Original Article

Peganum Harmala L. Extract Reduces Oxidative Stress and Improves Symptoms in 6-Hydroxydopamine-Induced Parkinson’s Disease in Rats

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Abstract

Parkinson’s disease is one of the most common neurodegenerative disorders. There are many documents about the effects of oxidative stress in Parkinson’s disease etiology. Angiotensin II activates NADPH dependent oxidases and causes superoxides formation. Peganum harmala L. extract, which has angiotensin converting enzyme (ACE) inhibitory effect, is considered to evaluate oxidative stress inhibition and Parkinson’s disease improvement.

Male rats weighting 200-250 g were divided into 5 groups: Control, Neurotoxin (injection of 6-hydroxydopamine into left hemisphere substantia nigra), Peganum harmala’s seeds aqueous extract (10 mg/kg) and captopril (5 mg/kg). Peganum harmala and captopril were injected intraperitonealy -144, -120, -96, -72, -48, -24, -2, 4 and 24 h relative to 6-hydroxydopamine injection time. Muscle stiffness, apomorphine induced unilateral rotation, amount of brain’s protein oxidation and lipid peroxidation, ACE activity and histology of substantia nigra were assayed in all groups.

Peganum harmala improved Muscle stiffness and one-direction rotation behavior significantly. It also reduced brain’s lipid and protein oxidation levels in neurotoxin-injected rats significantly. In Peganum harmala group compared to control group, brain’s ACE activity was significantly inhibited. In histological study, Peganum harmala prevented degeneration of dopaminergic neurons, too.

In conclusion, aqueous extract of Peganum harmala could prevent symptoms and reduced oxidative stress markers in rats with Parkinson’s disease induced by 6-hydroxydopamine.

Keywords: Peganum harmala; Parkinson’s disease; 6-hydroxydopamine; angiotensin converting enzyme; rat.

Introduction

Parkinson’s disease (PD) is the most prevalent neurodegenerative disease after Alzheimer’s disease (1). In PD the basal ganglia cells and substantia nigra (SN) cells are destroyed and then the level of dopamine is decreased (2).

Increasing evidence showed the role of oxidative stress as a pathogenic factor in PD (3). Oxidative stress is the release of reactive oxygen species (ROS). Some of the most important causes of oxidative stress are aging, genetic factors, metals, changing in vital macromolecules, diet,
Aqueous extraction of Peganum harmala seeds

100 grams of dried plant’s seeds was poured into 1 liter boiling water in a beaker and kept in room temperature for 2 h. After that the solution was filtered and freeze-dried.

Parkinsonism induction

Each rat was anaesthetized by i.p injection of 100 mg/kg ketamin and 5 mg/kg xylazine and then his head was fixed on stereotaxic device (Stoelting, USA). Stereotaxic parameters for SN: AP: -4.8 mm to brigma, ML (left): 2 mm, DV: -8.3 mm from the surface of scalp by Watson & Paxinos atlas. 4μL of toxin (2mg/mL 6-OHDA with 0.1% vitamin C in normal saline) was injected by Hamilton syringe at a rate of 1 μl/min (16).

Rotation test

We tested rats’ unidirectional rotation test induced by apomorphine hydrochloride (2.5 mg/kg) in PD rats. Whole (right-sided minus left-sided) rotation in a cylinder box (33 cm diameter, 35 cm height) was measured in an isolated room in a 60 min. period.

Murprogo’s test

This is a method to measure muscle stiffness (17), by laying the animal on a flat surface the rat received a score of 0.5 if it did not move when touched. After that the right paw of the rat was laid on the edge of a box with 3 cm height. If the animal did not take its paw off after 10 sec, it received a score of 0.5. The same method was used for the left hand. In the next step, only the right paw of the rat was placed on the edge of a box with a height of 9 cm. If the rat did not take its paw off after 10 s, it received a score of 1. The last step was repeated for the left hand of the rats. The sum of the scores of movement on the floor and movement of hands while being hanged on the edge of boxes with 3 cm and 9 cm heights was 3.5.

ACE enzyme activity in serum blood and brain tissue homogenate

Brains were kept in -80°C freezers until analysis time. Brain tissue was homogenated and 10 μl of homogenate was incubated with
40 μl substrate (hippuryl histidyl leucine) in a thermo-mixer (eppendorf- MTP model) for 30 min. in 37°C and 300 rpm. After that, 150 μl phosphoric acid (5M) was added to each well to stop the reaction. 20 μl of the reactant mixture was injected into HPLC (Shimadzu, pump: LC-10ADVP, control system: SCL-10AVP, detector: SPD-10AV) and area under the curve of hippuric acid (enzyme product) was detected in 228 nm with 1 mL/min flow rate of mobile phase consisting of 1:1 methanol: KH₂PO₄ 0.1M, pH = 3. One unit of enzyme activity was defined as: 1 nmol of hippuric acid produced in one mg of brain tissue protein in one min in 37°C.

Lipid peroxidation
Lipid peroxidation was tested by complex formation between malondialdehyde and thiobarbitoric acid. Thiobarbitoric acid reactive substances (TBARS) were measured by spectrophotometer at 532 nm (5).

Protein concentration was measured by Bradford method with BSA (bovine serum albumin) as standard (18).

Protein oxidation
Protein oxidation was tested by measuring the concentration of carbonyl groups of proteins. Carbonyl group content of protein was assayed by spectrophotometer at 370 nm (19). Carbonyl group concentration was calculated based on e = 22 mM⁻¹cm⁻¹.

Histology examination
After decapitation, 5 to 8 cut of SN was processed for the number of dopaminergic neurons. We counted Nissl-stained dopaminergic neurons in the substantia nigra pars compacta and substantia nigra pars reticulate region in left and right hemispheres at 200x zoom.

Chemicals used in experiments
1, 1, 3, 3-Tetraethoxy propane, 2, 4-Dinitrophenyl Hydrazine, Apomorphine hydrochloride, Cresyle violet acetate, Guanidine hydrochloride, Hippuryl-His-Leu, Streptomycin sulfate, Tritonx-100, Desferrioxamine, and 6-hydroxydopamine were purchased from Sigma-Aldrich. Ketamine, Xylazine, Magnesium acetate tetrahydrate, Sucrose, Thiobarbituric acid, and Trichloro acetic acid were obtained from Merck.

Statistical analysis
Because of failure of normality distribution, we used non parametric Kruskal-Wallis test, and comparisons between 2 groups were made by Mann-Whitney U test. All analysis were done by IBM SPSS Statistics ver. 20.

Results

Number of unilateral rotation
Left handed rotation in 1 h period in toxin group was significantly higher than the control and two treatment groups (Figure 1).
Carbonyl group content was studied as a protein oxidation marker in the study groups. Kruskal-Wallis test showed a significant (p = 0.012) difference between study groups.

**p < 0.01 compared to toxin group tested by Mann-Whitney U test.
*p < 0.05 compared to toxin group tested by Mann-Whitney U test.

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Figure 3. Malondialdehyde concentration of brain in study groups. Kruskal-Wallis test showed a significant (p = 0.001) difference between study groups.

**p < 0.01 compared to toxin group tested by Mann-Whitney U test.
*p < 0.05 compared to toxin group tested by Mann-Whitney U test.

Murprogo’s test
Rigidity was significantly higher in toxin group compared to other groups (Figure 2).

Lipid peroxidation
Malondialdehyde production as a lipid peroxidation index was significantly higher in toxin group than other groups (Figure 3).

Protein oxidation
Protein oxidation in *Peganum harmala* group was significantly lower than toxin group (Figure 4).

Brain ACE activity
*Peganum harmala* significantly inhibited ACE activity in the brain compared to toxin group (Figure 5).

Histology examination
There were no significant differences in the number of dopaminergic neurons in left and right hemispheres in control group, but in toxin, captopril and PHS groups these differences were significant (Figure 6 and Table 1).

Discussion
We studied the protective effect of AEPHS on male rats with Parkinson’s disease induced by 6-OHDA. Data showed AEPHS (10 mg/kg) improved movement criteria in diseased rats by lowering rigidity and apomorphine induced rotation. Oxidative stress markers such as lipid peroxidation and protein oxidation in the brain of *Peganum* group were significantly lower than toxin group. Brain ACE activity in the *Peganum*
Peganum harmala in Parkinson’s disease

Peganum harmala L. is a full source of β carboline alkaloids. Some of its important alkaloids are harmine, harmaline, and harmalol (37). Harmaline inhibits ACE comparable to captopril (14). In a study of 135 herbal medicines for their ACE inhibitory effect, Peganum harmala showed a complete inhibition (15). β carboline alkaloids are benzodiazepine antagonists and inhibitors of amine oxidases, too (38).

We showed that Peganum harmala extract had antioxidant and ACE inhibitory effect. Peganum harmala decreased lipid peroxidation and protein oxidation in the brain of rats with 6-OHDA induced PD, and increased vital neurons in SN, which improved PD symptoms.

Conclusion

These findings demonstrate that peganum harmala L. has protective effect on 6-OHDA induced hemi-Parkinsonism rats, which might be through ACE inhibition.

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