www.ThePharmaJournal.com

The Pharma Innovation

**ISSN: 2277- 7695**
TPI 2014; 3(7): 101-108

www.thepharmajournal.com
Received: 30-07-2014
Accepted: 15-08-2014

**Vivek Kahale**
*Department of Pharmacology, R.T.M. Nagpur University, ACP Wardha, Maharashtra, India.*

**Amrapali Mhaiskar**
*Department of Pharmacology, R.T.M. Nagpur University, ACP Wardha, Maharashtra, India.*

**Priyanka Shelat**
*Department of Pharmacology, R.T.M. Nagpur University, ACP Wardha, Maharashtra, India.*

**Pooja RU**
*Agnihotri College of Pharmacy, Bapuji wadi, ramnagar, Sindhi meghe, Wardha, MS India*

**Gaikwad NJ**
*University Department of Pharmaceutical Sciences, R.T.M Nagpur University, Nagpur, India*

**Mundhada DR.**
*Agnihotri College of Pharmacy, Bapuji wadi, ramnagar, Sindhi meghe, Wardha, MS India*

**Correspondence:**
**Vivek Kahale**
Department of Pharmacology, R.T.M. Nagpur University, ACP Wardha, Maharashtra, India.

# To determine the Effect of Berberine on 6-OHDA induced memory impairment in Parkinson's disease in rodents

**Vivek Kahale, Amrapali Mhaiskar, Priyanka Shelat, Pooja RU, Gaikwad NJ, Mundhada DR.**

**Abstract**
The relevant doses of Berberine administration in present preclinical study states that neurotoxicity induced by 6-Hydroxydopamine is remarkably neuroprotective in male wistar rats. 6-Hydroxydopamine induce Parkinson's disease (PD) which is a chronic and progressive neurodegenerative disease with loss of brown pigment neuromelanin with preferential loss of dopaminergic neurons of the substantia nigra pars compacta results in oxidative stress and mitochondrial disfunction. Berberine (BER) (2, 3-methylenedioxy-9, 10-dimethoxy protoberberine chloride) is an isoquinoline alkaloid from dried herb acts as a antioxidant and prevent reactive oxygen species (ROS) injury in various ways. Berberine treatments protects behavioral changes, significantly reduce oxidative damage & improved mitochondrial complex enzymes activities in different regions (striatum, cortex and hippocampus) of rat brain due to neurotoxicity. Administration of 6-Hydroxydopamine through I.C.V. produces hypo activity that resembles juvenile onset and advanced Parkinson's disease in rats. Berberine activity investigates the neuroprotective effect in animal model of Parkinson's disease.

**Keywords:** Parkinson Disease, 6-OHDA, I.C.V., Berberine, Memory enhancer.

## 1. Introduction

### 1.1 Parkinson Disease

Parkinson's disease was first described by James Parkinson in 1817 which is a chronic and progressive neurodegenerative disease with multiple motor and non-motor features that contribute to the impairment of health-related quality of life (QOL). [1] It is characterized by a preferential loss of the dopaminergic neurons of the substantia nigra pars compacta. It is also characterized by reduced movement, rigidity, and tremor. [2] Parkinson's disease (PD) was first associated with the loss of the brown pigment neuromelanin from the substantia nigra. Later, it was postulated that the progressive loss of dopamine-producing cells in the substantia nigra pars compacta of the ventral midbrain caused PD symptomatology. In addition, PD is also associated with the presence of intracytoplasmic inclusions known as Lewy Bodies (LBs), which are composed largely of alpha-synuclein (alpha-syn). [3] The concept of oxidative stress & antioxidant may be directly & indirectly involved in pathogenesis of Parkinson disease. There was a tight correlation between cognitive impairment in PD and cholinergic deficit. [5] The pathologic hallmark of Parkinson's disease is presence of lewy bodies, degeneration of brain stem nuclei & loss of dopaminergic neurons. In pathogenesis of Parkinson's Disease includes abnormalities in cellular protein transport, interaction between proteins & protein aggregation. The neurochemistry have shown involvement of excitotoxicity & oxidative stress in cell death. Parkinson's Disease is pathologically characterized by loss of catecholaminergic neurons in brain stem. The number of biochemical processes are involved in pathogenesis & progression of neurological disorder [4].
Parkinson's disease is also known as ―PARKINSONISM.

### A) Genetics

In Parkinson disease approximately 15 percent of people have a family history of this disorder. Familial cases of Parkinson disease can be caused by mutations in the LRRK2, PARK2, PARK7, PINK1, or SNCA gene, or by alterations in genes that have not been identified.

**B) Inheritance**

Among familial cases of Parkinson disease, the inheritance pattern differs depending on the gene that is altered. If the LRRK2 or SNCA gene is involved, the disorder is inherited in an autosomal dominant pattern, which means one copy of an altered gene in each cell is sufficient to cause the disorder. If the PARK2, PARK7, or PINK1 gene is involved, Parkinson disease is inherited in an autosomal recessive pattern. In general, the inheritance patterns of human disorders are identified by examining the way the disorders are transmitted in the family of the index patient.

## 2. Drug Profile

### 2.1 Inducer Drug

**A) 6-Hydroxydopamine**

6-Hydroxydopamine is a neurotoxin used to selectively kill dopaminergic and noradrenergic neurons to lesion dopaminergic pathways. 6-hydroxydopamine produce neurotoxicity is initiated via extracellular auto-oxidation and the induction of oxidative stress from the oxidative products generated. 6- OHDA shares some structural similarities with dopamine and norepinephrine, exhibiting a high affinity for several catecholamininergic plasma membrane transporter such as the dopamine. [6]

**B) Stereotaxic Surgery**

Each animal was mouted on stereotaxic apparatus. [5] Anesthetised the rat with 10 mg/kg ketamine + 3 mg/kg zylaxine (i.p.) with the nose oriented 11 ºC below the horizontal plane. [7] A 2 cm midsaggital skin incision is made on scalp & skin overlying the skull was cut to expose the skull & coordinates for substantia nigra (SNPC) was measured accurately (Anteroposterior-0.5 mm from bregma, mediolateral-2.1 mm from midline, and dorsoventral-7.7 mm from the skull). An infusion cannula consulting of a sterilized length of 30 gauge stainless steel tubing is stereotaxically placed via a hole in skull & the internalised tip is located within the nigrostrital pathway. A Right-unilateral lesion was made & the solution of 6-µg of 6-OHDA in 2 µL 0.2% ascorbic acid saline were infused into SNPC through 30 gauge stainless needle [5] at a rate of 1µL/min for 4.50 min., the syringe was left in place for 5 min. then slowly withdrawn and skin incision closed with stainless steel wound clips. [7]

### 2.2 Treatment Drug

**A) Berberine**

Berberine (BER) (benzyltetrahydroquinoline) C20H18NO4+, is a quaternary ammonium salt from the group of isoquinoline alkaloids(2,3-methylenedioxy-9,10-dimethoxy protoberberine chloride). The berberine alkaloid can be found in the roots, rhizomes, and stem bark of the plants. anxiolytic effect of berberine might be related to an increase in turnover rates of monoamines in the brain stem and decreased serotonergic system activity. Berberine exerts an inhibitory effect on catecholamine biosynthesis, e.g. on DA synthesis in neuronal cells. Destruction of Dopaminergic Neurons in the Midbrain by 6-Hydroxydopamine Decreases Hippocampal Cell Proliferation in Rats.

## 3. Materials and Methods

### 3.1 Animals

All the Male Wistar rats weighing between 250–300 g were used in this study (six per cage).They were acclimatized to condition in the animal housing unit at 23±2 ºC under 12:12 hrs light/dark cycle. Prior to the experiments the approval of the Institutional Animal Ethics Committee, was taken. (Proposal no.03 at dated 27/12/2012).Constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

### 3.2 Drugs and Chemicals

Berberine (25, 50, 100 mg/kg, oral) (Sigma-Aldrich Labs, Bangalore, India) & 6-OHDA (6-µg of 6-OHDA in 2 µL 0.2% ascorbic acid saline) (Sigma-Aldrich Labs, Bangalore, India) were used of analytical grade Chemicals & reagents. Other chemicals, reagents & dietary supplements were used in the present investigation were of analytical grade & provided by college/university.

### 3.3 Treatment Schedule

Animals were randomly divided into six groups of 6 animals in each case.

- 1 Group— treated with vehicle, received normal saline (i. p.);
- 2 Group— received 6-OHDA (6-µg in 2 µL 0.2% ascorbic acid saline i.c.v.) for14days;
- 3 Group— received Berberine (100mg/kg, oral) per se;
- 4, 5&6 Group— received Berberine (25, 50, 100 mg/kg, oral) + 6-OHDA (6-µg of in 2 µL 0.2% ascorbic acid saline) for 14 days.
- Berberine was administered 1 hr prior to 6-OHDA administration.

### 3.4 Behavioral tests

#### 3.4.1 Morris water maze test

We used a Morris water maze (MWM) task for animals in the differentiation paradigm. Berberine treatment affects hippocampus dependent memory function in animals given by 6-OHDA, The animals were given a task for 3 days. Each session consisted of six trials lasting 60 s each, separated by a 60 s inter-trial interval. At the start of each trial, rats were placed at one of four start locations at the limb of a circular pool (150 cm in diameter, water temperature at 26 °C) with their face toward the wall. Animals were required to escape to an invisible platform (10 cm in diameter, 1 cm below the water surface) fixed at a predetermined location. If animals could not reach the platform within 60 s, an experimenter gently led them onto the platform. Once animals got upon the platform, they were left on it for 15 s and then returned to a waiting cage.

#### 3.4.2 Novel object recognition

This test is based on the natural propensity of animals to spend more time exploring a new rather than a formerly encountered object. Memory was evaluated at two retention intervals (30 min and 24 h). Rats were transported from the animal vivarium to the testing laboratory and allowed to acclimatize to testing environment for at least 30 min before behavioral testing began. Testing was monitored by an overhead camera. The test was performed in the open field arena (60cm×40cm×28cm) as previously described. Each rat was exposed to three experimental conditions in the open field. In the initial trial (T1), one object-stimulus (O1) was placed in one corner of the open field and the rat positioned in the opposite corner of the arena, and time spent exploring the object (touching the object with paws or exploring it by olfaction with direct contact of the snout) was measured.

### 3.4.3 Passive Avoidance Test

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 cm X 27 cm X 27 cm) having three walls of wood and one wall of plexiglass, featuring a grid floor (made up of 3 mm stainless steel rods set 8-mm apart), with a wooden platform (10 cm X 7 cm X 1.7 cm) in the center of the grid floor. The box was illuminated with a 15-W bulb during the experimental period. Electric shock (20 V, AC) was delivered to the grid floor. Training (i.e. eighth day of drug treatment) was carried out in two similar sessions. Each rat was gently placed on the wooden platform set in the center of the grid floor. When the rat stepped-down placing all its paws on the grid floor, shocks were delivered for 15 seconds and the step-down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to the grid floor with all its paws on the grid floor. Animals showing SDL in the range of 2-15 seconds during the first test were used for the second session and the retention test. The second session was carried out 90 minutes after the first test. During the second session, if the animals stepped down before 60 seconds, electric shocks were delivered once again for 15 seconds. During the second test, animals were removed from the shock-free zone, if they did not step down for a period of 60 seconds and were subjected to the retention test. Retention (memory) was tested after 24 hours (i.e. ninth day, 24 hours after last dose) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut-off time of 300 seconds. Significant increase in SDL value indicated improvement in memory.

## 4. Results

### 4.1 Effect of daily treatment of berberine on 6-Hydroxydopamine induced alterations in various behavioral parameters

#### 4.1.1: Effect of berberine on spatial navigation task in 6-OHDA treated rats

**Table 1.**

| Control | 6-OHDA | Berberine | 6-OHDA+ Berberine(25mg/kg) | 6-OHDA+ Berberine(50mg/kg) | 6-OHDA+ Berberine(100mg/kg) |
|---|---|---|---|---|---|
| 20.00 | 79.00 | 23.00 | 53.00 | 32.00 | 34.00 |
| 15.00 | 66.00 | 30.00 | 33.00 | 52.00 | 19.00 |
| 30.00 | 70.00 | 15.00 | 46.00 | 39.00 | 24.00 |
| 22.00 | 58.00 | 28.00 | 37.00 | 28.00 | 21.00 |
| 13.00 | 60.00 | 9.00 | 28.00 | 34.00 | 16.00 |
| 10.00 | 50.00 | 13.00 | 44.00 | 36.00 | 10.00 |

**Fig 1 & 2** Represents the mean escape latency was decreased in 6-OHDA treated animals. However, Berberine (50 and 100 mg/kg, oral) treatment showed a significant improvement in memory performance.
Each value represents mean ± S.E.M. of 6 observations. (One-way ANOVA followed by Tukey's post hoc test).

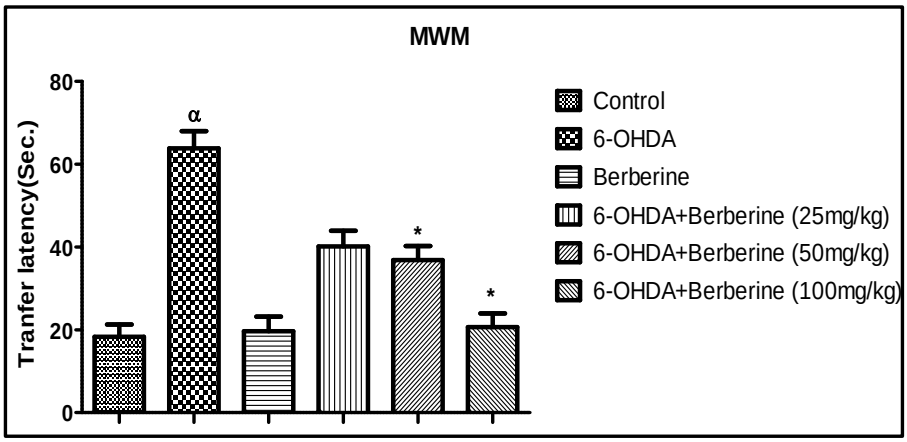


**Fig 1:** Influence of berberine treatment on transfer latency in Morris water maze.

**Table 2.**

| Control | 6-OHDA | Berberine | 6-OHDA+ Berberine(25mg/kg) | 6-OHDA+ Berberine(50mg/kg) | 6-OHDA+ Berberine(100mg/kg) |
|---|---|---|---|---|---|
| 40.00 | 40.00 | 69.00 | 48.00 | 43.00 | 42.00 |
| 58.00 | 50.00 | 64.00 | 34.00 | 40.00 | 55.00 |
| 51.00 | 45.00 | 66.00 | 52.00 | 52.00 | 67.00 |
| 52.00 | 29.00 | 63.00 | 30.00 | 35.00 | 58.00 |
| 59.00 | 18.00 | 62.00 | 37.00 | 43.00 | 60.00 |
| 69.00 | 33.00 | 45.00 | 59.00 | 28.00 | 48.00 |

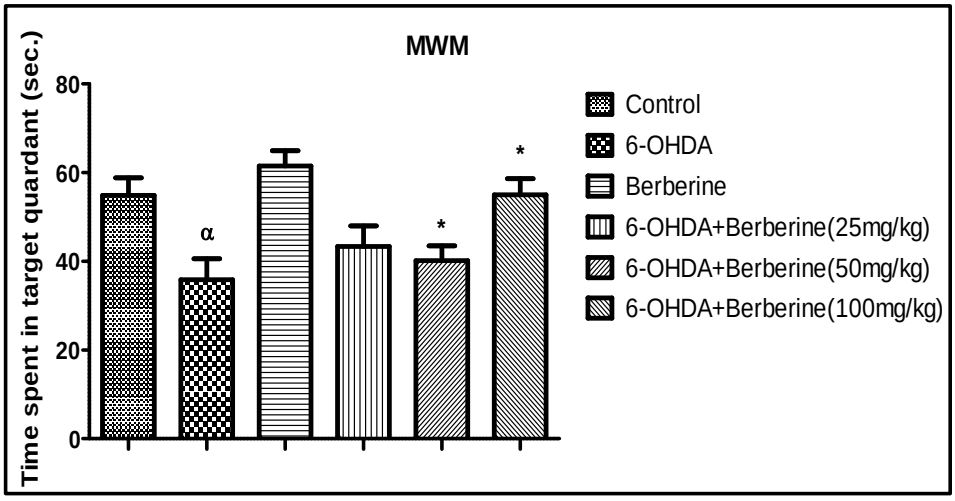


**Fig 2:** Influence of berberine treatment on time spent in target quadrant in Morris water maze.

### 4.1.2 Effect of berberine on Novel object recognition memory

**Table 3.**

| Control | 6-OHDA | Berberine | 6-OHDA+ Berberine(25mg/kg) | 6-OHDA+ Berberine(50mg/kg) | 6-OHDA+ Berberine(100mg/kg) |
|---|---|---|---|---|---|
| 6.00 | 11.0 | 10.00 | 4.00 | 10.00 | 15.00 |
| 3.00 | 6.00 | 15.00 | 9.00 | 12.00 | 18.00 |
| 8.00 | 5.00 | 9.00 | 5.00 | 14.00 | 13.00 |
| 14.00 | 9.00 | 4.00 | 7.00 | 16.00 | 19.00 |
| 9.00 | 4.00 | 8.00 | 10.00 | 18.00 | 17.00 |
| 8.00 | 2.00 | 13.00 | 13.00 | 11.00 | 16.00 |

**Figure 3 & 4:** Represents the 6-OHDA-induced Parkinson's disease rats explored less to the novel object compared to non Parkinson's rats. However, berberine (50 and 100 mg/kg, oral) treated Parkinson's disease rats explored more to novel objects as compared to vehicle treated group at both retention trials (30 min & 24 hrs).
Each value represents mean ± S.E.M. of 6 observations. (One-way ANOVA followed by Tukey's post hoc test).

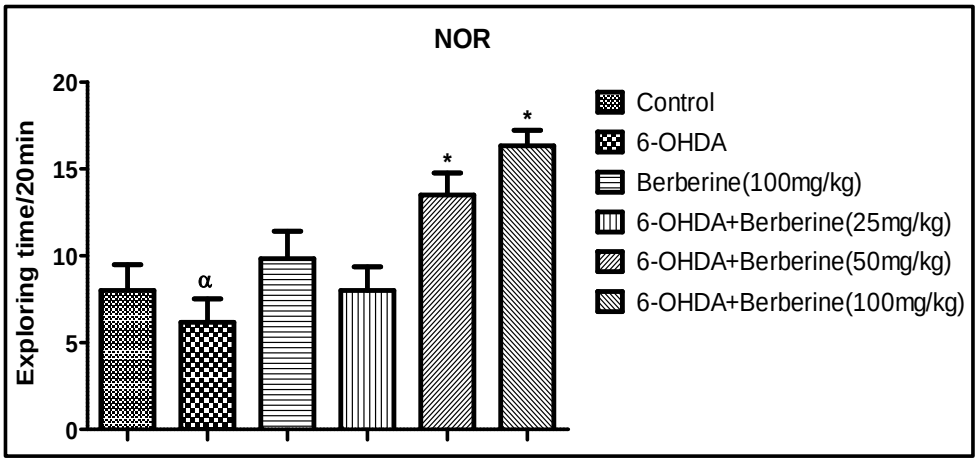


**Fig 3:** Influence of treatment of berberine on the Exploring time for the retention intervals (30 min), for the object recognition test.

**Table 4.**

| Control | 6-OHDA | Berberine | 6-OHDA+ Berberine(25mg/kg) | 6-OHDA+ Berberine(50mg/kg) | 6-OHDA+ Berberine(100mg/kg) |
|---|---|---|---|---|---|
| 5.00 | 3.00 | 9.00 | 11.00 | 11.00 | 14.00 |
| 4.00 | 5.00 | 15.00 | 9.00 | 10.00 | 15.00 |
| 8.00 | 11.00 | 8.00 | 7.00 | 13.00 | 22.00 |
| 6.00 | 4.00 | 6.00 | 4.00 | 18.00 | 18.00 |
| 11.00 | 7.00 | 7.00 | 14.00 | 14.00 | 17.00 |
| 3.00 | 2.00 | 4.00 | 6.00 | 12.00 | 16.00 |

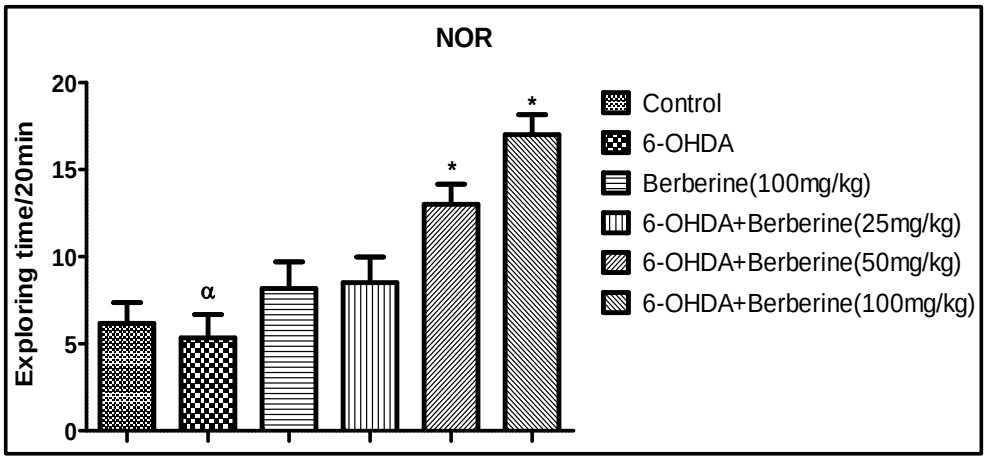


**Fig 4:** Influence of treatment of berberine on the Exploring time for the two retention intervals (24 h), for the object recognition test.

### 4.1.3: Effect of berberine on passive avoidance test

**Table 5.**

| Control | 6-OHDA | Berberine | 6-OHDA+ Berberine(25mg/kg) | 6-OHDA+ Berberine(50mg/kg) | 6-OHDA+ Berberine(100mg/kg) |
|---|---|---|---|---|---|
| 5.00 | 3.00 | 9.00 | 10.00 | 11.00 | 10.00 |
| 4.00 | 5.00 | 10.00 | 9.00 | 9.00 | 14.00 |
| 7.00 | 6.00 | 8.00 | 7.00 | 13.00 | 21.00 |
| 10.00 | 4.00 | 6.00 | 8.00 | 18.00 | 18.00 |
| 8.00 | 9.00 | 7.00 | 12.00 | 14.00 | 9.00 |
| 14.00 | 2.00 | 15.00 | 4.00 | 12.00 | 16.00 |

**Figure 5 & 6** Represents the 6-OHDA-induced Parkinson's disease rats explored less to the passive avoidance compared to non Parkinson's rats. However, berberine (50 and 100 mg/kg, oral) treated Parkinson's disease rats explored more to passive avoidance as compared to vehicle treated group at both retention trials (30min & 24 hrs).
Each value represents mean ± S.E.M. of 6 observations. (One-way ANOVA followed by Tukey's post hoc test).

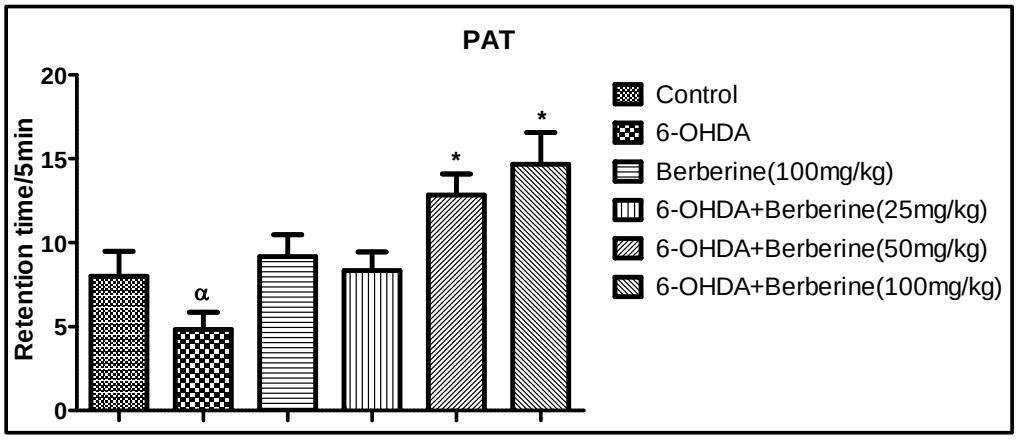


**Fig 5:** Influence of treatment of berberine on the Retention time for the two retention intervals (30 min), for the passive avoidance test.

**Table 6.**

| Control | 6-OHDA | Berberine | 6-OHDA+ Berberine(25mg/kg) | 6-OHDA+ Berberine(50mg/kg) | 6-OHDA+ Berberine(100mg/kg) |
|---|---|---|---|---|---|
| 5.00 | 3.00 | 9.00 | 10.00 | 11.00 | 14.00 |
| 4.00 | 5.00 | 15.00 | 9.00 | 10.00 | 15.00 |
| 7.00 | 6.00 | 8.00 | 7.00 | 13.00 | 19.00 |
| 6.00 | 11.00 | 6.00 | 8.00 | 20.00 | 23.00 |
| 12.00 | 9.00 | 7.00 | 13.00 | 14.00 | 17.00 |
| 9.00 | 2.00 | 4.00 | 5.00 | 12.00 | 16.00 |

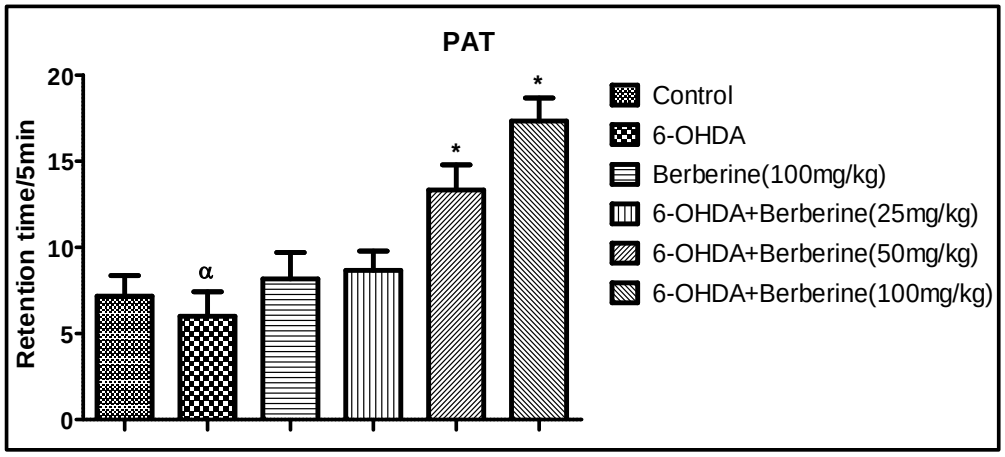


**Fig 6:** Influence of treatment of berberine on the Retention time for the two retention intervals (24 h), for the passive avoidance test.

**4.2 Effect of daily treatment of Berberine on 6-Hydroxydopamine induced alterations in various biochemical parameters**

**4.2.1 Effect of Berberine on nitrite concentration**

I.C.V. administration of 6-OHDA significantly increased nitrite concentration in striatum as compared to vehicle treated group. However, Berberine (50 and 100 mg/kg) treatment significantly attenuated nitrite concentration as compared to 6-OHDA treated group ($P < 0.05$). However lower dose of Berberine (25 mg/kg) did not produce any significant effect on these oxidative stress parameters in 6-OHDA treated group.

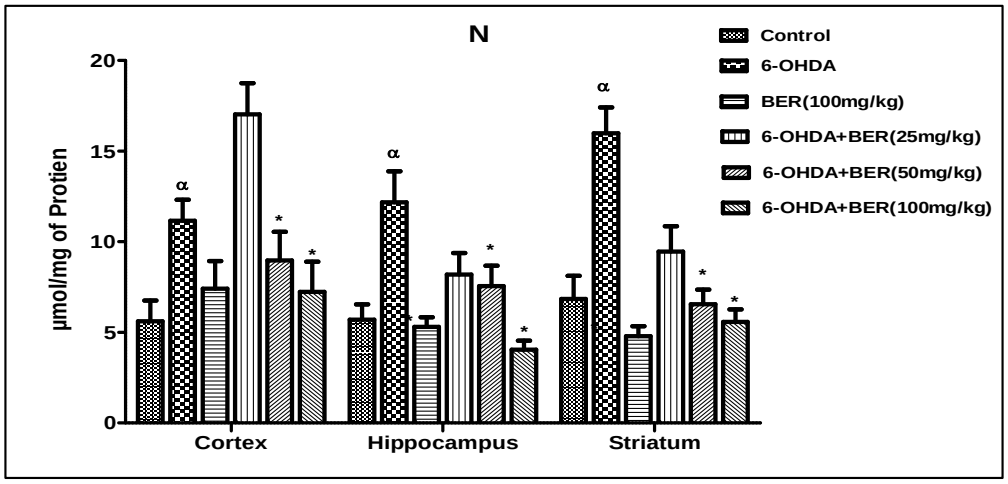


**Fig 7:** Influence of treatment with Berberine on nitrite concentration in striatum, cerebral cortex and hippocampus of rat brain (mean ± S.E.M. of 6 observations) in the different groups of rats.

**4.2.2: Effect of berberine on acetylcholine esterase levels in 6-OHDA treated rats**

The changes in ChE activity in striatum, cerebral cortex and hippocampus after administration of berberine are presented in figure 8. It can be observed that, ChE activity was significantly increased in the striatum, cortex and hippocampus of Parkinson control group compared to the non-Parkinson control group. Treatment with berberine (25, 50 and 100 mg/kg) significantly decreased the ChE activity in striatum, cortex and hippocampus compared to Parkinson control rats ($P< 0.01$). Berberine treatment in non-Parkinson rats did not influence the ChE activity as compared to non-Parkinson control rats ($P>0.05$).

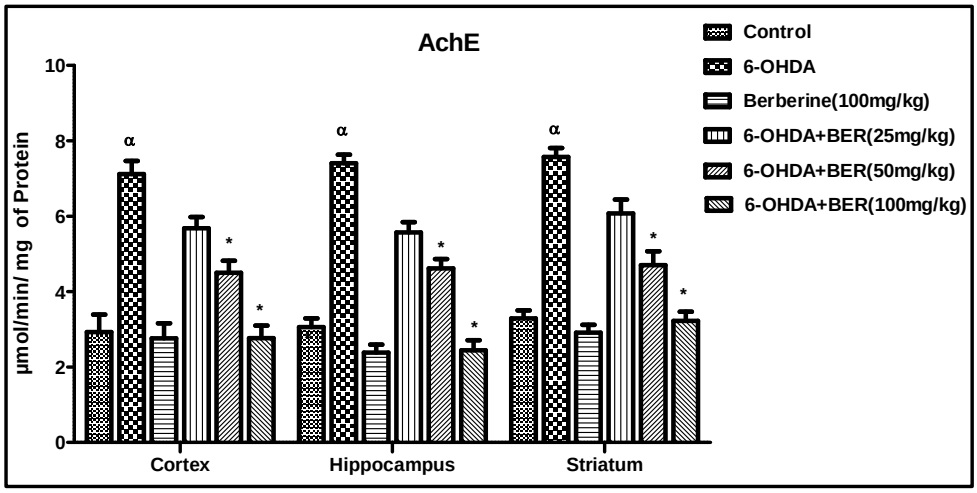


**Fig 8:** Effect of treatment with Berberine on Acetylcholinesterase level in striatum, cerebral cortex and hippocampus of rat brain (mean ± S.E.M. of 6 observations) in the different groups of rats.

## 5. Discussion

In the present study we studied 6-Hydroxydopamine induced neurotoxicity & the administration of relevant doses of berberine is remarkably neuroprotective in rats. We have chosen the dose of berberine (25, 50 and 100 mg/kg., oral) according to the previous studies done in our laboratory (8). There are no previous reports on the protective effect of berberine in 6-Hydroxydopamine induced neurotoxicity, an animal model for Parkinson's disease. In the present study, berberine attenuated various behavioral and biochemical alterations due to 6-Hydroxydopamine and thus providing the first evidence regarding its beneficial effect in Parkinson's disease.

In the present study, the administration of 6 µg of 6-OHDA into the right unilateral ventricle led to a decrease in DA levels of approximately 50–60% in the right striatum of rats at all ages. Nigral DA levels were reduced to a slightly less extent. In contrast to more severe bilateral lesions, the patterns of changes in body weight after surgery were similar between the vehicle and lesioned animals [9].

We studied 6-OHDA as animal model of Parkinson disease. 6-OHDA induce nigrostrital dopaminergic lesion via the generation of hydrogen peroxide and derived hydroxyl radicals. 6-OHDA could induce catecholaminergic cell death by three main mechanisms: reactive oxygen species generated by intra or extracellular auto-oxidation, hydrogen peroxide formation induced by MAO activity or direct inhibition of the mitochondrial respiratory chain. These events lead to strong oxidative stress amplified by cytoplasmic free calcium and to a decrease in cellular ATP availability, both leading to cell death. Unilateral 6-OHDA-induced SNpc degeneration produces an asymetric and quantifiable motor behavior after unilateral lesion induced by systemic administration of either DA receptor agonists, l-dopa or dopamine releasing drugs (amphetamine).This allows easy and reliable control of the extent of the lesion and the potential benefits of therapeutic treatments [10].

6-Hydroxydopamine administration decreased the ambulatory movements (in actophotometer) and causes a delay in retention time of the passive avoidance test (in passive avoidance test apparatus), thus representing the motor abnormalities. Daily treatment with Berberine for 14 days dose-dependently attenuated 6-Hydroxydopamine-induced hypolocomotion and motor incoordination.

I.C.V. administration of 6-Hydroxydopamine also decreased the SOD levels in the whole brain, suggesting mitochondrial damage and pretreatment with Berberine attenuated this decrease in SOD levels. These results show that Berberine may prevent mitochondrial deterioration and maintain synaptic integrity against damage induced by 6-Hydroxydopamine. It is known to produce hypoactivity that resembles juvenile onset and advanced Parkinson's disease in rats. It produces significant motor and behavioral abnormalities including bradykinesia, muscles weaknesses and rigidity. These findings are in agreement with earlier reports which also observed a variety of neurobehavioral abnormalities and motor deficit in rats following 6-OHDA administration.

Cholinergic neurotransmission is a central process underlying memory and cognitive function. Cholinergic basal forebrain neurons in the nucleus basalis magnocellularis innervate the cerebral cortex, amygdaloid complex and hippocampus, and are essential for learning and memory formation. One of the most important mechanisms responsible for correct cholinergic function is performed by enzyme choline esterase (ChE). In the present study, treatment with Berberine partially decreased the levels of ChE in cerebral cortex and hippocampus of PD rats.

These study demonstrate that daily treatment with Berberine protects against various behavioral and biochemical alterations induced by 6-Hydroxydopamine in rats. However, further studies are required to understand the exact mechanism involved in its neuroprotective role in this animal model of Parkinson's disease.

## 6. Conclusion

We investigate that the neuroprotective effect of Berberine in animal models of Parkinson’s disease. The results show that berberine could be used as an effective therapeutic agent in the management of Parkinson's disease and related conditions.

Berberine treatment protects behavioral changes, and significantly attenuated oxidative damage and improved

mitochondrial complexes enzyme activities in different regions (striatum, cortex and hippocampus) with memory improvement of rat brain against 6-OHDA induced neurotoxicity.

To get a detailed about the Berberine in neuroprotection in Parkinson's disease further research & confirmatory studies are required.

**5. References**


1. Mohsen I, Hossein H. Pharmacological and Therapeutic Effects of Berberis vulgaris and its Active Constituent Berberine. Phytotherapy research 2008; 22:999-1012.
2. Sontakke AV, Nikam S, Nikam P, Ahaley SK. Oxidative stress in Parkinson's disease. Indian journal of Clinical Biochemistry 2009; 24(1):98-101.
3. Ciobica A, Hritcu L, Artenie V. Biochemical estimation in 6-OHDA induce rat model of Parkinson disease. Analele Ştiinţifice ale Universităţii, Alexandru Ioan Cuza, Secţiunea Genetic Biologie Moleculară, Vol 8, Romania, 2007, 97-100.
4. Ciobica A, Paduraria M, Hritcu L. The effect of short term nicotine administration on Behavioural & oxidative stress deficiencies induced in rat model of Parkinsons Disease. Psychiatria Danubina 2012; 24:194-205.
5. Bhutada P, Mundhada Y, Bansod K, Tawari S, Patil S, Dixit P *et al*. Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav Brain 220; 2011:30-41.
6. Lee B, Sur B, Shim I, lee H, Habm D. Phellodendron amurense & its major alkaloid compound, Berberine Ameliorates scopalamine induced neuronal impairment and memory disfunction in rat. Korean J Physiol Pharmacol 2012; 2:79-89.
7. Chen CM, Chang HC. Determination of berberine in plasma, urine and bile by high performance liquid chromatograpy. J Chromatography 1995; 665(1):117-123.
8. Bhutada P, Mundhada Y, Bansod K, Tawari S, Patil S, Dixit P *et al*. Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav Brain 2011; 220:30-41.
9. Wayne A, Laura E, Michael P. Reductions in spontaneous locomotor activity in aged male, but not female, rats in a model of early Parkinson's disease. Brain Research 2005; 1034:153-161.
10. David B, Sakina T, Nathalie L, Marie-F, Alim-L, Remy S *et al*. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Progress in Neurobiology 2001; 65:135-172.