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RESEARCH ARTICLE

Evaluation Of Plant-Derived Flavonoids For Neuroprotective Activity In An Experimental Model Of Parkinson's Disease

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Abstract: In the case of Parkinson disease, a neurodegenerative disorder that progresses with the loss of dopamine-producing neurons in the substantia nigra, oxidative stress and neuroinflammation are major contributors. Well-known plant-derived flavonoids are believed to possess neuroprotective properties, and researchers investigated their effects in a parkinsonism model in rats. Parkinsonism was induced in the rats through the use of 6-hydroxydopamine, and various doses of flavonoid-rich plant extracts were given after 21 days. The scientists looked at the rats' motor activities with three different behavioral tests: rotarod, open-field and catalepsy, while checking on oxidative stress levels of malondialdehyde, reduced glutathione, catalase and superoxide dismutase, as well as conducting histopathological study of the substantia nigra and striatum, which indicated the status of the neurons and potential protective effects of the fluoride treatment. Results showed that there was a significant boost to the rats' motor coordination and an increase in the levels of the antioxidant enzymes, and supported by a conservative look at the state of the neurons, the researchers found that the plant-based flavonoids appear to have a compelling neuroprotective effect.

Keywords: Flavonoids, Neuroprotection, Parkinson diseases, 6-Hydroxydopamine, Oxidative stress, Dopaminergic neurons, Antioxidant activity.

INTRODUCTION

We often think of Parkinson's disease, we're primarily thinking about its effects on the motor system. Parkinson disease is caused by the progressive destruction of the dopaminergic neurons within the part of the brain, the substantia nigra pars compacta, which produces dopamine. The resulting loss will result in a substantial reduction in the amount of dopamine in the striatum, which causes the typical motor symptoms of the disease, including tremors at rest, muscle rigidity, slowed movements, and poor balance. In addition to these motor disorders, there are also other non-motor symptoms such as cognitive failure, sleep disorders, depression, and autonomic dysfunction, which can be experienced by patients. That can severely affect the quality of life of patients. [1].

The causes of PD are multifactorial and complicated, with both genetic mutation and exposure to environmental influences, and age alterations. Critical factors associated with dopaminergic neuron degeneration are the oxidative stress, dysfunction of mitochondria, abnormal protein aggregation especially that of alpha-synuclein and chronic neuroinflammation. One of them, oxidative stress, is the primary element since it promotes the formation of reactive oxygen species (ROS), which damage lipids, proteins and nucleic acids and eventually induces neuronal apoptosis [2]. Moreover, the build-up of iron in the substantia nigra and the impaired antioxidant defense mechanisms contribute to the acceleration of the oxidative damage (Figure 1).

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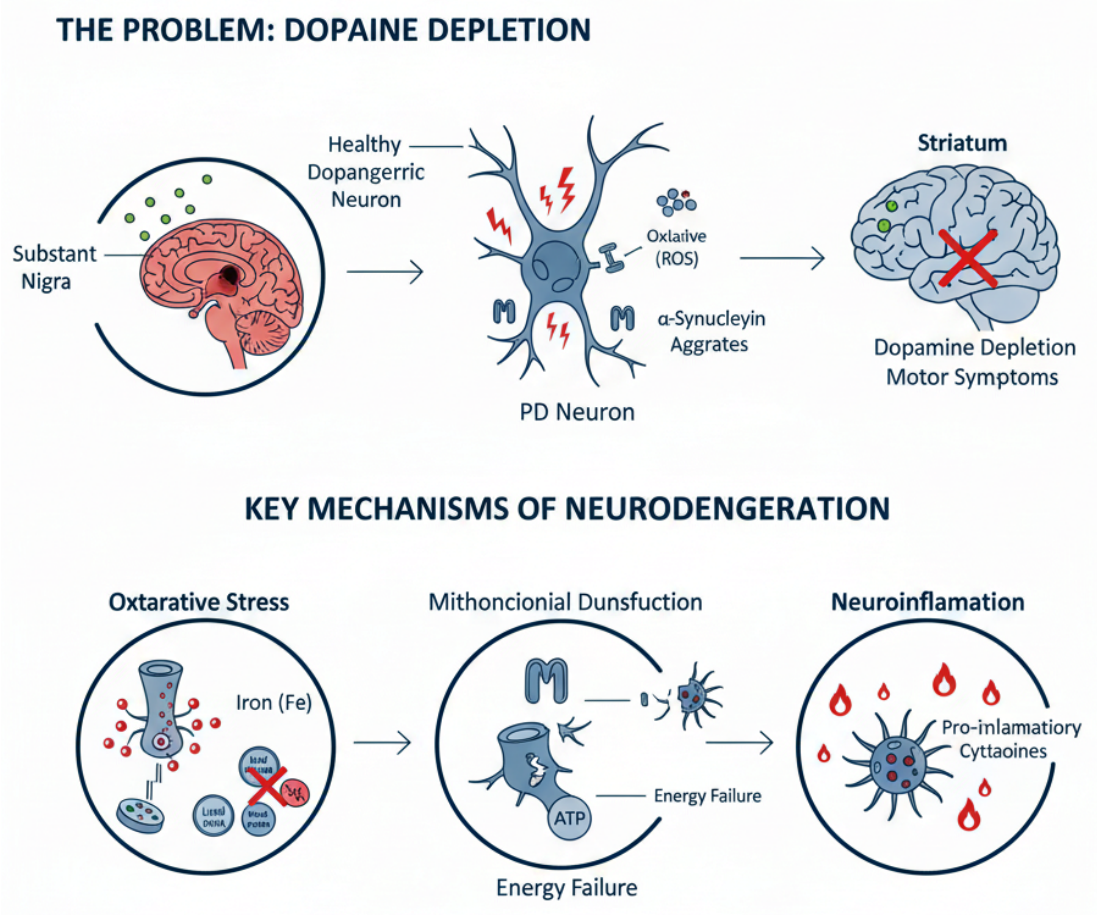


Figure 1: Pathogenesis of PD

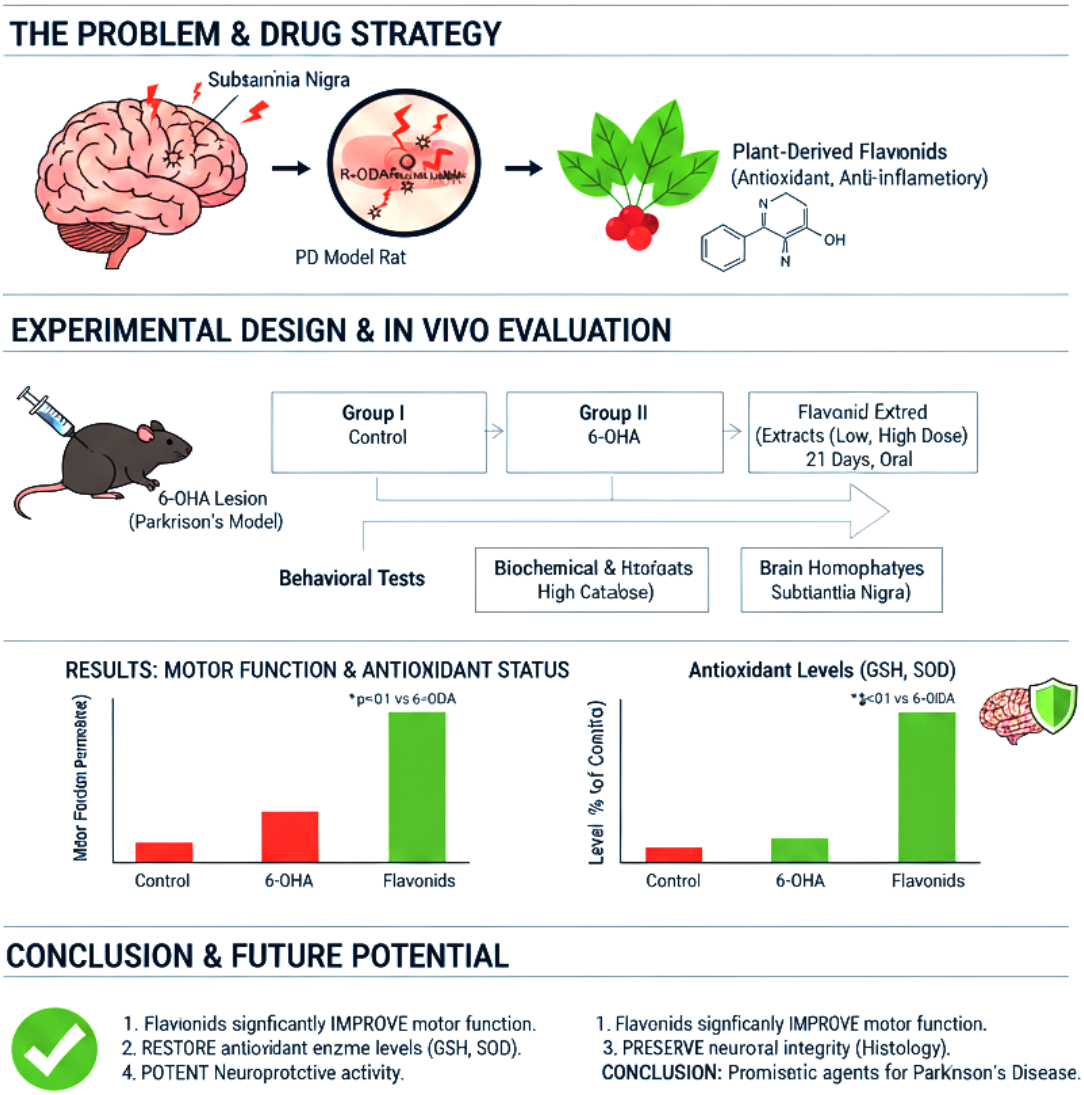
Existing therapies of Parkinson disease such as levodopa, dopamine agonists, monoamine oxidase-B inhibitors, and catechol-O-methyltransferase inhibitors are mostly used to treat the disease symptomatically by improving dopaminergic transmission. These treatments however do not stop the neurodegenerative processes. The motor complications are common with long-term use (dyskinesia and changes in drug response), which is why there is an urgent necessity to find disease-modifying therapies [3]. Natural compounds with possible neuroprotective properties such as antioxidant, anti-inflammatory, and mitochondrial-stabilizing action have therefore become of interest to research.

Flavonoids are a heterogeneous family of natural polyphenols existing in fruits, vegetables, tea, and medicines whose pharmacological activity is very diverse. These are free radical scavenging, regulation of metal ions and the regulation of cell signaling. Notably, the flavonoids have an ability to penetrate the blood-brain barrier and provide the neuroprotective effect through control of oxidative stress, neuroinflammation, and apoptotic pathways [4]. Examples of experimental studies that have shown flavonoids like quercetin, rutin, catechin, and naringenin to prevent dopaminergic neuronal loss and ameliorate motor functions in several models of PD are provided.

The 6-hydroxydopamine (6-OHDA) neurotoxin is typically applied to cause experimental Parkinsonism in animals. Simulating the pathological characteristics of PD in humans, 6-OHDA specifically kills dopaminergic neurons via oxidative stress and mitochondrial dysfunction, which renders it an appropriate model of assessing neuroprotective interventions [5].

It is against this background that the current study was aimed at investigating the neuroprotective nature of the plant flavonoids on 6-OHDA induced PD in rats. The research was directed at behavioral measurements, oxidative stress parameters, and histomorphologic analyses of the substantia nigra and striatum after the administration of flavonoids. The results will help to explain the therapeutic potential of flavonoids as natural agents in preventing and treating PD, as well as the information about the underlying mechanism of action [6].

Graphical abstract:



INTRODUCTION

RESULTS AND OBSERVATIONS:

Chemicals and Reagents

Chemicals of analytical grade used were the 6-hydroxydopamine hydro bromide (6-OHDA), ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), reduced glutathione (GSH) and others that were acquired at Sigma-Adlrich (USA) [7]. The reagents and standard drugs of the biochemical analysis were bought in reputable commercial sources. New and used solutions were made.

Plant Material and Preparation of Flavonoid Extract

The selected plant with a high concentration of flavonoids was picked, identified with the help of a professional botanist, and dried in the shade. The dry contents were pulverized then extracted in a Soxhlet apparatus consecutively with ethanol. A rotary evaporator was then used to concentrate the resulting extract under reduced pressure and store the extract at 4 o C [8]. The overall amount of flavonoid was determined spectrophotometrically with the colorimetric system of aluminum chloride and expressed in quercetin equivalents (mg QE/g extract).

Experimental Animals

The weight of the healthy adult male Wistar rats was 180-220 g. Animals were kept under the normal laboratory conditions (temperature 25 +2 C, relative humidity 5060, 12 h light/dark cycle) with free access to normal pellet food and water. The Institutional Animal Ethics Committee (IAEC) approved all the experimental procedures and followed the guidelines of CPCSEA [9].

Induction of Parkinson's Disease Model

Unilateral intracerebral inoculation of 6-OHDA into the right striatum triggered the development of parkinsonism. Ketamine (80 mg/kg) and xylazine (10 mg/kg) were used to anesthetize animals, and 8 0HDA in 4 0L of 0.02% ascorbic acidsaline solution was stereotaxically injected. Animals that were fed by the sham were given an equal amount of vehicle. Animals were given one week to recover after surgery, and then the treatment began [10].

Experimental Design

Rats were randomly divided into five groups (GP) (n = 6 per group) [11]:

- *GP I:* Normal control (saline)
- *GP II:* 6-OHDA control (vehicle-treated)
- *GP III:* 6-OHDA + Standard drug (Levodopa + Carbidopa, 10 mg/kg, orally)
- *GP IV:* 6-OHDA + Flavonoid extract (low dose, 50 mg/kg, orally)
- *GP V:* 6-OHDA + Flavonoid extract (high dose, 100 mg/kg, orally)

Treatments were administered daily for 21 consecutive days following induction.

Behavioral Assessment

Motor coordination and behavioral performance were evaluated using the following tests [12]:

- *Rotarod Test:* To determine muscle coordination and balance, the animals were put on a rotating object (20 rpm) and the time taken to fall was recorded.
- *Open Field Test:* The measure of locomotor activity was the number of line crossings and rearings in a specific arena (5 minutes) [13].
- *Catalepsy Test:* The bar test was used to assess the duration for which an abnormal posture was maintained.

Biochemical Estimations

The behavioral testing was followed by the sacrifice of animals, after which, the brain regions such as the striatum and substantia nigra were isolated and homogenized. The biochemical parameters that were measured included the following ones [14]:

- *Lipid Peroxidation (MDA):* Assessed using the thiobarbituric acid reactive substances (TBARS) method.
- *Reduced Glutathione (GSH):* Determined using Ellman's reagent (DTNB) method.
- *Catalase (CAT):* Measured by monitoring the rate of hydrogen peroxide decomposition.
- *Superoxide Dismutase (SOD):* Evaluated through inhibition of pyrogallol autoxidation [15].

Histopathological Examination

Brain sections were fixed, paraffined and cut to a thickness of 5 mm. The extent of neuronal degeneration, necrosis and inflammatory alterations of the substantia nigra and striatum were assessed by the methods of hematoxylin and eosin (H and E) staining under light microscope [16].

Statistical Analysis

The data are shown in the form of mean and SEM. One-way ANOVA with post hoc test of Tukey was used to compare groups with each other. A p-value of less than 0.05 was deemed to be statistically significant (Figure 2) [17].

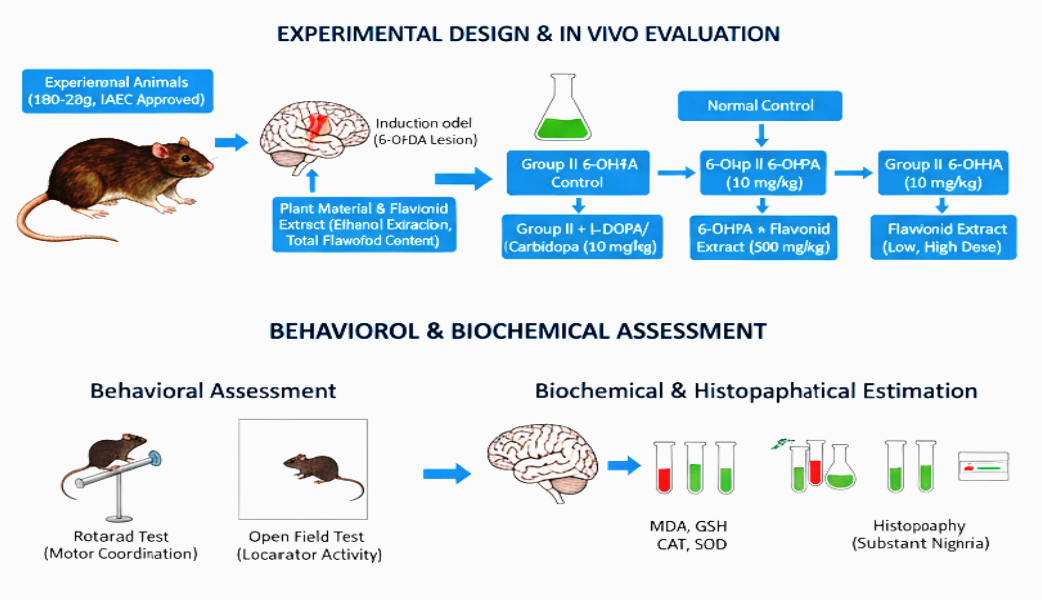


Figure 2: Experimental Design

RESULTS

Effect on Behavioral Parameters

Administration of 6-hydroxydopamine (6-OHDA) induced marked motor impairments in rats, evidenced by significant reductions in rotarod performance, decreased locomotor activity in the open-field test, and prolonged

catalepsy compared to the normal control group ($p < 0.001$). Treatment with plant-derived flavonoid extract for 21 days led to a dose-dependent improvement in motor coordination and spontaneous activity. Notably, the high-dose flavonoid group (100 mg/kg) exhibited motor performance comparable to the standard levodopa + carbidopa group, showing enhanced balance and reduced cataleptic behavior (Table 1, Figure 3).

Table 1. Effect of Plant-Derived Flavonoids on Behavioral Parameters in 6-OHDA-Induced Parkinson's Rats

Group	Rotarod Performance (sec)	Open Field Activity (line crossings/5 min)	Catalepsy Duration (sec)
Normal Control	120.4 ± 5.6	85.3 ± 4.2	8.2 ± 1.3
6-OHDA Control	45.6 ± 3.8***	32.5 ± 2.7***	42.7 ± 4.6***
Standard (Levodopa + Carbidopa, 10 mg/kg)	110.8 ± 4.5###	80.6 ± 3.9###	10.1 ± 1.5###
Flavonoid Extract (50 mg/kg)	89.2 ± 4.1##	65.8 ± 3.5##	18.3 ± 2.8##
Flavonoid Extract (100 mg/kg)	104.7 ± 4.3###	76.5 ± 3.8###	12.4 ± 2.0###

Values are expressed as mean ± SEM, n = 6;
***p < 0.001 vs. normal control; ##p < 0.01, ###p < 0.001 vs. 6-OHDA control.

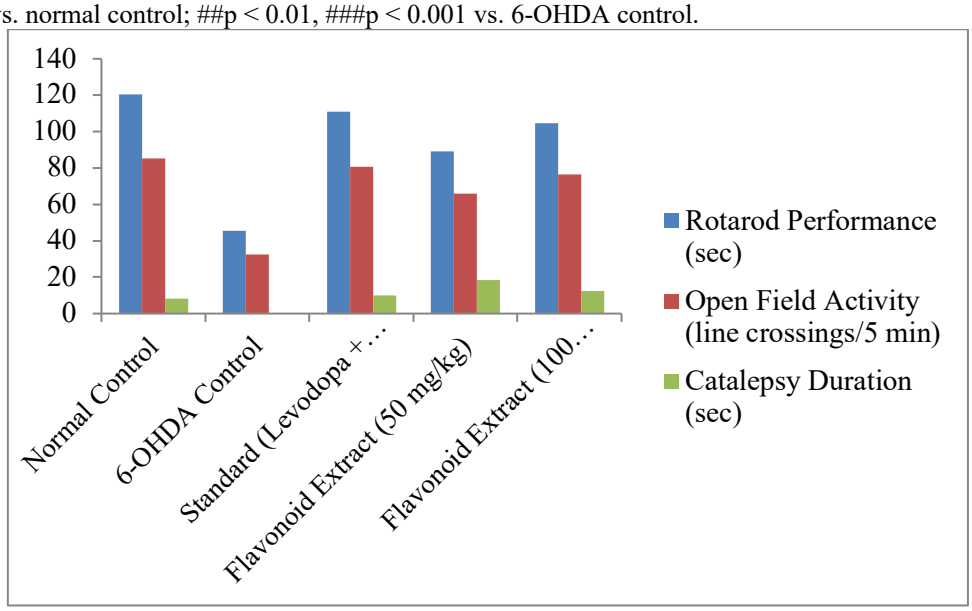


Figure 3: Graphical presentation of Plant-Derived Flavonoids on Behavioral Parameters in 6-OHDA-Induced Parkinson's Rats

Effect on Oxidative Stress Markers

Biochemical analysis revealed that 6-OHDA administration significantly increased malondialdehyde (MDA) levels, indicating elevated lipid peroxidation, while the activities of endogenous antioxidant enzymes - superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were markedly decreased ($p < 0.001$). Treatment with flavonoid extract significantly lowered MDA levels ($p < 0.01$) and restored antioxidant enzyme activity toward normal levels. The high-dose group demonstrated the greatest effect, reflecting the strong antioxidant potential of the flavonoids in mitigating 6-OHDA–induced oxidative stress (Table 2).

Table 2. Effect of Plant-Derived Flavonoids on Oxidative Stress Markers in Brain Tissue

Group	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Normal Control	1.45 ± 0.08	9.85 ± 0.35	6.42 ± 0.24	5.76 ± 0.22
6-OHDA Control	3.98 ± 0.15***	3.42 ± 0.28***	2.18 ± 0.19***	1.92 ± 0.18***
Standard (Levodopa + Carbidopa, 10 mg/kg)	1.68 ± 0.09###	8.72 ± 0.31###	6.05 ± 0.21###	5.31 ± 0.19###
Flavonoid Extract (50 mg/kg)	2.12 ± 0.11##	7.26 ± 0.29##	4.85 ± 0.23##	4.42 ± 0.17##
Flavonoid Extract (100 mg/kg)	1.79 ± 0.10###	8.24 ± 0.32###	5.76 ± 0.25###	5.08 ± 0.21###

Values are expressed as mean ± SEM, n = 6;
***p < 0.001 vs. normal control; ##p < 0.01, ###p < 0.001 vs. 6-OHDA control.

Histopathological Findings

Histological examination of the substantia nigra and striatum in the 6-OHDA control group showed extensive neuronal degeneration, vacuolation, and loss of dopaminergic neurons. In contrast, flavonoid-treated groups exhibited preserved neuronal architecture with minimal cellular damage and reduced inflammatory infiltration. The high-dose flavonoid group displayed nearly normal histology, comparable to the standard drug-treated group.

Statistical Analysis

Statistical analysis using one-way ANOVA followed by Tukey's post hoc test confirmed that the differences between treatment and disease control groups were statistically significant ($p < 0.05$). Overall, the findings demonstrate that plant-derived flavonoid extract exerts a dose-dependent neuroprotective effect against 6-OHDA induced neuronal damage.

DISCUSSION

As the current research shows, plant-based flavonoids have a huge neuroprotective effect on the 6-hydroxydopamine (6-OHDA)-induced experimental model of the Parkinson's disease (PD). The popular 6-OHDA model has been known to simulate dopaminergic neuronal death and oxidative damage of PD, thus is a good model to test possible neuroprotective treatments [18].

On the behavioral measures, 6-OHDA treatment resulted in severe motor impairment with low rotarod scores, reduced locomotor activity scores during the open-field test, and increased catalepsy. The study under consideration clearly demonstrated a dose-dependent and significant improvement of behavioral abnormalities that are characteristic of Parkinson. This positive outcome can probably be explained by the fact that the flavonoids have a neuroprotective effect, and they preserve the dopaminergic neurons and also sustain the levels of dopamine in the striatum [19].

One cause of the death of dopaminergic neurons in Parkinson disease is oxidative stress. The 6-hydroxydopamine (6-OHDA) administration in the current study was observed to have a significant effect in raising the level of lipid peroxidation, represented by a heightened level of malondialdehyde (MDA), and reducing the activity of endogenous antioxidants like superoxide dismutase(SOD), catalase(CAT), and glutathione (GSH). These alterations prove the induction of oxidative stress in the experimental PD model [20]. The treatment with flavonoid significantly improved lipid peroxidation and recovered the lost antioxidant enzyme activities to normal levels, indicating their capability of neutralizing free radicals and increasing the defense of cell to oxidative damage. The recovery of SOD and CAT functions also indicate an enhancement of mitochondrial functions since the two enzymes play important roles in ensuring redox homeostasis in cells.

The behavioral and biochemical results were supported by histopathological analysis. The sections of brains of 6-OHDA-treated rats exhibited extensive neuronal loss, vacuolization, and glial activation of the substantia nigra and striatum. On the other hand, the animals treated with flavonoids had maintained neuronal morphology and less neuroinflammation, especially when using the high dose (100 mg/kg) [21]. These results suggest that flavonoids are effective in the

protection of dopaminergic neurons but probably due to their antioxidant and anti-inflammatory properties.

The neuroprotective properties in the given study are in line with prior studies which contradict that flavonoids were capable of regulating signal transduction pathways affecting oxidative stress, inflammatory and neuronal survival such as Nrf2, MAPK, and NF-KB. The flavonoids promote the expression of cytoprotective enzymes through the activation of the Nrf2 pathway and the release of pro-inflammatory cytokines through the inhibition of NF- 0B, thus preventing neuronal damage [22].

In general, the findings indicate that flavonoid of plant materials ameliorate behavioral effects, inhibit oxidative stress, and maintain neuronal intactness in PD models. It seems that their neuroprotective effects resemble free radical scavenging, anti-inflammatory effects, and mitochondrial stabilization. The results of this study demonstrate the possibility of flavonoid-containing plant extracts as an adjunct or alternative treatment to the prevention and treatment of PD. Prospective research on molecular studies and clinical trials should be done to explain these mechanisms more and how they can be applicable in humans. [23].

CONCLUSION

The present study demonstrates that plant-derived flavonoids possess significant neuroprotective potential against 6-hydroxydopamine-induced Parkinsonism in rats. Administration of flavonoid-rich extracts markedly improved behavioral performance, enhanced the activity of antioxidant enzymes, and reduced lipid peroxidation, thereby mitigating neuronal damage caused by oxidative stress. Histopathological analyses further confirmed the preservation of dopaminergic neurons and a reduction in neuroinflammation in the flavonoid-treated groups.

These findings suggest that the neuroprotective effects of flavonoids are largely mediated through their potent antioxidant, anti-inflammatory, and free radical-scavenging activities. The study provides strong experimental evidence supporting the therapeutic potential of flavonoid-rich plant extracts for the prevention and management of Parkinson's disease.

Future research should focus on isolating the active constituents, elucidating the underlying molecular mechanisms, and conducting clinical trials to validate flavonoids as effective neuroprotective agents for neurodegenerative disorders.

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