemical, St. Louis, Mo., USA) injection (left side) was performed through a 5  $\mu$ l Hamilton syringe on anesthetized rats (ketamine 100 mg/kg and xylazine 5 mg/kg, i.p.) using stereotaxic apparatus (Stoelting, USA) at the coordinates: L -3 mm, AP 9.2 mm, V. 4.5-5 mm from the center of the interaural line, according to the atlas of

Paxinos and Watson (Paxinos, Watson, 1986). 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  $\mu$ l of 0.9% saline containing 2.5  $\mu$ g of 6-hydroxydopamine-HCL (6-OHDA, Sigma) and 0.2% ascorbic acid (W/V) at a rate of 1  $\mu$ l/min. The sham group received an identical volume of ascorbate-saline solution. Hesperetin was administered i.p. at a dose of 10 mg/kg one hour before surgery (Choi, Ahn, 2008).

### 2.1. Behavioral Testing

The animals were tested for rotational behavior by apomorphine hydrochloride (0.5 mg/kg, i.p.) one week before (baseline) and two weeks after the surgery. The rotations were measured according to a method as described previously (Roghani, Behzadi, 2001). Briefly, the animals were allowed to habituate for 10 min and then 1 min after the injection of drugs, 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 quiet isolated room. Net number of rotations was defined as the positive scores minus the negative scores.

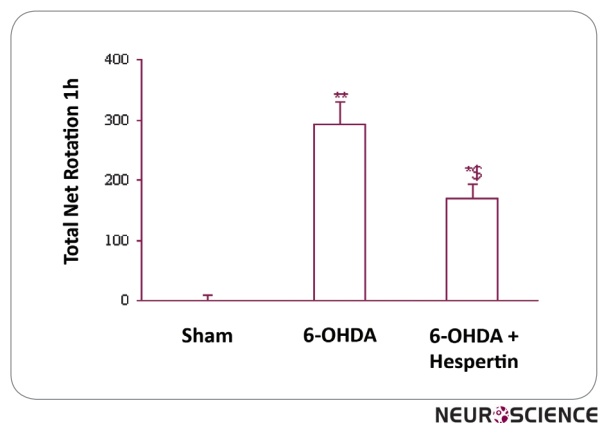
### 2.2. Statistical Analysis

All data were expressed as mean  $\pm$  S.E.M. For the drug-induced rotational behavior, non-parametric Kruskal-Wallis test was applied. In all analysis, the null hypothesis was rejected at 0.05 level.

## 3. Results

All animals well tolerated surgical operations and except for two rats that were excluded from the study due to morbidity, there was no mortality due to treatments. There was also no significant changes in the animals' weights, in each group.

The beneficial effect of hesperetin was evaluated on apomorphine-induced rotations for a one hour period (Fig. 1). There were no significant differences among the groups at baseline (before surgery). Statistical analysis of the total net number of rotations made over a 60-min period 2 weeks after the surgery showed that apomorphine caused a very significant contra lateral turning in the rats of 6-OHDA group ( $P < 0.0001$ ) and induced less significant rotations in hesperetin-treated lesion group ( $P < 0.001$ ) in comparison with Sham group. Moreover, group 6-OHDA + hesperetin showed a significant reduction of rotations ( $P < 0.05$ ) when compared to 6-OHDA group.



**Fig. 1:** Total net number of rotations (mean  $\pm$  S.E.M.) induced by apomorphine (0.5 mg/Kg, i.p.) at second week following the surgery over a period of 60 min in experimental groups. Note that the positive values indicate turns contralateral to the side of lesion. 6-OHDA stands for 6-hydroxydopamine.

\*  $p < 0.001$ , \*\*  $p < 0.0001$  (Versus Sham group)

#  $P < 0.05$  (versus 6-OHDA)

#### 4. Discussion

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 up-regulation of dopaminergic postsynaptic receptors at the same side. These changes produce a prominent functional and motor asymmetry which can be evaluated by direct acting (apomorphine) and indirect-acting (amphetamine) dopaminergic agonists (Sauer, Oertel, 1994; Schwarting, Huston, 1997). These rotations, especially those induced by apomorphine are considered as reliable indicators of nigrostriatal dopamine depletion (Schwarting, Huston, 1997). In the present study, a significant attenuation of the apomorphine-induced rotational behavior was observed in hesperetin-treated 6-OHDA-lesioned group after two weeks. The observed attenuation of rotational behavior in treated lesioned group in this study could be attributed to possible protective effect of hesperetin against nigral neurodegeneration and maintenance of striatal dopamine at a level that is not accompanied with a marked turning behavior. It is also possible that hesperetin may have preserved SNC neurons against neurodegenerative effects induced by the neurotoxin 6-OHDA which needs further studies. In this respect, it has been reported that reactive oxygen radicals are involved in the toxicity of 6-OHDA-induced nigrostriatal lesions that is used as an experimental model of unilateral Parkinsonism (Roghani, Behzadi, 2001). Oxidative stress is also an important factor that could affect the survival of dopaminergic neurons in PD (Choi, Ahna, 2008). Neuronal cells mostly depend on energy produced by mito-

chondria and are simultaneously faced with high levels of reactive oxygen species (ROS) as well as increased levels of free iron, which can promote OH formation (Choi, Ahna, 2008). Overload of the free radical formation may lead to cell death (Wu, Frucht, 2005); thus, free radical scavengers might be helpful in prolonging survival time of dopaminergic neurons (Algeri, 2002). In this respect, hesperetin may have attenuated neuronal damage and loss in our study through counteracting oxidative stress and in this way caused an improvement in rotational asymmetry in hemiparkinsonian rats. Hesperetin has been reported to have neuroprotective effect through attenuation of oxidative stress, which indeed proves the mentioned hypothesis to be right (Chi, Ahn, 2008). Nevertheless, further studies are warranted to test these hypotheses.

Taken together, hesperetin administration attenuates behavioral abnormality in hemiparkinsonian rats and this may be of benefit, along with other therapies, in neurodegenerative disorders including PD.

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