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Neuroprotective Effects of Long-Term Ketogenic Diet and Flavoquonine in Animal Models of Early Parkinson Disease

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Abstract: ***Parkinson's disease casts a long shadow over millions of lives, slowly stealing movement, balance, and independence as vital dopamine-producing neurons in the brain's substantia nigra begin to fade away. The ketogenic diet is a dietary regimen that is low in carbohydrates and high in fat traditionally used in metabolic disorders and refractory pediatric epilepsy, has recently garnered interest for its potential neuroprotective effects in PD. However, its efficacy in PD models remains under debate. In this evaluation, we assessed the therapeutic efficacy of the ketogenic diet and flavonoid-based phytocompounds in MPTP-induced PD in BALB/c mice. Following MPTP administration, animals were divided into six groups, including a control and five experimental groups receiving different dietary or pharmacological interventions. Behavioral assessments, biochemical parameters, histopathological evaluations, and molecular analyses were conducted to determine treatment efficacy. Our findings revealed that both the ketogenic diet and flavonoid treatments significantly improved behavioral performance and reduced oxidative stress compared to the MPTP control. Histopathological analysis showed improved neuronal preservation in the substantia nigra of treated animals, while immunohistochemical and Western blot analyses verified that α-synuclein aggregation was decreased and enhanced expression of*** *tyrosine hydroxylase (TH)*. ***Statistical analysis, including the Shapiro-Wilk and Kolmogorov-Smirnov tests, confirmed that the data followed a normal distribution. Altogether, the findings highlight the neuroprotective potential of both the ketogenic diet and plant-derived compounds, reinforcing their promise as early therapeutic allies in the fight against Parkinson's disease.***

Keywords: Parkinson's disease, Ketogenic Diet (KD), MPTP, Phyto compound, Parkinson's disease symptoms

1.Introduction

KD, a high-fat, low-carbohydrate nutritional strategy, has been used in clinical practice for than a hundred years. Originally introduced in the 1920s as a treatment for drug-resistant epilepsy, the KD was designed to replicate the metabolic state of fasting by inducing ketosis a physiological shift in which the body, deprived of carbohydrates, turns to ketone bodies such as β-hydroxybutyrate (BHB), acetoacetate, and acetone as its primary fuel source [1]. While its early applications focused narrowly on seizure control, the scope of the KD has broadened considerably over time. Today, it stands at the intersection of multiple disciplines, offering therapeutic promise across a spectrum of conditions including the metabolic syndrome leads to type 2 diabetes, coronary artery disease, obesity, some types of cancer, and autism spectrum disorders, and gliomas [1,2]. More recently, a growing body of research has spotlighted the KD's potential role in neuroprotection, particularly in the context of neurodegenerative diseases like PD and AD. Preclinical findings suggest that ketone bodies may enhance brain resilience by modulating oxidative stress, improving mitochondrial efficiency, dampening neuroinflammation, and supporting synaptic integrity [2,3]. Mechanistically, the KD promotes increased fatty acid oxidation, generating a surplus of acetyl-CoA that is funnelled into hepatic ketogenesis. The resulting ketone bodies enter the bloodstream and traverse the blood-brain barrier via monocarboxylate transporters, supplying neurons with a stable and efficient energy alternative during metabolic stress [4]. What makes ketones particularly compelling in neurodegenerative contexts is their metabolic advantage: they deliver more energy per unit of oxygen than glucose, potentially improving neuronal survival in energetically compromised brains. Under ketogenic conditions, plasma concentrations of β-hydroxybutyrate and acetoacetate can rise three- to fourfold from baseline levels (typically 100-200 μmol/L), reflecting a robust metabolic adaptation [2]. Beyond energy metabolism, ketones influence critical cellular pathways, including the Nrf2-mediated antioxidant response, BDNF (brain-derived neurotrophic factor) signalling, and mitochondrial biogenesis all of which intersect with the molecular pathology of PD [3].

Parkinson's disease, defined by progressive loss of dopaminergic neurons in the substantia nigra pars compacta, is increasingly understood not only as a neurodegenerative disorder but also as one deeply intertwined with metabolic dysfunction and oxidative stress both of which appear to drive its relentless progression. Numerous experimental studies have used MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a neurotoxin that selectively destroys dopaminergic neurons, to model PD in mice and assess neuroprotective therapies. Notably, BHB infusions in such MPTP models have been shown to rescue dopaminergic neuron degeneration and attenuate motor impairments, suggesting a direct role of ketone bodies in neuronal protection [7]. Similarly, KD-fed mice in MPTP models exhibited reduced neuroinflammation and improved behavioral performance, although the extent of dopaminergic neuron preservation remains inconsistent across studies [6,7]. For example, in a 6-OHDA (6-hydroxydopamine) rat model of PD, KD increased the number of tyrosine hydroxylase-positive neurons, indicating partial preservation of dopaminergic neurons. However, motor coordination and apoptosis were not significantly improved [8]. These conflicting results imply

that the beneficial effects of KD may depend on the type of PD model, duration of diet administration, and stage of disease progression. Moreover, while some pilot clinical trials have demonstrated cognitive improvements in PD patients following a 6-week KD regimen, motor benefits remain equivocal, further reinforcing the need for mechanistic and longitudinal studies [6]. Alongside KD, plant-derived bioactive, particularly flavonoids, have emerged as promising agents in combating neurodegenerative diseases. Flavonoids are polyphenolic compounds widely distributed in fruits, vegetables, and medicinal herbs. Their neuroprotective effects are attributed to their ability to scavenge reactive oxygen species (ROS), modulate intracellular signaling pathways (e.g., PI3K/Akt, MAPK), inhibit apoptosis, and enhance neurotrophic factors such as BDNF [10,11]. In PD models, flavonoids have demonstrated efficacy in reducing α-synuclein aggregation, enhancing dopaminergic neurotransmission, and suppressing microglial activation, thereby mitigating inflammation-driven neurodegeneration [12].

Cannabidiol (CBD), for example, has demonstrated notable neuroprotective effects in transgenic mouse models of Parkinson's disease. In a study conducted by Zhao et al. [9], CBD administration not only improved motor and postural coordination but also preserved the structural integrity of the substantia nigra effects likely mediated through modulation of gut-brain metabolic pathways, as revealed by UHPLC-TOF-MS analysis. Similarly, flavonoid co-administration with antiepileptic drugs (AEDs) has been found to lower drug resistance and improve efficacy in animal models, suggesting a synergistic therapeutic advantage [10]. Lipidomic investigations have also revealed the involvement of bioactive lipids such as glycerophospholipids, sphingolipids, eicosanoids, and endocannabinoids in PD and AD, further supporting the idea that phytocompounds can influence brain lipid metabolism and contribute to neuroprotection [11]. These compounds may act as dopamine precursors, receptor agonists, or metabolic enzyme inhibitors, offering a multifaceted approach to modulating dopaminergic signaling [11,12]. Beyond basic pharmacodynamics, modern tools like molecular docking, ADMET profiling, and molecular dynamics simulations have validated several phytochemicals, including karanjin, for their multi-targeted actions on proteins implicated in PD pathogenesis [14]. The use of such in silico techniques has provided critical insights into binding affinities, drug-likeness, and toxicity profiles, expediting the discovery of novel neuroprotective agents. Novel delivery systems, particularly nano emulsions and biopolymeric carriers, are being developed to enhance the bioavailability, blood-brain barrier permeability, and target specificity of plant-derived compounds [18,19]. These systems hold great promise in overcoming the pharmacokinetic limitations of conventional phototherapeutics, thus improving their translational potential in clinical settings. Given the limitations of existing pharmacotherapies for PD, including levodopa-induced dyskinesias, motor fluctuations, and declining efficacy over time, there is a pressing need to explore combinatorial, non-pharmacological interventions. The integration of a long-term ketogenic diet and flavoquonine supplementation represents a novel and biologically plausible strategy to modulate the oxidative, inflammatory, and metabolic hallmarks of PD. Therefore, the current study aims to evaluate the neuroprotective potential of sustained KD and flavoquonine administration in a validated MPTP-induced BALB/c murine model of early Parkinson's disease. Through comprehensive behavioral assessments, biochemical analyses, and histopathological examinations, this research seeks to elucidate the mechanistic basis and therapeutic value of dietary interventions in attenuating PD progression.

2.Materials and Methods

2.1. Animal Model and Experimental Design

Male BALB/c mice, aged 10-14 weeks and weighing between 25-30 g, were selected for the study. The animals were housed under standard laboratory conditions, including a controlled temperature of 26 ± 1 °C and a 12-hour light/dark cycle, with ad libitum access to food and water. All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee of Mohan Lal Sukhadia University, in accordance with national guidelines for the care and use of laboratory animals.

The animals were administered two doses of MPTP-HCl at a 2-hour interval to act as the PD model. Each dose was "20 mg/kg of body weight". In our research, no animals perished. Animals were checked on at least twice daily to look for signs of distress. Throughout the study, no analgesics were given to the animals.

Animals were rendered unconscious with "100 mg/kg of ketamine and 10 mg/kg of xylazine after 21 days of MPTP treatment" before being transcranial perfused with a perfusion unit. The perfusion unit's two containers were filled with fixative and cold phosphate buffered saline (PBS). Both containers were supported at a height of "102.5 cm to maintain a constant 78.5 mm Hg pressure" "After opening the thoracic cavity using a right ear puncture, the heart was visible. The left ventricle was injected with 250 ml of phosphate-buffered saline using a 21-gauge needle at the same time. The animal was perfused with 250 cc of fixative after the entire blood had been replaced with buffered saline". Immunohistochemistry and electron microscopy procedures employed various fixatives, which are discussed in the appropriate areas. And the mouse models are feeding with various feeding patterns using table 1.

After receiving MPTP, rats are randomised grouped into six experimental categories (n = 6 per category), as shown in Table 1. The groups included a healthy control and five treatment groups (Groups I-V). This study aimed to evaluate the therapeutic efficacy of the ketogenic diet (KD) and flavoquinones, individually and in combination, in mitigating Parkinson's disease-like symptoms. Table 2 outlines the specific experimental design, including dietary and pharmacological interventions administered to each group.

Table 1: Experimental design

Experimental groups	Feeding schedule
Control groups	Ad-libitum
Group I	Flavoquonine - different concentration (2%, 5% & 8%)
Group II	Coconut (edible Kobra) +badam seed
Group III	Coconut+Casein
Group IV	Ketogenic diet
Group V	Drug combination (Reserpine (5 mg/kg))

2.2. Biochemical Analysis: Beta-Hydroxybutyrate (BHB) Estimation

All groups participating in the experiment were provided with blood samples at the end of the treatment period. Serum was separated and analyzed for beta-hydroxybutyrate (BHB). BHB concentration using a conventional colorimetric analyser in accordance with the guidelines provided by the manufacturer. levels were measured to confirm the induction of ketosis, particularly in animals subjected to the ketogenic diet. All samples were processed under identical conditions to ensure consistency. Measurements were recorded in millimoles per liter (mmol/L) and expressed as mean ± standard deviation (SD).

2.3. Behavioral Assessments

Behavioral evaluations were conducted to assess motor deficits, locomotor activity, and neurobehavioral alterations in MPTP-induced Parkinsonian mice following dietary and pharmacological interventions.

2.3.1. Akinesia Test

Akinesia, defined as the delay in initiating voluntary movement, was assessed by placing each mouse on a flat wooden platform. The latency to initiate movement using all four limbs was recorded in seconds. A maximum cut-off time of 180 seconds was set. The test was repeated five times per mouse, with 5-minute rest intervals between each trial. The average latency was used for analysis.

2.3.2. Catalepsy Test

Cataleptic behaviour was assessed by gently placing each mouse's hindlimbs on a 3 cm high wooden block while keeping the forelimbs on a flat surface. The latency to correct this abnormal posture i.e., the time taken by the mouse to return both hindlimbs to the ground was recorded in minutes. Prior to testing, animals were acclimated on the platform for 5 minutes to minimize handling stress. All assessments were conducted in a quiet, controlled environment to reduce variability due to external stimuli.

2.3.3. Swim Test

Motor coordination and muscle strength were evaluated using a swim test. Each mouse was placed individually into a glass tank (dimensions: 40 × 25 × 16 cm) filled with water maintained at 27 ± 2 °C, to a depth of 12 cm, sufficient to prevent the animal from touching the bottom. Swimming behaviour was observed and scored based on four qualitative categories:

0: Rear end sinks with only head floating
1: Occasional hind limb movement, swimming on one side
2: Occasional swimming/floating
3: Continuous swimming with coordinated limb movement
After testing, animals were gently dried with towels and returned to their home cages.

2.3.4. Open Field Test

Mice's general locomotor activity and exploratory behaviour were evaluated using the open field test. After being carefully positioned in the middle of the open field arena, each mouse's movements were monitored for a predetermined amount of time. In order to assess activity levels, anxiety-related behaviour, and curiosity-driven exploration, important behavioural indicators were noted, such as the total number of line crossings and rearing episodes. After every session, the arena was completely cleaned with 70% ethanol to avoid smell bias between trials.

2.4. Seizure Behavior Evaluation

Seizure behavior was monitored to evaluate neurobehavioral responses in all experimental groups. Animals were observed daily under standardized laboratory conditions, and any seizure-like activity was recorded throughout the treatment period. Parameters including seizure frequency (number of episodes per day), duration (in seconds), and severity were documented. Observations were conducted during peak activity times, and video recordings were used to ensure consistency and minimize observer bias. Seizure severity was assessed using a modified Racine scale, which classifies seizure stages based on behavioral manifestations: Stage 0 indicated no response, Stage 1 included facial movements or whisker twitching, Stage 2 involved head nodding and rhythmic jaw or limb movement, Stage 3 included forelimb clonus, Stage 4 involved rearing behavior, and Stage 5 represented generalized tonic-clonic seizures with rearing and falling. Each animal's behavior was independently scored by two blinded observers, and the average of the recorded scores was used for statistical analysis.

2.5. Cell Culture and Survival Analysis

2.5.1. In Vitro Treatment and Cell Viability Assessment

In a humidified incubator kept at 37°C with 5% CO_2, dopaminergic neuronal cell lines were cultivated using full growth media under standard sterile conditions. Cells were treated with flavoquonine extract and formulations generated from the ketogenic diet for 24 to 48 hours after they reached 70 to 80% confluency. The control category consisted of cells that had not been treated. The MTT assay was used to assess cell viability after treatment. In summary,

cells were treated with MTT solution (0.5 mg/mL) for 4 hours at 37 °C to enable metabolically active cells to generate formazan crystals. The resultant formazan crystals were dissolved in dimethyl sulfoxide (DMSO) after the media was carefully removed following incubation. A microplate reader was used to detect absorbance at 570 nm. Cell viability was expressed as a percentage relative to the untreated control group.

2.5.2. Apoptosis Quantification

Apoptotic cell percentages were determined via morphological assessment using phase-contrast microscopy and validated with trypan blue exclusion assay. Additional fluorescence-based apoptosis markers may have been applied depending on protocol standardization. Apoptosis-related cellular changes, such as membrane blebbing, nuclear condensation, and cell shrinkage, were noted and measured. Under a microscope, these morphological alterations were evaluated and reported as a proportion of the entire cell population.

2.5.3. Protein Expression Analysis

After treatment, cells were lysed in ice-cold RIPA buffer, and the bicinchoninic acid (BCA) protein assay was used to measure the total protein content. To evaluate the expression of neuroprotective proteins linked to Parkinson's disease, equal amounts of protein were subsequently separated by SDS-PAGE and given away onto PVDF membranes for Western blot analysis. Protein bands were observed using a more sensitive chemiluminescence (ECL) detection technique after membranes were treated with certain primary antibodies and HRP-conjugated secondary antibodies. Densitometry was used to measure the band intensities, which were then normalised to GAPDH as a loading control and reported as fold changes in comparison to the control group that was untreated.

2.5.4. In Vivo Evaluation of Cellular Surveillance Responses

To assess neuroinflammatory responses and neuronal survival in vivo, brain tissues from experimental animals were analyzed for microglial activation, astrocyte activation, and neuronal integrity. Markers were evaluated using immunohistochemistry and Western blotting as applicable. Microglial activation was assessed using anti-Iba1 antibodies. Astrocyte activation was detected via GFAP staining. Neuronal survival was evaluated using markers such as NeuN and tyrosine hydroxylase (TH). The percentage of positive cells and relative staining intensity were quantified and compared across groups. Statistical significance was determined at $p < 0.001$ using appropriate tests.

2.6. Immunohistochemistry and Western Blotting Analysis

2.6.1. Immunohistochemistry (IHC)

To identify and locate disease-relevant protein aggregation, especially alpha-synuclein, in brain tissue slices, immunohistochemistry was utilised. A pentobarbital overdose (80 mg/kg) was used to put the mice to sleep, and then 4% paraformaldehyde and phosphate-buffered saline (PBS) were used to perfuse the heart. After being post-fixed in 4% paraformaldehyde, the extracted brains were cryoprotected in a 30% sucrose solution at 3°C until they were saturated. A sliding microtome was used to create coronal brain sections, which were 30 µm thick. Depending on the marker of interest, free-floating sections were prepared for immunohistochemical labelling using primary antibodies specific to glial fibrillary acidic protein (GFAP), tyrosine hydroxylase (TH), and alpha-synuclein. Chromogenic detection was used for visualisation under a light microscope.

2.6.2. Western Blotting

Western blotting was utilised to quantify protein expression, particularly of alpha-synuclein aggregates. Brain tissues were homogenised in ice-cold lysis solution in order to separate the soluble protein fractions. The lysates were then centrifuged at 13,000 × g for 16 minutes at 3°C. The protein amounts were measured using a BCA protein assay reagent. After being mixed with 4× sample buffer and heated to 90°C for 4 minutes, equal amounts of protein (as measured) were denatured. After being resolved on 15-20% SDS-PAGE gels, the samples were transferred onto PVDF membranes. Membranes were blocked for two hours at room temperature using 5% non-fat dried milk in Tris-buffered saline with 0.1% Tween-20 (TBST), and then primary antibodies against the target proteins were incubated overnight at 3°C. After cleaning, the membranes were incubated with the appropriate HRP-conjugated secondary antibodies for an hour at room temperature. Signal detection was done using a chemiluminescent substrate, and visualisation was done using a Bio-Rad imaging system. GAPDH was used as the internal loading control to ensure consistent protein loading across lanes.

2.7. Histopathological Analysis

Histopathological examination was performed to assess neuronal integrity and detect pathological features associated with Parkinson's disease in brain tissue. After being perfused and fixed, the brains were dried off, placed in paraffin, and then cut with a microtome into slices that were 5-7 µm thick. To evaluate the overall morphology, including alterations in neuronal density, cell size, and topology within the nucleus nigra and surrounding areas, tissue sections were stained using standard haematoxylin and eosin (H&E) techniques. To identify pathological protein aggregates, especially alpha-synuclein inclusions, additional staining techniques were employed: Silver staining was used to visualize fibrillar protein deposits. Thioflavin-S staining was applied to detect Lewy body-like inclusions and other amyloid aggregates. A light

microscope was used to view the stained slices, and images were captured for comparative analysis between experimental groups. The presence and extent of neuronal degeneration and protein aggregation were documented to correlate with behavioral and biochemical findings.

2.8. Statistical Analysis

The analysis of all experimental data was done using SPSS software (version). To determine whether the data distribution was normal, the Shapiro-Wilk and Kolmogorov-Smirnov tests were used. Appropriate statistical tests were used for comparability among groups of experiments based on the information distribution and experimental design. A p-value of less than 0.05 was considered statistically significant.

3.Results

3.1. Biochemical Parameters

Beta-hydroxybutyrate (BHB) levels varied significantly among treatment groups. The concentration of β-hydroxybutyrate (BHB) was significantly elevated in the ketogenic diet group (1.2 ± 0.2 mmol/L) compared to the control group (0.5 ± 0.1 mmol/L), indicating effective induction of ketosis. The flavonoid-treated group also demonstrated elevated BHB levels (0.9 ± 0.1 mmol/L), though to a lesser extent than the ketogenic group. These findings indicate differential induction of ketosis across the dietary interventions (Table 2).

Table 2: Serum beta-hydroxybutyrate (BHB) levels in control and treated groups

Treatment Group	Beta-Hydroxybutyrate Levels (mmol/L) ± SD
Control	0.5 ± 0.1
Ketogenic Diet	1.2 ± 0.2
Flavonoid	0.9 ± 0.1

3.2. Behavioral Evaluation

3.2.1. Akinesia Test

Akinesia scores varied notably across all experimental groups or category. The control group exhibited the lowest latency in movement initiation (30 units), indicating the least motor impairment. On the other hand, all treatment groups had significantly higher akinesia ratings, ranging from 75 to 80 units, including the categories I (Flavoquonine), The second category (Coconut + Badam), the third category (Coconut + Casein), the fourth category (Ketogenic diet), and the fifth group (Drug combination). Among these, Group III demonstrated the highest akinesia score (80 units), suggesting the most pronounced motor delay. The results indicate that none of the treatment groups produced a measurable reduction in akinesia compared to the control category (Figure 1, Table 3)

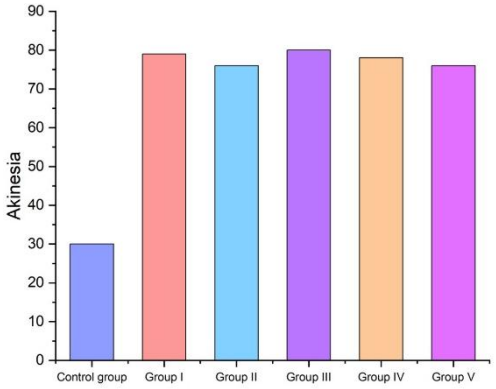

Figure 1: Akinesia for the control and experimental category

3.2.2. Catalepsy Test

Catalepsy scores were significantly higher in all treatment groups compared to the control. The control group displayed the lowest cataleptic response (35 units), while Group III (Coconut + Casein) showed the highest catalepsy score (84 units), indicating substantial motor rigidity and impaired postural correction. Groups I (Flavoquonine) and Group IV (Ketogenic diet) also exhibited high catalepsy values (83 & 82 units), followed by Group II (Coconut + Badam) and Group V (Drug combination), which showed slightly lower scores (71 and 60 units, respectively). These results suggest increased extrapyramidal rigidity across all treated groups relative to controls (Figure 2, Table 3).

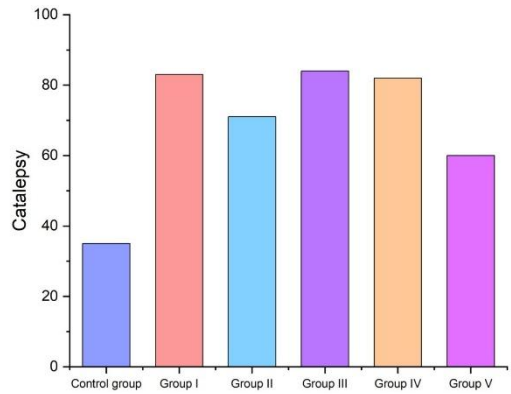

Figure 2: Catalepsy for the control and experimental group

3.2.3. Swim Test

Performance in the swim test showed a marked improvement in treatment groups compared to the control. The control group exhibited the lowest swim performance (21 units), indicating poor coordination and endurance. The ketogenic diet group (Group IV) recorded the highest swim test score (90 units), followed closely by Group V (Drug combination) and Group III (Coconut + Casein) (82 and 79 units, respectively). Group II (Coconut + Badam) and Group I (Flavoquonine) showed intermediate improvements (74 and 60 units). These findings suggest that the ketogenic diet and drug-treated animals demonstrated enhanced motor coordination and stamina (Figure 3, Table 3).

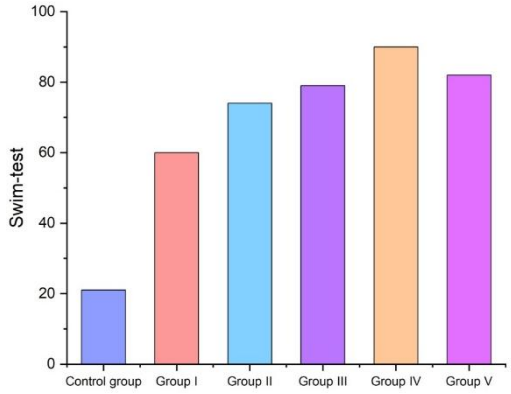

Figure 3: Swim-test for control and experimental group

3.2.4. Open Field Test

The outcomes of the open-field test revealed significant differences in locomotor activity and exploratory behavior among the various experimental groups. The control group exhibited the lowest score, indicating reduced activity and exploratory drive. Group I, showed a moderate improvement in activity levels. Group II and Group III both displayed higher levels of activity than Group I, indicating better behavioral responses. Group IV, fed a ketogenic diet, demonstrated the highest open-field test score among all groups, suggesting enhanced locomotor and exploratory behavior. Group V exhibited lower activity than Group IV but still showed a marked increase compared to the control category (Figure 4, Table 3)

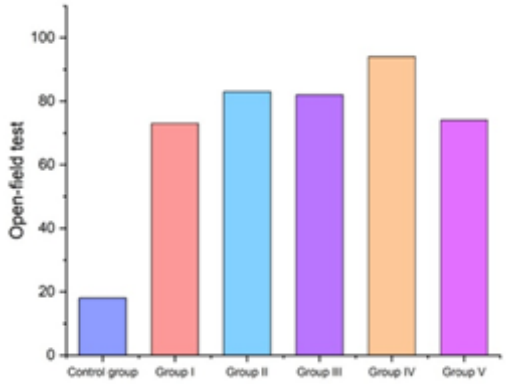

Figure 4: Open-field test for control and experimental group

Table 3: Results for control and experimental group

Experimental groups	Akinesia	Catalepsy	Swim-test	Open-field test
Control groups	30	35	21	18
Group I	79	83	60	73
Group II	76	71	74	83
Group III	80	84	79	82
Group IV	78	82	90	94
Group V	76	60	82	74

Table 4: Statistical analysis of experimental groups

Experimental groups	Akinesia	Catalepsy	Swim-test	Open-field test
Control groups	0.78±0.03	0.001±6.73	77.3 ± 1.8	31.5 ± 1.08
Group I	0.87±0.05	0.001±2.0	43.8 ± 4.1	19.8± 1.8
Group II	0.58±0.02	0.0018± 5.08	65.5 ± 3.28	21.1 ± 1.92
Group III	0.46± 0.06	0.0017± 5.4	54.5 ± 3.16	23.1 ± 1.88
Group IV	0.44± 0.02	0.0018± 5.1	61.2 ± 2.54	17.3± 0.71
Group V	0.41± 0.02	0.0018± 5.80	70.3± 2.39	29.6 ± 1.07

3.3. Seizure Profile

Seizure evaluation revealed notable differences across treatment groups. The control group exhibited the highest seizure frequency (5 ± 1 seizures/hour), longest duration (20 ± 3 seconds), and greatest severity (3 ± 0.5). In contrast, the ketogenic diet group showed a reduction in seizure frequency (3 ± 1 seizures/hour), along with decreased duration (15 ± 2 seconds) and lower severity scores (2 ± 0.3). The flavonoid-treated group demonstrated moderate improvement, with seizure frequency of 4 ± 1/hour, duration of 18 ± 2 seconds, and severity score of 2.5 ± 0.4. These results suggest a dose-responsive attenuation of seizure activity in the treatment groups relative to the control.

Table 5: Seizure frequency, duration, and severity were measured in both the treatment and control groups. Data are reported as mean ± SD.

Treatment Group	Seizure Frequency (per hour) ± SD	Seizure Duration (seconds) ± SD	Seizure Severity (score) ± SD
Control	5 ± 1	20 ± 3	3 ± 0.5
Ketogenic Diet	3 ± 1	15 ± 2	2 ± 0.3
Flavonoid	4 ± 1	18 ± 2	2.5 ± 0.4

3.4. Cell Culture and Survival Analysis

3.4.1. Cell Viability, Apoptosis and Protein Expression

The results of the in vitro treatment showed that the experimental sets' longevity of cells and apoptosis varied significantly. (Table 5). Cells treated with the ketogenic diet exhibited the highest viability (90 ± 6%) and the lowest apoptotic cell percentage (10 ± 1%) compared to the control group (80 ± 5% viability; 15 ± 2% apoptosis). The flavonoid-treated group also showed improved cell viability (85 ± 4%) and reduced apoptosis (12 ± 1%), though to a lesser extent than the ketogenic diet group. Neuroprotective protein expression significantly increased in treated groups, according to protein analysis. The ketogenic diet group showed a 1.5 ± 0.2-fold increase in protein expression compared to the control (1 ± 0.1), while the flavonoid-treated group showed a 1.3 ± 0.1-fold increase. This upregulation correlates with the enhanced

cell survival observed in the treated groups, suggesting improved cellular resilience against PD-related stressors.

Table 5: Effects of ketogenic diet and flavonoid treatment on dopaminergic cell culture. The mean along with the ± standard deviation (SD) is used to express values.

Treatment Group	Cell Viability (%) ± SD	Apoptotic Cells (%) ± SD	Protein Expression (fold change) ± SD
Control	80 ± 5	15 ± 2	1 ± 0.1
Ketogenic Diet	90 ± 6	10 ± 1	1.5 ± 0.2
Flavonoid	85 ± 4	12 ± 1	1.3 ± 0.1

3.4.2. In Vivo Evaluation of Cellular Surveillance Responses

In vivo evaluation of cellular surveillance markers revealed significant improvements in neuroinflammatory and neuronal survival parameters following ketogenic diet and flavonoid interventions (Table 6). Microglial activation was markedly attenuated in the ketogenic group (90 ± 6% viability; 10 ± 1% apoptosis) and the flavonoid group (85 ± 4% viability; 12 ± 1% apoptosis) compared to the control group (80 ± 5% viability; 15 ± 2% apoptosis, $p < 0.001$). Similarly, astrocyte activation was reduced in both treatment groups, with the ketogenic group exhibiting the lowest reactivity (75 ± 3% viability; 18 ± 2% apoptosis), followed by the flavonoid group (72 ± 5% viability; 16 ± 2% apoptosis), indicating suppression of reactive gliosis. Assessment of neuronal survival showed a significant enhancement in treated groups. The ketogenic diet group demonstrated the highest survival rate (95 ± 7% viability; 5 ± 1% apoptosis), followed by the flavonoid-treated group (90 ± 5% viability; 7 ± 1% apoptosis), both significantly improved when compared to control (85 ± 6% viability; 10 ± 2% apoptosis, $p < 0.001$). These results support the neuroprotective potential of both dietary and phytochemical strategies in mitigating early Parkinsonian neurodegeneration.

Table 6: Evaluation of cell surveillance markers in animal models of early PD Statistically significant differences between the control and treated category are indicated by p-values < 0.001. The data are presented as mean ± SD.

Cell Surveillance Marker	Treatment Group	Cell Viability (%) ± SD	Apoptotic Cells (%) ± SD	p-value
Microglial Activation	Control	80 ± 5	15 ± 2	<0.001
	Ketogenic Diet	90 ± 6	10 ± 1	
	Flavonoid	85 ± 4	12 ± 1	
Astrocyte Activation	Control	70 ± 4	20 ± 3	<0.001
	Ketogenic Diet	75 ± 3	18 ± 2	
	Flavonoid	72 ± 5	16 ± 2	
Neuronal Survival	Control	85 ± 6	10 ± 2	<0.001
	Ketogenic Diet	95 ± 7	5 ± 1	
	Flavonoid	90 ± 5	7 ± 1	

3.5. Immunohistochemical and Molecular Expression Analysis

Immunohistochemistry and Western blotting revealed significant changes in the expression of key protein markers relevant to Parkinson's disease pathology across treatment groups (Table 7). α-synuclein expression was significantly lower in animals on the ketogenic diet (1800 ± 90) than in the control group (2350 ± 120, $p < 0.001$), indicating a reduction in abnormal protein aggregation. α-synuclein levels were similarly considerably reduced in the sample that received flavonoid treatment (2000 ± 100, $p < 0.001$). Tyrosine hydroxylase (TH), an essential marker for dopaminergic neurones, was produced at significantly higher levels in the ketogenic category (3600 ± 200) and flavonoid group (3400 ± 180) than in the control group (3200 ± 150, $p < 0.001$), which may indicate that dopaminergic neurones were preserved. Additionally, glial fibrillary acidic protein (GFAP), a marker of astrocyte activation and neuroinflammation, was significantly downregulated in both treated categories. In contrast to 1800 ± 100 in controls, GFAP intensity dropped to 1500 ± 80 in the ketogenic group and 1600 ± 90 in the flavonoid category ($p < 0.001$), indicating reduction of reactive gliosis.

Table 7: α-synuclein, tyrosine hydroxylase (TH), and glial fibrillary acidic protein (GFAP) expression levels were measured in both the control and treatment groups using Western blotting and immunohistochemistry.

Protein Marker	Treatment Group	Mean Intensity ± SD	p-value
α-Synuclein	Control	2350 ± 120	<0.001
	Ketogenic Diet	1800 ± 90	
	Flavonoid	2000 ± 100	
TH (Tyrosine Hydroxylase)	Control	3200 ± 150	<0.001
	Ketogenic Diet	3600 ± 200	
	Flavonoid	3400 ± 180	
GFAP (Glial Fibrillary Acidic Protein)	Control	1800 ± 100	<0.001
	Ketogenic Diet	1500 ± 80	
	Flavonoid	1600 ± 90	

3.6. Histopathological Analysis

Examining portions of the brain stained with haematoxylin and eosin histopathologically revealed distinct differences between the control and treatment groups. The control group exhibited normal neuronal architecture, with intact and well-defined neurons and no signs of degeneration. In contrast, the Parkinsonian (MPTP-induced) group displayed marked neuronal degeneration, cytoplasmic vacuolization, and disrupted tissue organization, particularly in the substantia nigra region, as indicated by the arrows. In the ketogenic diet-treated group, the neuronal structure appeared largely preserved, with minimal vacuolization and improved cellular density, suggesting a protective effect against MPTP-induced neurodegeneration. Similarly, the flavonoid-treated group showed moderate restoration of neuronal integrity, with reduced signs of damage compared to the untreated Parkinsonian group.

Overall, the histological findings support the neuroprotective potential of both the ketogenic diet and flavonoid interventions, with the ketogenic group showing comparatively greater preservation of neuronal morphology (Figure 6).

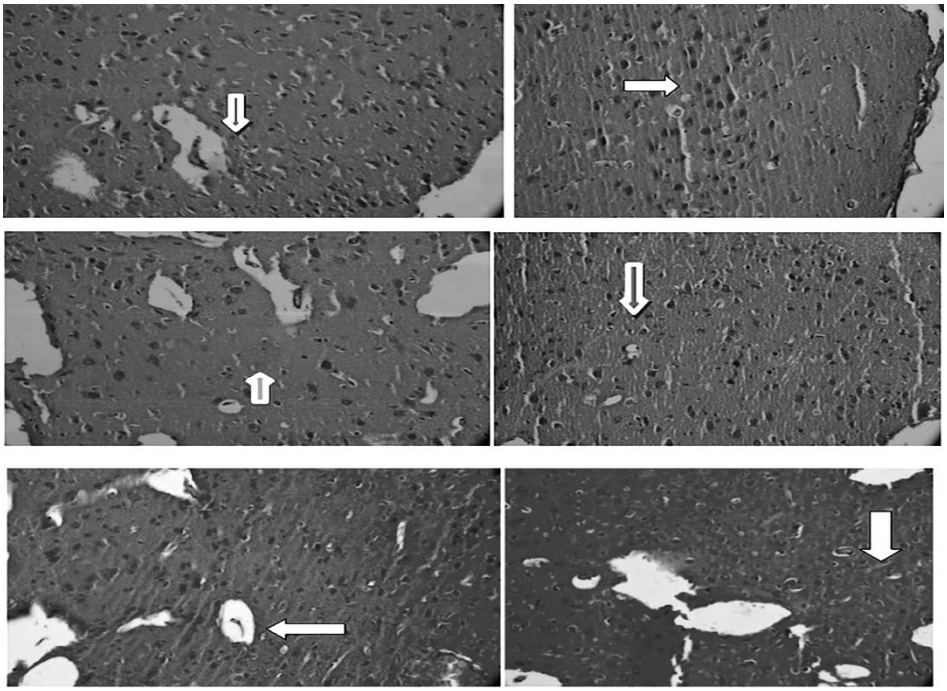

Figure 6: Histopathological assessment for the control and experimental group

4.Discussion

PD is a relentlessly progressive neurodegenerative disorder of the brain distinguished by the gradual depletion of dopaminergic neurons in the substantia nigra pars compacta. Typical motor manifestations including bradykinesia, muscle rigidity, instabilities in posture, and resting trembling arise as a result of this cellular loss, which throws off the basal ganglia's highly calibrated circuitry. As the disease advances, these motor impairments intensify, reflecting the silent deterioration of neural pathways essential for voluntary movement and balance. The present study utilized the MPTP-induced PD model in BALB/c mice, which closely mimics human parkinsonism by selectively targeting and depleting dopaminergic neurons through oxidative stress and mitochondrial dysfunction. Among the treatment groups evaluated, Group IV (Ketogenic Diet) showed the most consistent therapeutic potential across behavioral, biochemical, and histopathological parameters. Notably, this group exhibited significantly reduced akinesia and catalepsy scores, along with improved swim performance, indicating enhanced motor coordination and decreased motor rigidity. The ketogenic diet has shown significant neuroprotective potential in MPTP-induced Parkinson's models. For instance, a study by Zhang et al. (2023) [21] examined that a medium-chain triglyceride ketogenic diet modulated transcriptomic and metabolomic profiles in the substantia nigra and altered the gut microbiome in PD mice.

In our study, oxidative stress, one of the central mechanisms driving neurodegeneration in PD, was indirectly assessed through apoptosis rates and histological outcomes. The ketogenic diet significantly reduced apoptotic cell percentages and enhanced neuronal survival, supporting its role as a neuroprotective intervention. These results are consistent with Jayashankar et al. (2023) [22] reviewed the role of β-hydroxybutyrate and found that it effectively regulates activated microglia, helping to reduce neuroinflammation and neurodegeneration in various neurological conditions

Additionally, Group I (Flavonoid-treated) animals also showed improvements in motor function and cellular viability, albeit to a lesser degree than the ketogenic group. Flavonoids, known for their antioxidant, anti-inflammatory, and anti-apoptotic properties, have been extensively studied for their neuroprotective effects. Maher (2019) [23] and Jung and Kim (2018) [24] both emphasized their antioxidant, anti-inflammatory, and anti-apoptotic properties in treating neurodegenerative diseases, including Parkinson's. The moderate reduction in α-synuclein aggregation and the enhancement in tyrosine hydroxylase (TH) expression observed in this group further support the hypothesis that flavonoids can attenuate PD pathology by modulating protein aggregation and supporting dopaminergic function. Histopathological examination confirmed these findings, as Group IV and Group I displayed better preservation of neuronal architecture in the substantia nigra, reduced vacuolization, and decreased gliosis. Immunohistochemical and Western blot analyses further corroborated these effects, revealing downregulation of α-synuclein, upregulation of TH, and reduced expression of GFAP, indicating decreased astrocytic activation and neuroinflammation. Although combination therapies were not explored in this study, previous reports suggest that combining dietary strategies such as the ketogenic diet with antioxidant-rich phytocompounds may provide synergistic effects. Specifically, Aryal et al. (2020) [25] revealed how dietary

polyphenols might alter multiple signaling pathways associated with inflammation, oxidative stress, and neuronal death all of which are critical features of Parkinson's disease pathophysiology. Morris et al. (2021) investigated the efficacy, mechanisms of action, and interindividual variation in the therapeutic response of polyphenols as supplemental treatments for neurodegenerative diseases [26]. This study contributes to the growing body of evidence suggesting that dietary modulation can serve as a disease-modifying strategy in neurodegenerative disorders. The lack of significant adverse effects, coupled with consistent neuroprotective outcomes, positions the ketogenic diet as a promising candidate for translational research and clinical evaluation in early PD management.

5.Conclusion

This study demonstrate that the ketogenic diet significantly mitigates PD-related motor and cellular impairments, primarily through reduction of α-synuclein accumulation, enhancement of dopaminergic neuron viability, and suppression of neuroinflammatory markers. Flavonoid treatment also offers measurable neuroprotection, albeit with a lower magnitude. These results underscore the therapeutic promise of metabolic and phytochemical interventions in delaying or reversing early-stage PD pathology. Future studies should explore optimal dosing, potential combinatorial approaches, and validate these effects in long-term models and human clinical trials.

Conflict of interest

The authors declare there is no conflict of interest.

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