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Research Article

Neuroprotective and Partial Agonistic Effect of 4-(2-phenyl-6, 7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl) morpholine (PP-43) in Rotenone-Induced Parkinson's Disease in mice

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Abstract: This study investigates the neuroprotective and partial agonistic effects of PP-43 against rotenone-induced Parkinson's disease using C57BL6/J mice. PP-43 Administration of PP-43 (10 mg/kg and 20 mg/kg) significantly attenuated neurotoxicity by improving behavioural performance and reducing oxidative stress markers. Treated mice exhibited reduced cataleptic behaviour, enhanced motor coordination, and increased locomotor activity. Biochemical analysis revealed that PP-43 elevated dopamine and glutathione levels while decreasing neuroinflammatory markers, including MPO, IL-1β, and IL-6. Additionally, PP-43 minimized lipid peroxidation and acetylcholine dysregulation, preserving neuronal integrity. Histopathological assessments confirmed reduced glial cell congestion and neuronal damage in PP-43-treated groups. While levodopa showed stronger neuroprotective effects, PP-43 effectively alleviated Parkinsonian symptoms and neuroinflammation. Observations of pyknotic nuclei in rotenone-treated mice further indicated dopaminergic neuronal apoptosis. These findings suggest that PP-43 holds promise as a neuroprotective agent for Parkinson's disease, warranting further research to elucidate its precise mechanism and therapeutic potential.

Keywords: PP-43, Rotenone Induced Parkinson's Disease, Neuroprotective, Partial Agonist, Neurodegeneration, Oxidative stress

INTRODUCTION

Parkinson's disease is the second most common neurodegenerative disorder worldwide. It occurs due to the vital loss of dopaminergic nigral neurons, leading to brain dopamine deficiency. The primary symptoms of dopamine deficiency are kinetic tremors, hypokinesia, bradykinesia, and muscle cramps [1,2]. The etiology of Parkinson's disease has long been thought to involve both genetic and environmental factors [3]. Specifically, mutations in α-synuclein, parkin, UCHL1, DJ1, PINK1, and LRRK2 cause PD with a Mendelian inheritance pattern. DJ1 and PINK1 are mitochondrial proteins, and over-expression of α-synuclein and parkin induces mitochondrial defects [4]. Pesticides consist of multiple classes and subclasses of insecticides, herbicides, fungicides, fumigants, and others and exhibit a vast array of chemically diverse structures. Rotenone and MPTP are the most potent pesticides that cross BBB and damage the dopaminergic neurons in the CNS [5]. As per the WHO report published, 8.5 million people are associated with PD globally, and 5.8 million people adjusted to the disability, which increased by more than 81% from the year 2000 (WHO report 9th August 2023). The current gold therapy of PD is the drugs levodopa and carbidopa, but it has several complications. Starting Levodopa therapy within a year produces dyskinesia symptoms in 13.5% and wearing ON-OFF symptoms in 55.9% of patients [6]. Problems with L-dopa and carbidopa therapy are numerous adverse effects such as postural hypotension, nausea, dizziness, headache, and somnolence[7]. Increasing carbidopa is recommended to relieve nausea, and domperidone can be helpful if additional carbidopa is ineffective, an abrupt withdrawal or dose reduction of L-dopa is associated with a risk of Neuroleptic Malignant Syndrome (NMS), Hyperthermia, and involuntary movement observed. These motor complications present in about 50% of levodopa patients for 5 to 10 years. Currently, an alternate monotherapy or treatment is necessary for PD. In our research, we synthesized a molecule, N-(4-bromophenyl)-2-phenyl-6,7-dihydro-5H-cyclopenta[d] pyrimidin-4-amine (PP-43) for the treatment of glioblastoma and the molecule shows better percentage inhibition score 82.91 ± 0.54 and 45.55 ± 1.46 for 100 µM and 10 µM, respectively in the glioblastoma cell line [8]. The morpholine ring with the active pharmacophore shows antioxidant, anti-inflammatory, anti-microbial, anti-cancer, anti-psychotic, anti-hypertensive, and fungicidal activity. In the present study, we have done an animal study in the C57BL6/J mice; continuous exposure to rotenone in the mice for up to 35 days damages Substantia Niagara in the brain; it damages dopaminergic neurons. Rotenone is a highly lipophilic pesticide that crosses

the human blood-brain barrier, inhibits the mitochondrial complex-I system, and inhibits NADPH oxidation. Rotenone is commonly used in models in the field of life science. Chronic exposure to rotenone in mice causes biochemical, behavioral, and histopathological changes in the brain [9]. Rotenone increases oxidative stress in the brain, leading to nerve cell apoptosis.

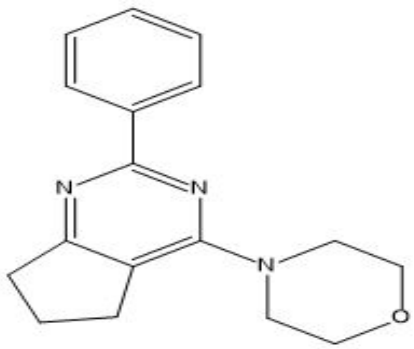
2. MATERIAL AND METHODS:

2.1 Animals:

Healthy male C57BL6/J mice 25-30gms were obtained from Global Bioscience, Pune. The animals were housed in standard cages and maintained at temperature (18 to 29 °C) and relative humidity (30% to 70%) with natural day-and-night cycles (12:12 h light and dark cycles). Lights of the animal room were put on at 6 A.M. and were put off at 6 P.M. Animals were allowed free access to food (standard laboratory rodent's chow) and water during the study. All experiments were carried out between 09:00 and 16:00 h. Animals were grouped, one to eight per cage, and were allowed a one-week habituation period in the animal room before testing. All procedures were conducted as per the guidelines of the CCSEA.

2.2 Drugs and Chemicals:

Levodopa and carbidopa were purchased from Sigma Aldrich St. Louis, MO 63178, United States of America, and Rotenone was purchased from GLP Bioscience, Montclair, CA 91763 USA. Dopamine, acetylcholine, IL-1Beta, IL-6, glutathione, myeloperoxidase, Lipid peroxidation, superoxide dismutase, and nitric oxide ELISA kits were purchased from Krishgen Biosystem, Worli, Mumbai. 4-(2-phenyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl) morpholine synthesized as per the procedure mentioned in the [8]



2.3 Structure:

4-(2-phenyl-6,7-dihydro-5H-cyclopenta[d] pyrimidin 4-yl) morpholine, PP-43.

2.3.1 Drug-Likeness:

Sr. No	Properties	Score	Acceptable Range
1.	Log P	3.05	>5
2.	Molecular Weight	281.35g/mol	>500 Dalton
3.	Hydrogen Bond Donor	0	>5
4.	Hydrogen Bond Acceptor	3	>10
5.	Topological Polar Surface Area	38.25 Å^2	>140 Å^2
6.	Log S	-3.08	(>1 µM)

Data is generated online from the SwissADME database

2.3.2 Molecular Docking:

Molecular docking was done using Schrodinger software. The X-ray crystallographic structures were downloaded from Protein Data Bank, PDB ID:7CMV, missing hydrogen bonds were added, and disulfide bonds were formed. In the final refinement stage, the OPLS3 force field was applied for complete energetic optimization, with the RMSD of heavy atoms set to 0.3 Å. The 3D structures of all synthesized compounds were generated using the LigPrep panel, ensuring proper ionization states at a physiological pH of 7.2±0.2. The receptor's active site grid was defined by centralizing the cognate ligand within the crystal structure using default box dimensions. Finally, molecular docking was performed in Maestro's workspace using Schrödinger's Glide, where the optimized 3D ligand structures and receptor grid files were loaded, and docking was carried out with extra precision (XP) methodology.

2.4 IR, NMR, and mass spectroscopy description:

Reaction time: 7 h; white solid; yield: 71%; melting point: 136−140°C; ^{1}H-NMR (500 MHz, CDCl3) δ ppm 2.09 (qnt, 2H cyclopentyl-CH2, J = 7.75 Hz), 2.96 (t, 2H, cyclopentyl-CH2, J = 7.9 Hz), 3.02 (t, 2H, cyclopentyl-CH2, J = 7.3 Hz), 3.81 (s, 8H, morpholine-CH2), 7.41−7.45 (m, 3H, aromatic-H), 8.34−8.36 (m, 2H, aromatic-H); 13C-NMR (500 MHz, CDCl3) δ ppm 22.37, 31.71, 34.22, 46.15, 66.94, 114.19, 127.98, 128.23, 129.84, 138.54, 160.20, 162.30, 174.47; ESI-MS m/z: 282.3 [M+H]+; IR (ATR, cm−1): 2938.87 (aliphatic C-H stretch stretch), 1745.26 (C=N stretch), 1589.06, 1544.70 (Ar-C=C stretch).

2.5 Experimental Methodology:

C57BL6/male mice weighing (25-30gms) were randomly assigned for the rotenone-induced PD. There were seven groups designed for the study, and each group contained 8 animals. (n=8), Group 1 the vehicle control group received drinking water 5mg/kg+ Sunflower oil(p.o), Group 2 received rotenone 2mg/kg in sunflower oil 1ml/kg, s.c for 35 days, Group 3 received L-dopa and carbidopa before 1 hr of rotenone administration, 2mg/kg/s.c for 35 days, Group 4 received PP-43,10mg/kg before rotenone 2mg/kg/s.c administration for 35 days, Group 5 received PP-43,20mg/kg before rotenone 2mg/kg/s.c administration for 35 days, Group 6 received PP-45,10mg/kg before rotenone 2mg/kg/s.c administration for 35 days, Group 7 received PP-45,20mg/kg before 2mg/kg/s.c administration for 35 days. All the animals were trained in the behavioural models before the 5-day start of the experiments. Animals' behavioural assessments were done for 0,7,14,21,28,35 days, except for the inclined plane test, which was done after 35 days. Animals were sacrificed, and brain homogenate was used for the biochemical estimation and histopathological evaluation. Grip strength, locomotor activity, exploratory behaviour,

and catalepsy were done at 7-day time intervals; till 35 days, animals were immediately sacrificed for dopamine, acetylcholine, LPO, MPO, NO, IL-1Beta, IL-6, and SOD estimation[9]

2.7 Acute Toxicity Study (OECD 425):
Female mice were kept at 22 °C (± 3°C), and relative humidity was 30 % and not more than 70%. Animals were fasted before dosing. The drug's toxicological information was available, and the dose started at 175mg/kg. Three animals were tested for each dose, but only single female mice were tested at once and carefully observed for 48 hours to the next 14 days. The progression factor was considered 3.2, and LD 50 was calculated at 175mg/kg as per OECD 425 calculations.

2.7 Behavioural studies:
2.7.1 Catalepsy:
Catalepsy was evaluated after 35 days of rotenone treatment. The 2.5cm and 4.5cm wooden blocks were used for the test, and the catalepsy was observed between 0,7,14,21,28,35 days. The score was 0 to 3.5, and the score was calculated based on the stages of the animals. When the mouse moved typically or was placed on the table, the score was given 0; when the mouse was moved or pushed the wooden block, the score was given 0.5; when the mouse was placed on the table with front paws set alternatively on the 2.5cm wooden high block fails to correct the posture in 10cm, the score was given 0.5 for each paw with a total of 1 score. When the mouse failed to keep the front paw 4.5cm block, the score was 1 for each paw [10]

2.7.2 Inclined Plane Test:
The inclined plane test was carried out on the 35th day of the experiment. The plane consists of two rectangular plywood boards connected at one end by a hinge. One board is the base; the other is the movable inclined plane. Two plywood side panels with degrees marked on their surface are fixed on the base. A rubber mat with ridges 0.2 cm in height was fixed to the inclined plane set at 45 degrees-angle, and the falling time of the animal was calculated in seconds [11]

2.7.3 Locomotor activity
Locomotor activity was evaluated by using an Actophotometer. Each animal was kept inside the Actophotometer for five minutes, and the number of light beam crosses was measured[12,13]. The test was conducted after the 0,7,14,21,28,35 days.

2.7.4 Muscle strength:
The muscle strength of the animals was evaluated by rota-rod; the apparatus consists of a horizontal metal rod of 3cm diameter, attached to a motor speed set at 25 rpm, and the cut of time was 300sec.and the height of the rod was kept at 50cm from the tabletop [13].The test was conducted after the 0,7,14,21,28,35 days.

2.7.5 Exploratory Behaviour:
The animals' exploratory behavior was checked using an elevated plus maze apparatus. The elevated plus maze consists of two open and closed arms: the open arm is (16 ×5 cm), and the closed arm is (16×5×12). The instrument's elevation was considered 25cm for the mice. The mice were placed individually at the center of the EPM with their heads facing towards the open arm during the 90-second-5-minute period.

2.8 Biochemical Studies:
Preparation of tissue homogenate: The animal brain was removed and placed in phosphate buffer saline at 7.4; excess blood was removed from the brain portion, minced into the brain, and homogenized in PBS. Centrifugation was conducted at 2000-3000rpm, and the supernatant was collected for biochemical estimation.

2.8.1 Lipid peroxidation estimation: The 100 ul standard solution was added to the wells, and the plate was covered, sealed, and incubated for 80 minutes and 37 degrees Celsius. Aspirate and wash the plate 4 times with diluted Wash Buffer (1X) and blot residual buffer by firmly tapping the plate upside down on absorbent paper. Pipette 100 ul Biotinylated Lipid Peroxide, LPO Antibody Working Solution to all wells, and wells should be covered for 50 minutes at 37°C. Aspirate and wash the plate 4 times with diluted Wash Buffer (1X). Pipette 100 ul Streptavidin: HRP Conjugate Working Solution to all wells and mixed well. Cover the plate with a sealer and incubate for 50 minutes at 37C. Aspirate and wash the plate 4 times with diluted Wash Buffer (1X), Pipette 100 ul TMB Substrate in all the wells. The plate was kept at 37°C for 10 minutes. Pipette 100 ul of stop solution to all wells. The wells should turn from blue to yellow. The absorbance was read at 450 nm with a microplate within 10-15 minutes after adding the stop solution.

2.8.2 Estimation of NO, MPO, SOD, Reduced glutathione, Dopamine and Acetylcholine:
All the biochemical parameters were performed per the procedure mentioned in the Manual of Krishgen Biosystem, Worli, Mumbai.

2.8.3 Histopathological Analysis:
Histopathological evaluation was done using the brain's section and eosin staining. The substantia niagra section was stained 7-9mm using a crystal violet stain. stained sections were observed under the Zeiss microscope for histopathological evaluations.
the section was stained 7-9mm using crystal violet stain. stained sections were observed under the Zeiss microscope for histopathological evaluations.

2.8.4 Statistical Analysis:
The data were expressed in each value representing mean ± SEM (n = 8). Two-way ANOVA analyzed the behavioral and biochemical estimation data using Bonferroni's test. Graph pad prism version 8 was used for the analysis of the result. P-values <0.05 were considered statistically significant for all comparisons.

3.0 RESULT:

Docking interaction of the synthesized compounds with the dopamine D3 receptor(7CMV) revealed that compound PP-43 (docking score: -6.684 kcal/mol) was more effective as compared to Dopamine (docking score: -6.681 kcal/mol). Π- Π stacking interaction has also been observed between the phenyl ring of compound PP-43 with PHE 346 residue of the dopamine D3 receptor. However, quaternary ammonia of dopamine exhibited salt bridge and hydrogen bond interaction with Asp-110 and Π- Π stacking between phenyl ring with Ser-196. The second most potent compound of the series, i.e., PP-43, had the Π- Π stacking interaction with Phe-346.

Figure: PP-43 docking with D3 (7CMV) receptor

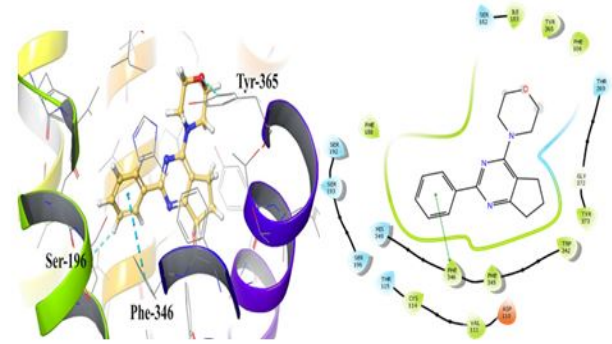
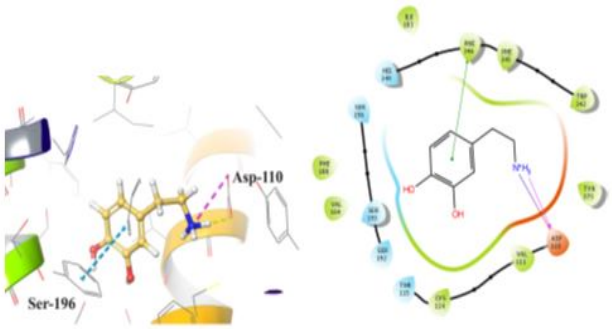


Figure: Dopamine docking with D3 (7CMV) receptor.



Molecules	Docking score (Kcal/mol)
PP-43	-6.684
Dopamine	-6.681

3.2 Body weight:

The animal's body weight was measured using two-way ANOVA using the Bonferroni test. The statistically significant interaction was found between weeks (1-5 weeks) and the groups of the body weight ($F_{(30,210)}$=6.59), P<0.001. We found that an overall comparison was carried out with the different groups. In the treated group, 2mg/kg, s.c administration, the body weight was significantly decreased as compared to vehicle control and positive control, that is, levodopa and carbidopa treated group. The positive treated group had constant body weight observed compared to vehicle control, PP-43 (10,20mg/kg) significantly reduced body weight after 21 days, and PP-45(10,20mg/kg) significantly reduced body weight after 14 to 35 days.

3.3 Catalepsy:

The Two-way ANOVA found a significant cataleptic score; there was a statistically significant two-way interaction between weeks (1-5 weeks) and groups for catalepsy,
($F_{(30,210)}$ =394), P<0.001. In the statistics, multiple comparisons were carried out; in the rotenone-treated group, higher catalepsy was observed compared to PP-43(10,20mg/kg), levodopa-treated animals. The catalepsy score was ranging between 0 to 3.5.

3.4 Motor coordination:

Motor coordination was observed in the rota-rod apparatus. Two-way ANOVA was performed by using the ANOVA test, and there was a statistically significant two-way interaction between weeks (1-5 weeks) and groups for motor coordination ($F_{(30,210)}$ =9.25), P<0.001. The PP-43 group decreased motor coordination significantly compared to the rotenone-treated group, and PP-43 was non-significant to the levodopa-treated group.

3.5 Locomotor activity:

Locomotor activity in the mice observed by Actophotometer apparatus, locomotor activity was performed Two -way ANOVA test, and there was a statistically significant Two-way interaction between weeks (1-5 weeks) and groups for locomotor activity, ($F_{(30,210)}$ =7.88), P<0.001. PP-43(10,20mg/kg) concentrations significantly increased locomotor activity compared to the rotenone-treated group.

3.6 Exploratory Behaviour:

Exploratory behavior of the mice observed in the elevated plus maze, the test was performed Two-way ANOVA by Bonferroni's test, and there was a statistically significant Two-way interaction between weeks (1-5 weeks) and groups for locomotor activity, ($F_{(06,33)}$ =8.80), P<0.001. PP-43(10,20mg/kg) concentrations were observed to significantly decrease closed-arm entries in multiple attempts compared to the rotenone-treated group, and the result was significant.

3.7 Dopamine and acetylcholine estimation:

Brain dopamine level in the whole brain was derived by the ELISA method; the test was performed by one-way ANOVA by Bonferroni's test; the treatment between group and weeks (1-5) was found ($F_{(2.283,11.42)}$ =2576), P<0.001 for dopamine, and ($F_{(6,35)}$ =270), P<0.001 for acetylcholine. The dopamine level increased in the PP-43 (10,20mg/kg) as compared to the rotenone-treated group, the acetylcholine level was found to increase in the PP-43-treated group as compared to a rotenone-treated group, acetylcholine and dopamine levels were found to be significant as compared to rotenone treated group, P<0.001.

3.8 IL-1 Beta and IL-6:

The estimation of IL-1 Beta and IL-6 was estimated by the ELISA method, and the test was performed by one-way ANOVA by Bonferroni's test; the treatment

between group and weeks (1-5) was found ($F_{(6,35)}$ =78.54) for IL-1Beta, ($F_{(6,35)}$ =92.42) for IL-6, both the results found significant, P<0.001. The IL-1Beta level decreased in the PP-43(10,20mg/kg) treated group compared to the rotenone-treated group. An increased level of IL-1 and IL-6 was found in the rotenone rotenone-treated group, P<0.001. The level of PP-43(10,20mg/kg) was significant compared to the rotenone-treated group, P<0.001.

3.9 Reduced Glutathione:

Reduced glutathione estimation was performed using the ELISA method. Statistically, results were evaluated by one-way ANOVA by Bonferroni's test; the treatment between group and weeks (1-5) was found ($F_{(7,40)}$ =265), P<0.001. The level of glutathione was decreased as compared to the rotenone-treated group.

3.10 Lipid Peroxidation and Myeloperoxidase:

LPO and MPO assays were performed by the ELISA method, the results were evaluated by one-way ANOVA by Bonferroni's test; the treatment between weeks (1-5) was found,

($F_{(6,35)}$ =448) for MPO, ($F_{(6,35)}$ =165.4) for LPO. The LPO level and Myeloperoxidase significantly increased in the rotenone-treated group compared to the PP-43(10,20mg/kg), P<0.001.

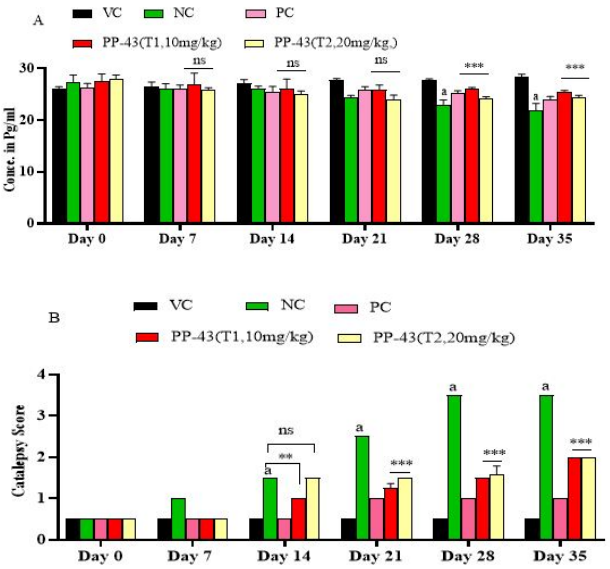


Fig.1. Effect of PP-43 (T1-10, T2-20mg/kg) in rotenone-induced PD, in graph (A) body weight observed from day 0 to day 35. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control, in graph (B) catalepsy scores are indicated from 0 to 3.5. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, ap<0.01 vs vehicle control; the data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control.

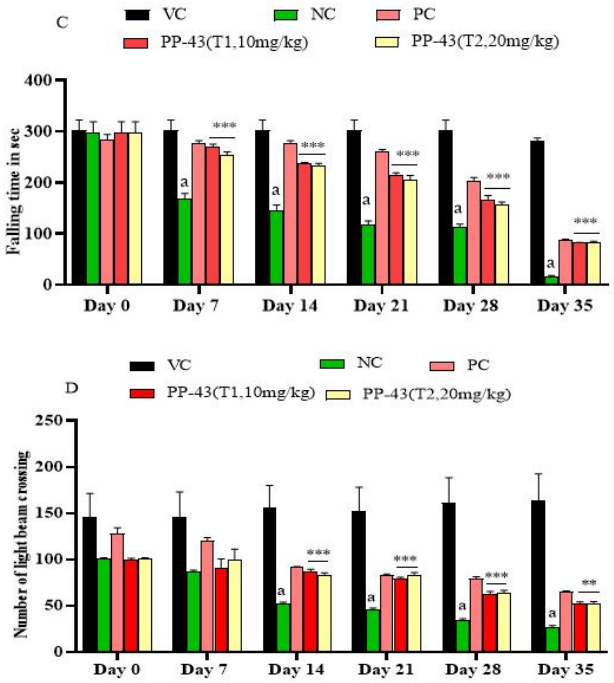


Fig.2. Effect of PP-43 (T1-10, T2-20mg/kg) was observed in rotenone-induced PD; in graph (C), motor coordination was observed from day 0 to day 35 by Rota-rod. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control, in graph (D) locomotor activity was observed from 0 to 3.5 by acto-photometer. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control, The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control

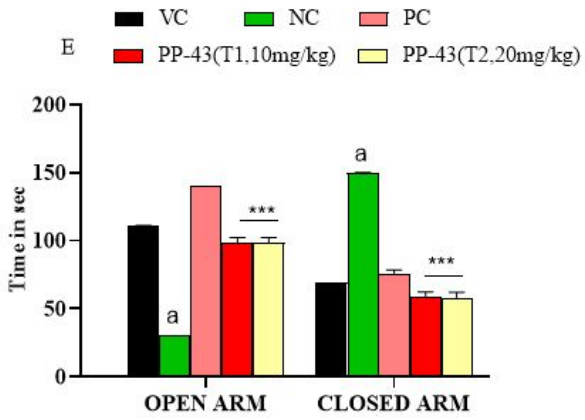


Fig.3. Effect of PP-43 (T1-10, T2-20mg/kg) in rotenone-induced PD, in graph (E) anxiety was observed after 35 days by Elevated plus Maze. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-

way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control.

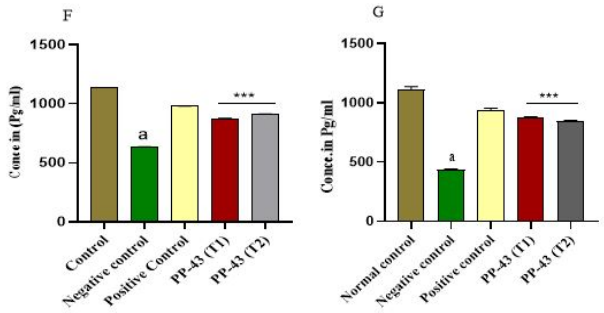


Fig.4. Effect of PP-43 (T1-10, T2-20mg/kg) in rotenone-induced PD, in the graph (F) dopamine and Acetylcholine (G) was observed after the 35 days by ELISA method. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control.

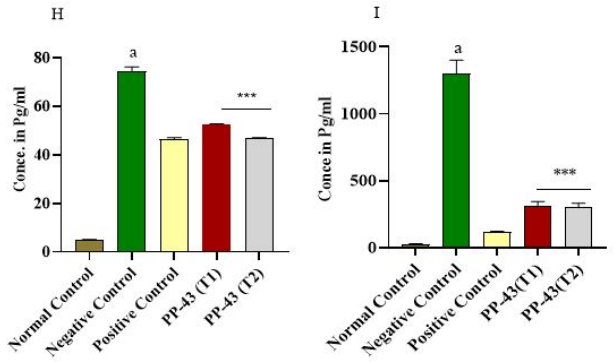


Fig.4. Effect of PP-43 (T1-10, T-20mg/kg) in rotenone-induced PD, in the graph (H) IL-1Beta and IL-6 (I) were observed after the 35 days by ELISA method. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control.

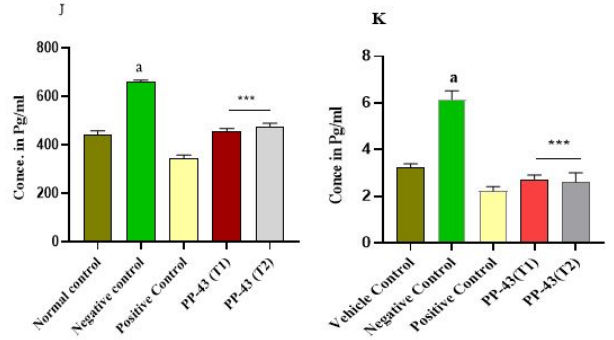


Fig.5. Effect of PP-43 (T1-10, T2-20mg/kg) in rotenone-induced PD, in the graph (J) Myloeperoxidase and Lipid Peroxidation (K) were observed after the 35 days by ELISA method. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control.

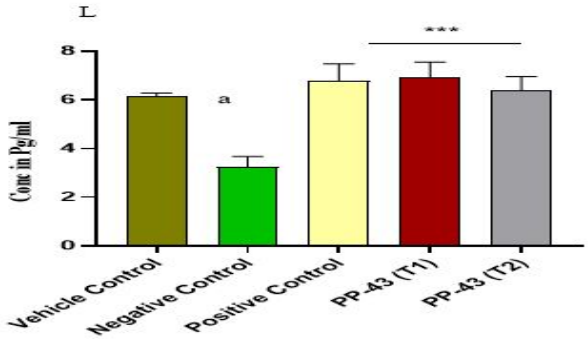
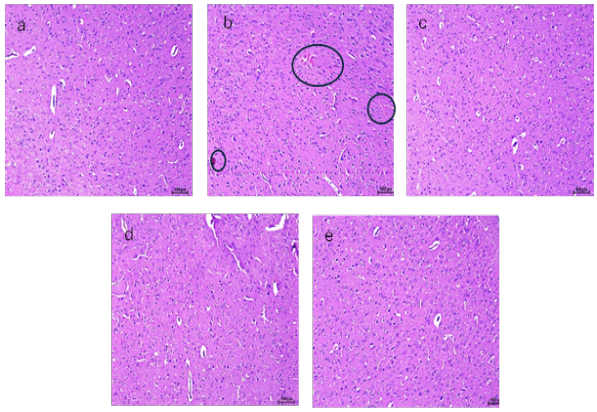


Fig.5. Effect of PP-43 (T1-10, T2-20mg/kg) in rotenone-induced PD, in the graph (L) reduced glutathione observed after the 35 days by ELISA method. The data were expressed with each value representing mean ± SEM (n = 8). The data were Analysed by Two-way ANOVA by Bonferroni test, *p< 0.05, **p<0.01, *** p<0.001 vs Negative control, [a]p<0.01 vs vehicle control.

3.11 Histopathology Result:



a) Vehicle control: No histomorphological changes were observed in the neurons, and neurons were found intact and uniform. **b) Negative control:** Pyknotic nuclei were observed, higher congestion in the glial cells was observed, and loss of vacuolated neuropil was observed. **c) Positive Control:** Less vacuolated neuropil was observed in the substantia niagra. The neuronal tissue and supporting matrix (neuropil) were intact and uniform.**d) PP-43 (T1):** Infiltration of glial cells was observed. The neuronal tissue and supporting matrix (neuropil) were intact and uniform **e) PP-43 (T2):** Infiltration of glial cells was observed.

DISCUSSION:

In the docking study, the docking interaction of the synthesized compounds with the human dopamine D3 receptor revealed that compound PP-43 (docking score: -6.684 kcal/mol) was more effective than compound Dopamine (docking score: -6.681 kcal/mol). Compound PP-43 showed two aromatic hydrogen bond interactions with the dopamine D3 receptor, more specifically between phenyl hydrogen and Ser-196 and morpholine ring and Tyr-365. The acute toxicity study (OECD-425) of the PP-43 was found to be 9.881 g/kg as per the Miller and Tainter method. The study evaluated the protective and partial

agonist effects of PP-43(10mg,20mg/kg) against rotenone-induced oxidative stress, which generates Parkinson's Disease (PD). The study is associated with biochemical and behavioral assessment in the C57BL6/J mice. Test compound PP-43 is a synthetic compound with anti-glioblastoma action in an in vitro study.[8]. The two doses were evaluated in the C57BL6/J mice, and both doses potentially show neuroprotective action in the rotenone model. Rotenone is potentially neurotoxic, which blocks the mitochondrial electron chain. In the present study, both the concentration of PP-43 attenuated in the rotenone-induced PD produces cataleptic behavior and decreased motor coordination and locomotor activity. [8] d In this rotenone model treatment, within the first 14 days, animals show the Parkinsonian effect; after the 35 days completion of rotenone treatment, the concentration of dopamine neurotransmitter significantly decreased in the mice brain as compared to levodopa treated group, due to exposure of the rotenone which damages dopaminergic neurons in the region of SN. Levodopa shows a neuroprotective effect in the mouse brain. The treatment of levodopa and PP-43(10,20mg/kg) significantly increased the glutathione concentration in the negative control group. The GSH is readily oxidized by the hydrogen peroxide radicals in oxidative stress, and increased GSH produces the interleukins level in the brain region [14]

Myeloperoxidase plays a vital role in neuroinflammation, which has a potential role in neurodegenerative disorders in the negative control group significantly increased the MPO level due to high oxidative stress, which led to neuroinflammation as compared to levodopa and PP-43 treated groups, also significantly increased the concentration of MPO found in PP-43 groups compared to levodopa group. PP-43 Both concentrations reduced the neuroinflammation of the brain compared to the treated group. LPO level is significantly increased in the rotenone-treated group; the peroxide radicals damage the lipid layer present outside of the neurons. The lipophilic nature of rotenone inhibits the mitochondrial complex I electron chain and produces peroxide ion generation, damaging the neurons' lipid layer. The concentration of acetylcholine significantly increased in the PP-43 group compared to the negative control group. The highest production of ROS occurred due to damage to neuronal mitochondria. The pro-inflammatory cytokines IL-1Beta and IL-6 levels were significantly lower in the PP-43 group compared to the rotenone-treated group.

The new findings proved that, in the PP-43 group, behavioural paradigms (Catalepsy, Rota-rod, Inclined plane test, Acto-photometer, and EPM) showed significantly better results in the PP-43 groups than rotenone-treated groups. Still, results were not found significant in the Levodopa-treated group. In the histopathological evaluation, In the region of substantial nigra pyknotic nuclei were observed in the rotenone-treated group, higher congestion of the glial cells was observed, and it was found to separate from neuropil. In the PP-43 group, only mild glial congestion was observed; in the Levodopa group, very little vacuolated neuropil was observed. No histomorphological changes were observed in the brain tissue in the vehicle control group.

4. CONCLUSION:

The study demonstrates that PP-43 exhibits significant neuroprotective and partial agonist effects against rotenone-induced oxidative stress, a model for Parkinson's disease in C57BL6/J mice. Both doses of PP-43 (10 mg/kg and 20 mg/kg) effectively attenuated rotenone-induced neurotoxicity by improving behavioural performance and reducing oxidative stress markers. PP-43 treatment mitigated cataleptic behaviour, improved motor coordination, and increased locomotor activity. Biochemical analysis further confirmed its neuroprotective role by enhancing dopamine and glutathione levels while reducing neuroinflammatory markers such as MPO, IL-1β, and IL-6. Additionally, PP-43 preserved neuronal integrity by minimizing lipid peroxidation and acetylcholine dysregulation. Histopathological evaluation supported these findings, showing reduced glial cell congestion and neuronal damage in the PP-43-treated groups compared to the rotenone group. While levodopa exhibited stronger neuroprotective effects, PP-43 significantly mitigated Parkinsonian symptoms and neuroinflammation. In the rotenone-treated group, pyknotic nuclei were observed, indicating the dopaminergic neurons' apoptosis. These findings suggest that PP-43 has potential as a neuroprotective agent for managing Parkinson's disease, though further studies are needed to explore its precise mechanism and therapeutic efficacy.

Conflict of Interest: The authors declare that they have no conflict of interest

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