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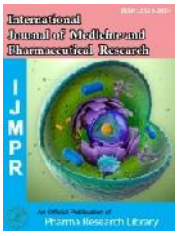

**Research Article** **Open Access**

# Neuroprotective role of Fenoprofen in experimental Parkinson's disease

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**A B S T R A C T**

Parkinsonism is a neurodegenerative disorder of the Central Nervous system in which the dopaminergic cells of substania nigra degenerate and hence dopaminergic output to the corpus stratium and putamen fails Dopaminergic cell death occurs due to intra cellular elevation of calcium levels due to Oxidative stress caused by Free-radicals. Parkinsonism is induced experimentally by stereotaxic injection of 6-OHDA. Estimation of brain dopamine levels was performed by HPLC. Iron localization in substantia niagra was performed by PERL'S DAB method. The cytosolic and mitochondrial iron content was determined by performing Bleomycin assay. The caspase 3 activation was determined by spectrophotometric assay. Sequencing of COX-2 gene in rat midbrain was performed. When compared with 6-OHDA control group the Fenoprofen and Levodopa treated groups showed significant increase in brain dopamine levels. The cytosolic and mitochondrial iron levels were significantly decreased in Fenoprofen treated group when compared with 6-OHDA control. The COX-2 gene expression was prominent in 6-OHDA control group whereas it was significantly less in Fenoprofen treatment group. The Fenoprofen was tested for anti-Parkinson's activity in 6-OHDA rat model. It showed reliable neuroprotection with respect to dopamine concentration, iron degeneration, inhibited caspase-3 activation and COX-2 gene expression. The investigations revealed that test drug could be a future molecule for treating clinical PD.

**Keywords:** Parkinsonism, Fenoprofen, Cytochrome C, COX-2 gene

**A R T I C L E I N F O**

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## 1. Introduction

As we enter the new century, Parkinson's disease ranks among the most common late life neurodegenerative diseases, affecting approximately 1.5% to 2.0% of the population older than age 60. According to the American Parkinson's Disease Association, there are approximately 1.5 million people in the U.S.Parkinson's disease (PD) is the second most common neurodegenetaive disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta.[(1)] It was first described by neurologist James Parkinson in 1817 that he called ‘‘Shaking palsy ‘or ‘‘Paralysis agitans’’. Parkinsonism is a clinical syndrome consisting of four cardinal features: bradykinesia (slowness of movement), muscular rigidity, resting tremor (which usually abates during voluntary movement), and an impairment of postural balance leading to disturbances of gait and falling. The rate of progression varies from person to person, as does the intensity of the symptoms.[(2)]

The effective treatment for Parkinson’s disease is still a mystery even though the advent and recent developments of levodopa therapy most of the PD patients experienced an intolerable drug related side effects, and continued disease progression, associated with development of clinical features that could not be controlled by levodopa therapy. Even though levodopa is considered to be a gold standard drug for the treatment of Parkinsonism but during its metabolism it converts to dopamine quinones (amidoquinones and paraquinones) in the neurons and these are highly reactive species and produces neurodegeneration and neuronal death.[(4)] Despite the advent of drugs like dopaminergic agonists, dopamine facilitator, virtually all patients developed an unacceptable level of disability and mortality rates. The application of new techniques in neurobiotechnology, genetics, neurochemistry and molecular biology in animal models has not proved to be clinically satisfiable and efficient.[(5)] More over the clinical trials with the above mentioned techniques are unacceptable and unapproachable. With these prejudiced scientific bases, we had an experimental attempt on already existing, marketed and clinically acceptable drug Fenoprofen for antiparkinsonian activity. [6,7] Hence, we hypothesized that, Fenoprofen could be used inhibit COX-2 enzyme in neuronal cells and may reduce the expression of inflammatory cytokines and there by prevent neuronal death through various molecular and cellular consequences.

## 2. Materials and Methods

**a) Chemicals (9)**

The chemicals which were used for the present study were procured from Sd-Fine Chemicals Mumbai, Sigma Aldrich USA, Loba chemie Mumbai, Merk chemicals Mumbai.

**b) Animals[(10)]**

Healthy, adult Wistar rats of both sexes (180-220g) were obtained from the Central animal house facility from J.S.S College of Pharmacy, Ootacamund, and Tamilnadu. The animals were kept in a well ventilated room and the animals had exposed to 12 hrs day and night cycle with a temperature between $20\pm3^0$C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum. All the experiments were performed after obtaining prior approval from CPCSEA and IAEC. The animals were housed in suitable environmental conditions. Approval No.: JSSCP/ IAEC/ M. PHARM/ PH. COLOGY/ 02/ 2012-13.

**c) Experimental design:[(11)]**

Animals were divided into four groups of 5 male and 5 female rats in each group.

Group I: Vehicle Control,

Group II: 6-OHDA Control,

Group III: 6-OHDA +L-DOPA (6mg/kg orally)

Group IV: 6-OHDA+ Fenoprofen (20mg/kg orally).

**d) Induction of Parkinsonism By 6-OHDA:[(12)]**

On the zero day desipramine (25mg/kg, i.p.) was administered 30 min before surgery, to protect noradrenaline containing terminals from the effects of 6-OHDA (Sigma). All animals were anaesthetised with ketamine (100 mg/kg, i.p.), xylaxine (15 mg/kg, i.m.) and then placed in a steotling stereotaxic apparatus (USA) (incisor bar: - 3.3 mm). The scalp was retracted and unilateral holes were drilled in the skull above the injection site. The needle of a 10µl Hamilton syringe (Bonaduz, Switzerland) was lowered to the appropriate coordinate [tooth bar: ±0.0mm; anterior/ posterior: -4.8 mm; medial/lateral: -2.2 mm; ventral/dorsal: -7.2 mm] were determined from bregma. Injection of 6-OHDA (30 µg of 6-OHDA hydrobromide in 4µl 0.9% saline with 0.02 µg/ml ascorbic acid) was then made over 5 min and the needle was left in place for a further 5 min. 6-OHDA lesions were made in an identical manner except that vehicle alone was injected. The scalp was then sutured closed; all efforts were made to minimize the animal pain and suffering. Three weeks after the lesions, the animals’ ability to rotate in response to apomorphine (0.5 mg/kg, s.c.) was tested. Contralateral rotations induced by apomorphine were measured 2 times at weekly intervals. Only animals showing at least 7 turns/ min in both tests were included in this study. After the induction of Parkinsonism the animals were treated with L-DOPA, Fenoprofen according to the groups of the animals at 09.00 hours upto 60 days.

**3. Biochemical and Molecular Parameters**

**i) HPLC Measurement of Dopamine:**

Dopamine content was analyzed according to the previously described method with some modifications. Dissected striata were immediately frozen on dry ice and stored at -80°C. Striatal tissues were sonicated in 0.1 M of perchloric acid (about 100 µL/mg tissue) containing paracetamol (100 µg/ml, $\lambda_{max}$-257) as the internal standard. The supernatant fluids were taken for measurements of levels of dopamine by HPLC. Briefly, 20 µL supernatant fluid was isocratically eluted through an 4.6-mm C18

column with a mobile phase containing 50 mM Ammonium phosphate pH 4.6, 25mM Hexane sulphonic acid pH 4.04, 5% acetonitrile and detected by a UV detector at 254 nanometer. The flow rate was 0.5 ml/min. Concentrations of DA was expressed as nanograms per milligram of protein. [13]

**ii) Localization of Iron In Substantia Nigra:**[14]

Brain tissues were stained for ferric ion using the perl's-DAB method. The isolated and partially frozen brains were cut horizontally to get 30-40 µm sections on a vibratome and then mounted on a glass slide. The sections were immersed in 2% potassium ferrocyanide and 2% hydrochloric acid for thirty minutes at room temperature and then rinsed with deionized water for five minutes. The perl's reaction was intensified by placing the tissue in 0.5% diaminobenzidine (DAB) in cold phosphate buffer (pH 7.4) for fifteen minutes. Next, 2ml of 1% hydrogen peroxide was added for every 200 ml of DAB solution. The sections remained in the solution for twenty five minutes. Following DAB treatment the sections were rinsed in deionized water for fifteen minutes. Counter stained the sections by using thionine solution. The slides of the section were made using a motic microscope (model Motic images plus 2.0), under the magnification of 40x equipped with camera (Li-Ping Liang et al., 2004).

$$\text{Iron asymmetry ratio} = \frac{\text{Ipsilateral densitometry value}}{\text{Contralateral densitometry value}}$$

iii) **Assay of Caspase-3 Activity** [15] **:** Activity of caspase-3 was determined spectrophotometrically as described previously by Gurtu et al. (1997) and Pei et al. (2003). Briefly, 100 µM of the peptide substrate N-acetyl Asp-Glu-Val- Asp-ρ-nitroanilide (Ac-DEVD-ρ-NA) was added to 100 µg of striatal extracts in the assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 10 mM dithiothreitol, 1 mM EDTA, 10% glycerol) and incubated at 37 ◦C for 1 h. Cleavage of the substrate was monitored at 405 nm. Enzyme activity was expressed as nmol/min/mg of protein (Naglaa I. Sarhan et al., 2011).

**4. Sequencing of the COX-2 Gene in Rat Midbrain** [16]

**1. Isolation of DNA from neuronal cell suspension**

Total DNA was extracted from rat brain tissue using the **XIT™ Mitochondrial DNA** Kit (***G-Biosciences, St Louis, MO. USA)***. The presence of DNA was verified by electrophoresis on 0.8% agarose gels containing 0.5 mg/ml ethidium bromide. Bands corresponding to full-length DNA were excised and extracted.

## 3. Results and Discussion

Fenoprofen is biologically important medicinal compound having diverse pharmacological activities. Owing to the importance of this compound, attempts are made to evaluate it for anti-Parkinson's activity.

**Effect of Fenoprofen on dopamine estimation using HPLC in rats**: Dopamine concentration in striatal region was measured by HPLC. When compared with sham control animals, 6-OHDA control showed more significant reduction in dopamine concentration, but levodopa showed higher degree of dopamine levels. When compared with 6-OHDA control, Fenoprofen treated groups showed significant (P<0.001) increase in dopamine concentration. Since dopamine is not elevated higher in Fenoprofen treated groups, it might be a good sign of neuroprotection. Dopamine over load is well evidenced with the production of oxidative abducts and will leads to neuronal loss in midbrain [17].

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**Effect of Fenoprofen on Caspase 3 activity in rats.**[19]

The caspase 3 activity was estimated from brain tissue homogenate. When compared with sham control animals the caspase 3 was significantly increased for 6-OHDA. When compared with 6-OHDA group, caspase 3 activity was significant increased for levodopa treated group but significantly decreased with Fenoprofen treated group with the value of (P<0.001).

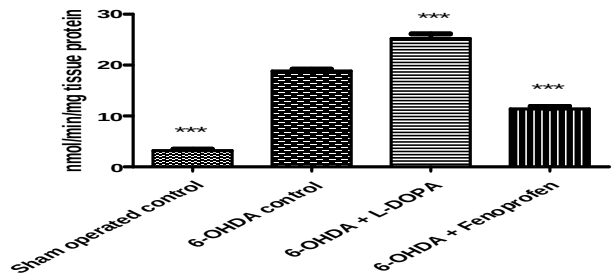


**Figure: 5** Effect of Fenoprofen on Caspase 3 activity

Values are mean ± SEM; n=6 in each group. ***P<0.001, when compared with 6-OHDA control; One-way ANOVA followed by Dunnets multiple comparision test.

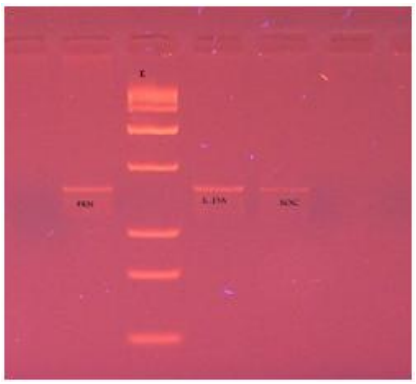

**Figure 6:** 1% Agarose Gel Plate Showing the Sequencing of the COX-2 Gene in Rat Midbrain

**Sequencing of the COX-2 gene in Rat Midbrain :**[20]

The sequencing of COX-2 gene was performed using polymerase chain reaction. The fluorescent bands were observed which are taken from 1% Agarose gel. The ladder value is from 18000 BP TO 2200 BP. there is pronounced band observed in the region for 6-OHDA treated group which indicates that the COX-2 gene is drastically mutated and over expressed in that region. The less pronounced bands were observed for sham control and Fenoprofen treated groups which indicate that fenoprofen treatment leads to cellular anti-inflammatory activity.

**Table 1:** Effect of Fenoprofen on Rat Brain Dopamine Levels

| SNO | Treatment group | Brain Dopamine Levels (ng/mg tissue protein) |
|---|---|---|
| 1 | Control | 5.233±0.03947*** |
| 2 | 6-OHDA control | 0.4117±0.05665 |
| 3 | 6-OHDA+L-DOPA | 3.345±0.04787*** |
| 4 | 6-OHDA+Fenoprofen | 2.135±0.04185*** |

Values are mean ± SEM; n=6 in each group ***P<0.001, when compared with 6-OHDA control; One-way ANOVA followed by Dunnets multiple comparision test.

**Table-2: Effect of Fenoprofen on Brain Iron Asymmetry Ratio (Perl's Dab)**

| SNO | Treatment group | Brain iron asymmetry ratio |
|---|---|---|
| 1 | Control | 0.8035±0.04217*** |
| 2 | 6-OHDA control | 1.766±0.06922 |
| 3 | 6-OHDA+L-DOPA | 1.625±0.008770 ns |
| 4 | 6-OHDA+Fenoprofen | 1.294±0.04053*** |

Values are mean ± SEM; n=6. *** P<0.001, non-significant (ns)when compared with 6-OHDA control; One-way ANOVA followed by post Dunnett's Multiple Comparison Test.

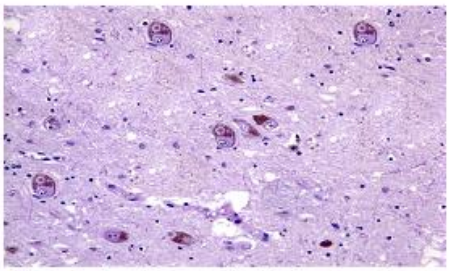

Figure 1: Iron degeneration of sham control rats

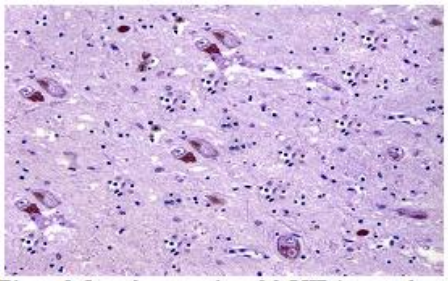

Figure 2: Iron degeneration of 6-OHDA control rats

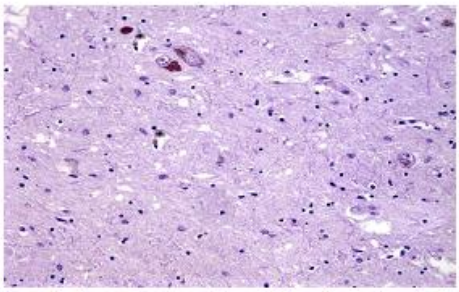

Figure 3: Iron degeneration of levodopa treated rats

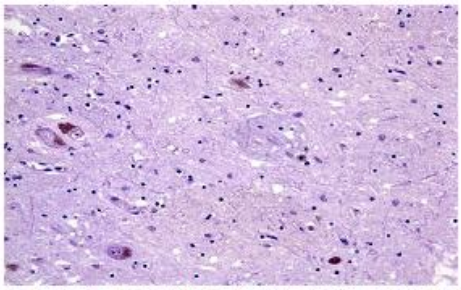

Figure 4: Iron degeneration of Fenoprofen treated rats

## 4. Conclusion

The Fenoprofen was tested for anti-Parkinson's activity in 6-OHDA rat model. It showed reliable neuroprotection with respect to dopamine concentration, iron degeneration, inhibited caspase-3 activation and COX-2 gene expression. The investigations revealed that test drug could be a future molecule for treating clinical PD. Reliability or clinical acceptability of these molecules for treating PD needs further toxicity and pharmacological investigation. Hence we suggest that, this molecule have to be further investigated to make it a clinically acceptable drug for Parkinson's disease in man.

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