Original Article

# Effect of Nelumbo nucifera rhizome extract in haloperidol induced Parkinson rat model

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## Abstract

Aim and Objective: In the present investigation, we tried to evaluate the anti-Parkinson activity of NN rhizome in haloperidol-induced Parkinson in mice.

Material and Method: Ethanolic extract was obtained from the rhizome of *Nelumbo nucifera* (NN). The solvent extractions were subjected to preliminary phytochemical screening. Neural degeneration was induced in a group (2, 3,4,5) by ip. Administration of Haloperidol at 1mg/kg in an animal. The standard drug L-Dopa 30 mg/kg ip. was ingested to group 3 animals before 30 of haloperidol challenge for 14 days. EENN at 250 and 500 mg/kg daily orally were given to G4, and G5 respectively before 30 min of haloperidol challenge for 14 days. A behavioral parameter was recorded on days 7 and 13 for bar test, days 8 and 14 for the rotarod test, and days 6 and 11 for locomotor activity. All statistical analyses were done with one-way and two-way ANOVA followed by Tukey's multiple comparison tests.

Result: EENN significantly ($p$<0.0001) improves the experimental rat's muscle coordination, locomotor, and catatonic behavior.
Conclusion: *Nelumbo nucifera* extract produces maximum (dose-dependent) significant action on Parkinson (dopaminergic neuron) in haloperidol-induced Parkinson in an animal.

**Keywords:** Actophotometer, Parkinson disease, Neurodegenerative, rota-rod, locomotor, muscle coordination.

## 1. INTRODUCTION:

Parkinson is the second most common idiopathic neurodegenerative disease after Alzheimer's disease worldwide, characterized by the progressive loss of dopamine level resulting from progressive degradation of the dopaminergic neuron in substantia nigra. The clinical symptom is manifested by motor balance dysfunctioning like rigidity, tremor, unstable posture, postural limbic balance [1,3]. Turn-up of PD in people is generally termed as catalepsy. Pathogenesis of PD involves genetic and environmental factors that progress to mitochondrial dysfunction, protein aggregation, apoptosis, inflammation, and excitotoxicity [4].

There are various neuroprotective drugs available, such as levodopa, selegiline, amantadine, carbidopa, and orphenadrine, which overcome the neural degradation process in the substantial nigar region [5]. Drug levodopa is then most effective for all concerned, but long-term ingestion proceeds motor complications like motor fluctuations and dyskinesia. Because of the side effects of the drug, the urge to search or screen natural resources has increased. These natural resources can potentially overcome the disease condition with an advantage on no or lesser side effects [6,7].

Nelumbo nucifera is an aquatic perennial monocotyledonous herb with a common name like Lotus (India), water lily (china), belongs to the Nelumbonaceae family. *N. nucifera* has wide geographical distribution. Scientific investigation confirms various pharmacological activities like anti-ischemic, astringent, anticancer, antioxidant, antipyretics, antiviral, immunomodulatory, anti-inflammatory, diuretic, hepatoprotective, antifungal, as well as gastroprotective activity [8,10].

Due to lack of investigation regarding the concerned disease, we tried to explore the antiparkinson activity evaluation of *N. nucifera* in experimental rats [9,20].

## 2. MATERIAL AND METHOD:

### 2.1 Collection and identification of the plant:

*N. nucifera* (Rhizome) was collected from the local region Prayagraj U.P. The sample specimen with reference No-BSI/CRC/SU/2020-21/236 was submitted to the botanical survey of India (BSI, department of Botany) Prayagraj, authenticated by Dr. Arati Garg, Scientist E, and head office, a regional botanical survey of India (BSI)[12,18,19,37].

### 2.2 Plant processing and extraction method:
The rhizome was shade dried further pulverized to coarse particles, and the resulting powder proceeds to the maceration process using solvent (methanol and water under ratio 1:1). The resulting was evaporated, concentrated & stored at cool temperature till further use [3,13,17,34].

### 2.3 Phytochemical evaluation:
Preliminary phytochemical screening tests were employed to confirm various chemical constituents like phenolic compounds, flavonoids, saponin, alkaloid, steroid, glycoside, etc [12,15,16,26,27,28].

### 2.4 Animal:
Wistar rats (180-200 gm.) of either sex was housed in a maintained ventilated propylene cage with optimum temp. 220c ± 20c with 12hr. Light/dark cycle, fedded with standard food pallet diet. All animals were acclimatized for one week before experimentation. All the experimental protocols were approved by IAEC under protocol No- IAEC (SIP-IAEC-01-1504) [5,6,8,15,29,30]

### 2.5 Acute toxicity study:
All animals exposed to this step fasted overnight. The *N. nucifera* extract was tested for acute toxicity, followed by OECD 423 (acute toxic class method). The dose was initiated from 50 mg/kg and proceeded further 300, 500, 750, and 2000mg/kg till suitable result according to protocol [8,9,14,31,32,33].

### 2.6 Experimental Design:
All animal was in 5 groups (n=6). **Group-I** (Normal Control)- was administered CMC (0.5%) orally/ once a day for two weeks. **Group-II** (Normal Control)- ip. administered haloperidol (1mg/kg, once a day for two weak. **Group-III** (standard Control)- ip. administered with L-Dopa (30 mg/kg once a day for two weak 30 min before haloperidol). **Group-IV, V** (Test Control 1, Test control 2) - oral administration of N. nucifera ( 250 mg/kg, 500 mg/kg once a day for two weak 30 min before haloperidol) in a respective animal group. After cataleptic induction, Behavioural parameter was recorded on day 7 and 13- for bar test, day 8 and 14- for a rota-rod test, day 8 and 11- for Locomotor activity [11,12,13,34,35,36].

### 2.7 Rota Rod test:
It is the most widespread method to access minimal neurological difficulties. Before the treatment challenge, all animals were prior trained for locomotor activity, performed by placing animals on the rota-rod (7 cm Diameter and speed up to 15rpm). Latency time to fall from the rod was documented for each rat. The cut-off duration was 180sec [7,8,21,22].

### 2.8 Catalepsy bar test:
Before 30 min. of Haloperidol challenge, all experimental animals received their respective dose of Vehicle, Standard Drug (L-dopa), or testing drug at definite dose and route. Cataleptic behavior was recorded at various intervals (0, 30, 60, 90, 120, and 180) in min. as average latency time. An animal's paws (for Paw) were placed on an elevated wooden bar (9cm height, 1cm diameter). The cataleptic end was considered when the movement of the animal head was in an exploratory manner. Cut-off time 180 min. was adopted [9,11,23,24].

### 2.9 Locomotor Activity (Actophotometer):
Actophotometer measures hypokinesia or akinesia. The apparatus consists of a cage with six light beam transmitters, and the receiver is placed as such in a way at a time only on photo beam was interrupted when animal cross the one photo beam [4]. One cell is completed when transmitted photo beam to acceptor photocell. The alteration was recorded when the animal interrupted the beam light. The activity counted as total photo beam interruption in 10 min per animal [4,6,25].

### 2.10Statistical analysis:
All analytical measures like cataleptic behavior (using elevated rod), muscle coordination behavior (using rotarod), locomotor activity (using actophotometer) were represented in the table, and the graph was denoted as mean ±S.E.M. (n=6). One and two-way ANOVA statistical method followed by Tukey's multiple comparisons was adopted. A significant difference was considered b/w group when $p<0.05$. All analyses were done with GraphPad Prism 8.0.2 software [9,10,11,24].

## 3. RESULT AND DISCUSSION:
### 3.1 RESULT:
### 3.1.1 Phytochemical analysis:
Preliminary phytochemical screening tests confirm various chemical constituents like phenolic compounds, flavonoids, saponin, alkaloid, steroid, glycoside, etc in *N. nucifera*.

### 3.1.2 Acute toxicity study:
All animals found to safe at 2000 mg/kg within experimentation (72 hr.). All behavioral and physical condition was at normal state (from 72hr to one week).

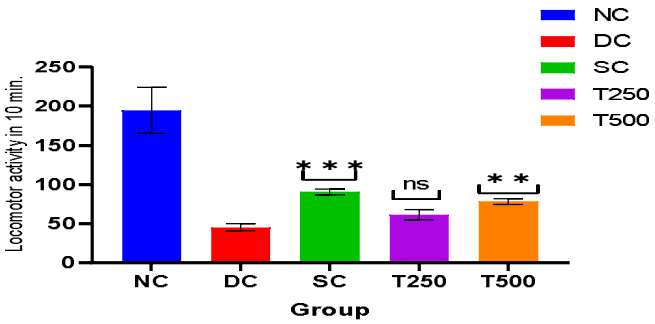
### 3.1.3 Locomotor activity (Actophotometer):

All the statistical data of locomotor activity are represented in table 3.1. DC (G2) showed a significant (P<0.0001) decrease in motor activity in comparison to normal control mice (G1). EENN 500 mg/kg show significant elevation (P<0.01) in locomotion function in contrast to G2 (DC); also, EENN 500 mg/kg (G3) showed a non-significant difference when compared to SC (G3).

**Table: 3.1** Locomotor activity (Actophotometer) in 10 min. (MEAN±SEM)

| Groups | Locomotor activity (Actophotometer) in 10 min. in MEAN±SEM |
|---|---|
| NC | 194.66±29.35 |
| DC (Haloperidol 1 mg/kg) | 45.33±4.48 |
| SC (L-Dopa 30 mg/kg) | 90.66±3.52*** |
| EENN 250 | 61.33±6.43## |
| EENN 500 | 78.33±3.48** |

Data are expressed as Mean ± SEM (n=6). DC=Disease Control, SC=Standard Control, EENN=ethanolic extract of *N. nucifera.*
***p<. 0.001, **p<. 0.01 compared with Disease control at same time
##p<. 0.01, and **ns** compared with Standard control at same time
All Statistical analysis was done with one -way ANOVA Followed by Tukey's multiple comparison test).




Data are expressed as Mean ± SEM (n=6).

***p<. 0.001 compared with Disease control at same time (one -way ANOVA Followed by Tukey's multiple comparison test).

**p<. 0.01 compared with Diabetic control at same time (one -way ANOVA Followed by Tukey's multiple comparison test).

**Fig: 3.1** Locomotor activity (Actophotometer) in 10 min. (MEAN±SEM)

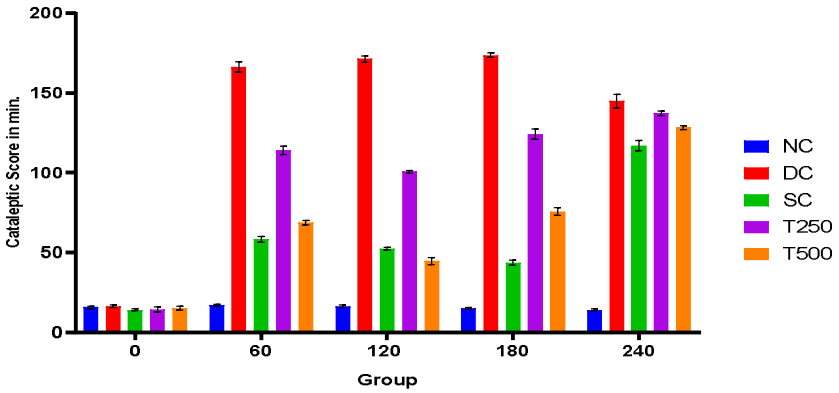
### 3.1.4 Cataleptic activity:

All the statistical data of cataleptic activity in different group are expressed in table 3.2 DC (G2) showed significant elevation (P<0.0001) in the cataleptic period at 60, 120, and 180 min. as compared to G1 (NC) animal. EENN AT 250 and 500 mg/kg G3 and G4, respectively, showed a significant reduction (P<0.0001) in cataleptic time compared to G2 (DC) at 60, 120, and 180 min. EENN 500 mg/kg showed a non-significant difference at 120 min. Compared to G3 (SC).

**Table 3.2** Effect of *N. nucifera* on cataleptic duration in minute in different group of animal

| Time Interval ( in min.) | MEAN±SEM (CATALEPTIC SCORE IN MIN.) | | | | |
|---|---|---|---|---|---|
| | NC | DC (Haloperidol 1 mg/kg) | SC (L-Dopa 30 mg/kg) | EENN 250 mg/kg | EENN 500 mg/kg |
| 0 | 15.66±0.88 | 16.33±0.66 | 14.00±0.57 | 14.33±0.57 | 15.00±1.15 |
| 60 | 17.00±0.00 | 166.33±3.28 | 58.33±1.76 **** | 114.00±2.51 ****#### | 68.66±1.45 ****## |
| 120 | 16.33±0.66 | 171.33±2.02 | 52.33±0.82 **** | 100.66±0.88 **** #### | 44.66±2.02 **** ns |
| 180 | 1500±0.57 | 173.66±1.45 | 43.66±1.45 ********## | 124.33±3.18 ****#### | 75.66±2.33 ********## |
| 240 | 1400±0.57 | 145.00±4.16 | 117.00±3.05 **** | 137.33±1.43 ns#### | 128.33±1.20 ****## |

All value in Mean ± SEM of six rat (n=6). EENN= Ethanolic Extract of *Nelumbo nucifera* rhizome. DC=Disease Control, SC=standard Control
****$p$<0.0001 compared with disease control.
#### p<0.0001, ## p<0.0001, and ns compared with standard control.
All Statistical analysis was done with one -way ANOVA Followed by Tukey's multiple comparison test).




**All value in Mean ± SEM of six rat (n=6). EENN= Ethanolic Extract of *Nelumbo nucifera* rhizome., DC=Disease Control, SC=Standard Control, T250= *Nelumbo nucifera* 250 mg/kg., T500= *Nelumbo nucifera* 250 mg/kg.,**

**Fig 3.2** Effect of *N. nucifera* on cataleptic duration in minute in different group of animal

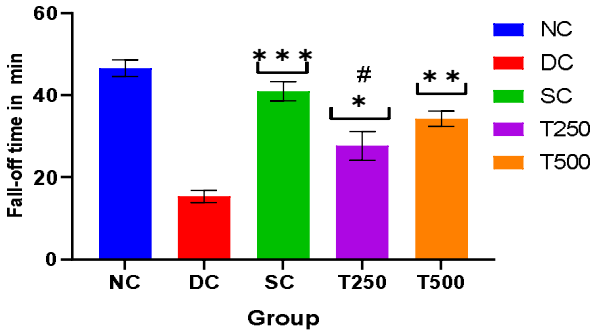
### 3.1.5 Rota-rod test:

The statistical result of muscle coordination behavior test (using rota-rod) are denote in table 3.3. G2 with haloperidol administration shows a significant (P<0.0001) decrease in fall-off time in contrast to G1. G3 (SC) shows significant (P<0.0001) motor coordination activity (increased fall-off time) when compared to G2. EENN 250 mg/kg (G4) show slightly higher muscle coordination activity, significantly (P<0.05) when compared G2. EENN 500 mg/kg (G5) significant increase in fall time in contrast to G2. EENN 500 mg/kg (G5) showed a nonsignificant difference in contrast to G2. EENN 250 mg/kg (G4) show a significant decrease (0.05) fall-of-time as compared to G3 (SC).

**Table 3.3** Effect of *N. nucifera* on muscle coordination behavior in various groups.

| Groups | Motor Co-ordination (rota rod) Fall-off time in min., in MEAN±SEM |
|---|---|
| NC | 47.66±2.02 |
| DC (Haloperidol 1 mg/kg) | 15.33±1.45 |
| SC (L-Dopa 30 mg/kg) | 41.00±2.30 *** |
| EENN 250 | 27.66±3.52 *# |
| EENN 500 | 34.33±1.85**ns |

Data are expressed as Mean ± SEM (n=6). DC=Disease Control, SC=standard Control ***p<. 0.001, **p<. 0.01, ***p<. 0.05 compared with Disease control at same time ##p<. 0.01, #p<. 0.05, and compared with Standard control at same time
All Statistical analysis was done with one -way ANOVA Followed by Tukey's multiple comparison test).




**Data are expressed as Mean ± SEM (n=6).**

*****p<. 0.001, **p<. 0.01, ***p<. 0.05 compared with Disease control at same time (one -way ANOVA Followed by Tukey's multiple comparison test).**

**##p<. 0.01, #p<. 0.05, and compared with Standard control at same time (one -way ANOVA Followed by Tukey's multiple comparison test).**

**Fig. 3.3** Effect of *N. nucifera* on muscle coordination behavior in various groups.

### 3.2 DISCUSSION:

PD is a neurodegenerative disease that shows duplication of dopaminergic neurons in the substantia nigra region of the brain, with clinical characteristics such as jaw, head tremor, rigidity of limbs resulting in impaired motor balance [3,4,5,6,8].
In the present evaluation, we tried to evaluate the neuroprotective property of EENN in Haloperidol-induced locomotor dysfunctioning. The haloperidol-induced locomotor abnormality model is generally used for the animal model to evaluate the antiparkinson activity. Haloperidol produces locomotor dysfunction by blocking nonselective D2 (Dopamine) receptors resulting in induction of Parkinson in experimental animals [13].
EENN contains verities of chemical compounds which cross the BBB to reach the resulting receptor and show the required response. Phytochemical screening shows the presence of flavanoid, carbohydrate, alkaloid, protein, and tannin. EENN may have a various significant properties like antioxidant, neuroprotective, anti-inflammatory properties, which alter the degeneration process of the dopaminergic neuron arising due to haloperidol [3,7,9,12].
In the diseased group (G2), there is significantly elevated catalepsy with sign increase in paw retention time on bar compared to other groups. EENN 500 mg/kg showed a significant reduction in retention time of paw on rod showed improved motor activity. Standard drug show maximum restoration near-normal motor activity compared to other groups. D2 (Haloperidol) Showed a decrease in retention time on the rota-rod apparatus, which clearly shows the muscle coordination property dysfunction due to dopaminergic depletion. Administration of EENN at 250 and 500 mg/kg showed a bar retention time of the rota-rod, confirming the improved muscle coordination behavior of experimental rats compared to diseased rats. Standard drug (L-dopa In G3) showed high retention time [11,13,15].
Administration of EENN at 250, 500 mg/kg showed improved locomotor activity compared to G2. G2 showed a significantly reduced locomotor activity than G1 (normal control).

## 4. CONCLUSION:

PD is a progressive dopaminergic degenerative disorder in the substantia nigra. Haloperidol is a more common way to induce a neural degradation model in experimental animals.
EENN at 100 and 200 mg/kg showed significant dose-dependent improvement in rota-rod actophotometer performance and minimized the catatonic response on elevated bar test. Hence the above result of EENN may be due to the neuroprotective, anti-inflammatory, and antioxidant properties of *N. nucifera*. Further investigation is required to establish and identify the single chemical compound that shows the neuroprotective response in the experimental animal. *N. nucifera* may be used as an aid to reduce the neural degeneration process [4,12].

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