Original Article

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Pharmacology Section

Comprehensive Evaluation of Neuroprotective Effects of Levetiracetam in C57BL/6J Male Mice Model of Parkinsonism: Preclinical Insights

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ABSTRACT

Introduction: Levetiracetam (LEV), an anticonvulsant used for epilepsy, exhibits neuroprotective effects by stabilising neuronal activity and reducing excitotoxicity. Parkinsonism, caused by the degeneration of dopaminergic neurons, is primarily managed symptomatically. LEV may offer both symptom relief and neuroprotection, presenting a potential alternative therapy.

Aim: This study aimed to evaluate the neuroprotective effects of LEV in a mouse model of Parkinsonism. It investigated whether LEV, an antiepileptic drug, can mitigate the neurodegenerative processes and improve motor function and oxidative stress markers in a mouse model induced with Parkinsonism using 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP), a neurotoxin that selectively destroys dopaminergic neurons.

Materials and Methods: The present experimental study was conducted at the Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, SRIHER, Chennai, Tamil Nadu, India, from October 2023 to March 2024. Thirty-six male mice were divided were divided into six groups: control (vehicle), control (MPTP-treated), LEV high dose, L-Dopa + MPTP, LEV low dose + MPTP, and LEV high dose + MPTP. The mice underwent various behavioural tests, including the Open Field Test (OFT), rota-rod test, and foot slips test. Biochemical assays, such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and nitrite levels, were performed to assess oxidative stress and antioxidant defences.

Results: The LEV-treated groups showed significant improvements ($p<0.05$) in locomotor activity, motor coordination, and exploratory behaviour compared to the MPTP-treated control group. LEV at a high dose of 54 mg/kg significantly enhanced antioxidant enzyme levels, with SOD at 0.373 U/mg, GPX at 3.436 mcg/mg/min, and Nitric Oxide (NO) at 3.482 mg/mL, indicating its neuroprotective potential.

Conclusion: LEV demonstrated significant neuroprotective effects in a mouse model of Parkinsonism. The improvements in both behavioural outcomes and biochemical markers suggest its potential as a therapeutic agent for neurodegenerative diseases.

Keywords: Antioxidant, Dopaminergic neurons, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Neurodegenerative

INTRODUCTION

Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta, leading to a range of motor and non-motor symptoms. The hallmark motor manifestations, including bradykinesia, rigidity, tremor, and postural instability, significantly impact the quality of life of affected individuals [1,2]. Currently, available treatments primarily focus on managing symptoms, but there remains a pressing need for disease-modifying therapies that can slow or halt the neurodegenerative process.

The pathogenesis of PD is complex and multifactorial, involving a combination of genetic and environmental factors. Oxidative stress, mitochondrial dysfunction, protein aggregation, and neuroinflammation are key contributors to neuronal death in PD [3]. While the exact mechanisms remain elusive, the interplay of these factors creates a cascade of events that ultimately culminates in the demise of dopaminergic neurons [4]. This intricate pathophysiology underscores the importance of exploring novel therapeutic targets that can intervene at multiple levels to provide neuroprotection.

LEV, an antiepileptic drug with a favourable safety profile, has recently garnered attention for its potential neuroprotective effects in various preclinical models of neurodegenerative diseases. Beyond its established anticonvulsant properties, LEV has demonstrated anti-inflammatory, antioxidant, and anti-apoptotic activities, suggesting its potential to modulate key pathways involved in PD pathogenesis [5]. Previous studies have reported promising results with LEV in schizophrenia, Alzheimer's disease, and convulsant disorders [6,7].

In PD, motor skills and cognitive abilities are affected and can only be treated with conventional therapeutic approaches that alleviate symptoms [8]. There is no drug currently available for the treatment of PD that provides permanent recovery for patients. The investigation of LEV, an antiepileptic drug, as a potential neuroprotective agent in PD opens up new avenues for drug repurposing and identifies novel therapeutic targets beyond traditional PD medications.

The present study aimed to conduct a comprehensive evaluation of the neuroprotective effects of LEV in a C57BL/6J mouse model of Parkinsonism induced by MPTP. MPTP is a neurotoxin that selectively targets dopaminergic neurons, leading to a Parkinsonian phenotype in mice [9]. The MPTP model [10] has been utilised to investigate the impact of LEV on various aspects of PD pathology, including motor function, oxidative stress, and neuroinflammation.

Through this comprehensive evaluation, the study aims to provide preclinical insights into the therapeutic potential of LEV for PD. By elucidating the mechanisms underlying its neuroprotective effects and demonstrating its efficacy in ameliorating motor deficits and oxidative stress in a relevant animal model, this study could pave the way for future clinical trials investigating the use of LEV as a disease-modifying therapy for PD. Furthermore, the findings may contribute to a broader understanding of the neuroprotective properties of LEV and its potential applications in other neurodegenerative diseases.

MATERIALS AND METHODS

This experimental study was conducted at the Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, SRIHER, Chennai, Tamil Nadu, India, from October 2023 to March 2024. The study was approved by the Institutional Ethics Committee of Sri Ramachandra Institution of Higher Education and Research (Approval No. IAEC/70/SRIHER/843/2023).

Inclusion and Exclusion criteria: Healthy adult male mice (C57BL/6J) were included in the study, while unhealthy or female mice (C57BL/6J) were excluded from the study. A total of 36 adult male mice (C57BL/6J) were included in this study.

Study Procedure

MPTP was procured from Tokyo Chemical Industry Co. Ltd., Chuo-ku, Tokyo, Japan. L-Dopa and LEV were obtained from Vijaya Scientific Company, Thuraipakkam, Chennai-600097, Tamil Nadu, India.

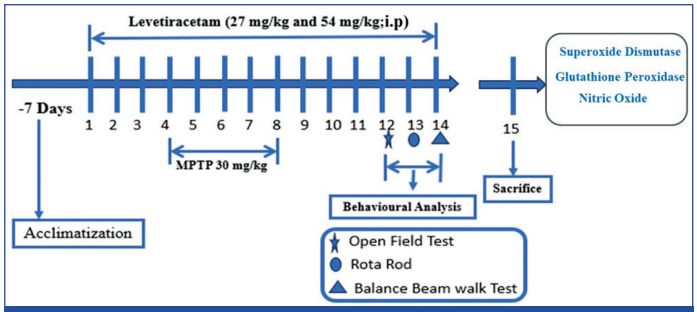
Animals and grouping: The study was conducted to evaluate the neuroprotective effects of LEV in a Parkinsonism model using C57BL/6J mice. A total of 36 adult C57BL/6J mice were divided into six groups (n=6 per group). group 1 (G1) received control treatment with normal saline administered intraperitoneally; group 2 (G2) was the control group treated with MPTP at a dosage of 30 mg/kg intraperitoneally [11]; group 3 (G3) was given LEV at a high dose of 54 mg/kg intraperitoneally [12]; group 4 (G4) received standard drug treatment of L-Dopa + MPTP 30 mg/kg intraperitoneally; group 5 (G5) was treated with LEV at a low dose of 27 mg/kg [12] + MPTP 30 mg/kg intraperitoneally; and group 6 (G6) received LEV at a high dose of 54 mg/kg + MPTP 30 mg/kg intraperitoneally.

Induction of parkinsonism: Parkinsonism was induced in mice using MPTP (30 mg/kg) administered intraperitoneally for five consecutive days [13]. MPTP is a neurotoxin that specifically targets and destroys dopaminergic neurons in the substantia nigra. This destruction results in a reduction of dopamine levels in the striatum, reproducing and mimicking the pathological characteristics of Parkinsonism [12]. The mice were monitored for signs of neurotoxicity and motor deficits.

Drug administration: LEV was administered intraperitoneally at doses of 27 mg/kg and 54 mg/kg [12] daily from day 1 to day 14 to evaluate its potential neuroprotective effect against MPTP-induced dopaminergic neurodegeneration. The treatment was initiated prior to MPTP administration, which was given from day 4 to day 8, as a preventive strategy to counteract the rapid and irreversible damage caused by MPTP to dopaminergic neurons in the substantia nigra. Starting LEV treatment from day 1 ensured that sufficient drug levels were present in the system before the onset of neurotoxic effects, thereby enhancing its ability to protect neural tissues. This early and continuous administration aimed to reduce oxidative stress, modulate excitotoxicity, and minimise neuroinflammation triggered by MPTP.

Continuing LEV until day 14 allowed for the evaluation of both its preventive and sustained protective effects during and after MPTP exposure. This approach reflects a clinically relevant model for investigating agents that may offer early intervention benefits in neurodegenerative diseases such as PD. The control group received an equivalent volume of normal saline. L-Dopa was administered at a dose of 20 mg/kg [14] as a positive control in combination with MPTP in one of the groups. The dosing regimen was designed to evaluate the neuroprotective effects of LEV in comparison to L-Dopa, a standard treatment for Parkinsonism [Table/Fig-1].

Behavioural assessments: A comprehensive approach integrating behavioural assessments, biochemical analyses, and histological evaluations was employed. Behavioural tests such as the OFT, Rotarod Test, and Balance Beam Walk Test were conducted to assess locomotor activity, motor coordination, and balance.



[Table/Fig-1]: A schematic representation of the experimentation design.

Biochemical assays were utilised to quantify antioxidant enzyme levels and oxidative stress markers. Additionally, histological examinations were performed to assess dopaminergic neuronal loss and neuroinflammation in the substantia nigra [15].

In the OFT, mice were placed individually in an open field arena measuring 40 cm×40 cm, which had a grid floor with a total of 16 equal squares. Their movement was tracked for 10 minutes using a video tracking system. Parameters measured included the number of squares crossed (total distance travelled), the number of entries into the centre square, and the frequency of rearing events [16].

In the Rotarod Test, mice were placed on a rotating rod (diameter 3 cm) that gradually accelerated from 4-40 rpm in five minutes. The time for which each mouse was able to remain on the rod before falling off was recorded. This test was repeated three times with at least 20-minute intervals between trials to ensure the evaluation of motor learning and coordination [16].

The Beam Walk Test is commonly used to evaluate motor coordination and balance in rodent models of locomotor dysfunction. It was conducted using a hardwood beam measuring 1 m in length and 10 mm in diameter, held at a height of 60 cm parallel to the bench top. The mouse was gently placed on one side of the rod and allowed to traverse the beam; the time taken to cover the one metre distance was recorded. Each mouse was permitted to walk twice, with an average reading recorded for analysis. Mice that dropped off or did not traverse the beam at all received a cut-off value of 120 seconds [15].

Tissue homogenate preparation: Mice were anaesthetised using a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg), followed by terminal euthanasia via cervical dislocation. The brains were carefully excised using sterilised surgical instruments under a dissecting microscope in a sanitised environment. The extracted brains were promptly rinsed with ice cold Phosphate-Buffered Saline (PBS). One cerebral hemisphere was fixed in 10% formalin for histopathological analysis, while the other was snap-frozen in liquid nitrogen and stored for subsequent antioxidant assays.

The homogenised tissue sample was used for antioxidant assays. For histopathology, the fixed brain was trimmed, processed, embedded in paraffin, sectioned, stained with Haematoxylin and Eosin, and the slides were examined.

Biochemical assays: Superoxide Dismutase (SOD) assay:

The SOD levels in tissue samples were estimated using a reaction mixture containing Phenazine Methosulphate (PMS) and sodium pyrophosphate (Kakkar P et al., 1984) [17]. A sodium pyrophosphate buffer (0.025 M) was prepared by dissolving 0.5575 g of sodium pyrophosphate in 50 mL of distilled water, with the pH adjusted to 8.3. A 186 µM stock solution of PMS was made by dissolving 3 mg of PMS in 10 mL of distilled water, and a 1:5 dilution was used for the working solution. An 800 µM solution of Nitroblue Tetrazolium (NBT) was prepared by dissolving 3 mg of NBT in 10 mL of the buffer solution. Additionally, 12 mg of NADH was dissolved in 20 mL of the buffer. Samples were homogenised and analysed in duplicates. Each tube received 50 µL of sample, 300 µL of buffer, 25 µL of PMS, 75 µL of NBT, and 75 µL of NADH. After incubation,

250 µL of glacial acetic acid and 2 mL of butanol were added, and the tubes were centrifuged. The absorbance of the supernatant was then measured at 560 nm.

Nitrite level: Nitrite changes, primarily occurring in the Substantia Nigra pars compacta (SNpc), contribute to oxidative damage in neurons, leading to dopamine deficiency in the striatum. To assess nitrite levels in mice brain SNpc samples, the method by Green LC et al., was employed [18]. A 10% tissue homogenate was prepared using ice-cold potassium chloride, and 0.2 mL of this homogenate was mixed with 1.8 mL of normal saline (0.9%) and 0.4 mL of 5-sulfosalicylic acid for protein precipitation. The mixture was centrifuged at 400 rpm for 10 minutes, and the supernatant was collected. From this, 1 mL was mixed with 2 mL of freshly prepared Griess reagent. The Griess reagent was composed of sulphanilamide, orthophosphoric acid, and naphthyl ethylenediamine. After 20 minutes, the mixture underwent absorption spectroscopy using a UV spectrophotometer (Thermo Fischer Scientific, USA), and absorbance was measured at 540 nm in a microplate reader to determine nitrite levels.

Glutathione content (GPX) assay: The activity of GSH was determined by quantifying the rate of oxidation of reduced glutathione. Glutathione content was estimated according to the method of Moron MS et al., (1979) [19]. A 10% cortex/hippocampal homogenate was added to an equal volume of ice-cold 5% TCA. To an aliquot of the supernatant, 0.2 M phosphate buffer (pH 8.0) and DTNB (0.6 mM) were added and mixed well. The absorbance was read at 412 nm using a microplate reader.

STATISTICAL ANALYSIS

Data analysis was performed using appropriate statistical tests to determine the significance of differences between groups. Analysis of Variance (ANOVA) was used for comparing multiple groups, followed by post-hoc Tukey's analyses where applicable to identify specific group differences. A significance level of $p<0.05$ was considered statistically significant. Statistical analyses were conducted using SPSS v.27 software.

RESULTS

Baseline demographics of the mice: The study involved the use of the C57BL/6J mouse strain. A total of 36 male mice, aged between six and eight weeks and weighing between 25 and 30 grams, were utilised for the experiment. These mice were in a healthy, pathogen-free condition and were housed under standard laboratory conditions, maintained at a temperature of 22±2°C, with a 12-hour light/dark cycle and humidity levels of 50-60%. Each experimental group consisted of six animals, ensuring a robust sample size. The mice had access to food and water ad libitum, promoting their well-being throughout the study.

Open Field Test (OFT): The [Table/Fig-2] shows the impact of different treatments on locomotor activity, measured by the number of squares crossed. The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.

S. No.	Groups	Average number of squares crossed (N)	p-value
1.	Control (Vehicle)	45.00±3.606	0.0078*
2.	Control MPTP (30 mg/kg, i.p)	13.50±3	
3.	LEV (54 mg/kg)	41.60±4.506	0.0068*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	47.75±10.56	0.0016*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	38.17±5.307	0.0015*
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	45.20±20.29	0.0021*

[Table/Fig-2]: Effect of Levetiracetam (LEV) on OFT (no. of squares crossed) in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). MPTP significantly reduced movement (13.5 squares) compared to the control (45 squares). LEV {Levodopa (L-Dopa)} at high doses, and L-Dopa + MPTP restored activity to 41.6 and 47.75 squares, respectively. LEV at a low dose + MPTP led to moderate recovery (38.17 squares), while LEV at a high dose + MPTP restored movement close to normal levels (45.2 squares). Significant p-values indicate the effectiveness of these treatments in improving movement impaired by MPTP.

The p-value was again determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). MPTP treatment significantly reduced entries (1 entry) compared to the control (4 entries). High doses of LEV (4 entries, p=0.0051) and L-Dopa + MPTP (3.75 entries, p=0.011) restored entries closer to control levels. LEV at a low dose + MPTP showed moderate improvement (2.833 entries, p=0.0962), while LEV at a high dose + MPTP (3.8 entries, p=0.0059) significantly improved centre square entries. The significant p-values indicate that these treatments reduce MPTP-induced Parkinsonism [Table/Fig-3].

S. No.	Groups	Average number of centre square entries (N)	p-value
1.	Control (Vehicle)	4.00±1	0.0101*
2.	Control MPTP (30 mg/kg, i.p)	1.00±1.155	
3.	LEV (54 mg/kg)	4.00±0.8165	0.0051*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	3.75±0.9574	0.011*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	2.83±1.169	0.0962
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	3.80±0.8367	0.0059*

[Table/Fig-3]: Effect of Levetiracetam (LEV) on OFT (no. of centre square) in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

The [Table/Fig-4] displays the number of rearing events (a measure of exploratory behaviour) observed in different experimental groups, likely assessing motor function under treatment conditions. The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). The control group (vehicle) shows an average of 4.33 rearing events. The group treated with MPTP (a neurotoxin) exhibits significantly fewer rearing events (0.75). L-Dopa and LEV treatments at various doses appear to partially recover the rearing behaviour, with statistical significance indicated by p-values (<0.05) compared to the MPTP group. LEV at a high dose shows similar recovery to L-Dopa, suggesting its potential neuroprotective effects.

S. No.	Groups	Average number of rearing (N)	p-value
1.	Control (Vehicle)	4.33±1.528	0.0085*
2.	Control MPTP (30 mg/kg, i.p)	0.75±0.5	
3.	LEV (54 mg/kg)	3.50±0.5774	0.0368*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	4.00±0.8165	0.0101*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	3.50±0.8367	0.0188*
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	4.00±2	0.0063*

[Table/Fig-4]: Effect of Levetiracetam (LEV) on OFT (no. of rearing) in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

Rota rod test: The [Table/Fig-5] illustrates the latency to fall (in seconds) as a measure of motor coordination across different treatment groups. The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.

S. No.	Groups	Average latency time of fall (sec)	p-value
1.	Control (Vehicle)	12.00±1	0.0003*
2.	Control MPTP (30 mg/kg, i.p)	3.50±2.646	
3.	LEV (54 mg/kg)	12.00±2.582	0.0001*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	9.75±1.708	0.0036*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	8.16±2.041	0.0209*
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	11.00±1.581	0.0003*

[Table/Fig-5]: Effect of Levetiracetam (LEV) on Rota Rod in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). The control (vehicle) group has a latency of 12 seconds, while the MPTP-treated group (neurotoxin) shows a significant decrease in motor coordination, falling at 3.5 seconds. Treatments with LEV and L-Dopa, particularly at high doses, improve motor function. The L-Dopa and high-dose LEV-treated groups show recovery in latency to fall (12 and 11 seconds, respectively), with p-values indicating statistically significant improvements compared to the MPTP group. These results suggest the treatments' efficacy in mitigating MPTP-induced motor deficits.

Balance beam walk test: The [Table/Fig-6] represents the time taken (in seconds) to traverse a beam, which is a measure of motor coordination and balance across different treatment groups. The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). The control (vehicle) group takes 13.66 seconds, while the MPTP-treated group shows significant impairment, requiring 37.35 seconds. LEV and L-Dopa treatments improve beam traversal times in the MPTP model. The L-Dopa + MPTP group (16.49 seconds) and LEV low-dose + MPTP group (18.16 seconds) significantly reduce traversal time compared to the MPTP group, suggesting recovery of motor function. The high-dose LEV + MPTP group also improves performance (23.86 seconds), indicating potential neuroprotective benefits.

S. No.	Groups	Average time to traverse on beam (sec)	p-value
1.	Control (Vehicle)	13.66±6.964	0.0002*
2.	Control MPTP (30 mg/kg, i.p)	37.35±6.547	
3.	LEV (54 mg/kg)	16.49±4.936	0.0002*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	18.16±4.555	0.0026*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	24.76±5.227	0.0239*
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	23.69±5.527	0.0172*

[Table/Fig-6]: Effect of Levetiracetam (LEV) on balance beam walk test (time to traverse on beam) in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

The [Table/Fig-7] compares the effects of different treatments on motor function, measured by foot slip time (in seconds), in a rodent model. The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). The control group (vehicle) shows a baseline foot slip time of 11.99 seconds. MPTP-treated animals display decreased motor function (31.67 seconds). The high-dose LEV group shows significantly improved motor function (10.33 seconds), indicating the highest recovery. LEV combined with MPTP, at both low and high doses, improves motor performance, although less than LEV alone. L-Dopa, a standard treatment for Parkinson's disease, results in moderate recovery (14.16 seconds). The p-values

S. No.	Groups	Average time of foot slips (sec)	p-value
1.	Control (Vehicle)	11.99±3.6	0.0033*
2.	Control MPTP (30 mg/kg, i.p)	31.67±5.5	
3.	LEV (54 mg/kg)	10.33±2.4	0.0003*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	14.16±3.3	0.0097*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	18.42±4.2	0.0237*
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	13.29±2.8	0.00183*

[Table/Fig-7]: Effect of Levetiracetam (LEV) on balance beam walk test (foot slip) in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

indicate significant differences between treatments, suggesting their efficacy.

This [Table/Fig-8] depicts the effect of different treatments on limb dragging duration, a measure of motor dysfunction. The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). The control group (vehicle) shows 20.51 seconds of limb dragging. MPTP (neurotoxin) exposure increases the limb dragging time to 37.85 seconds. LEV at a high dose without MPTP significantly enhances motor performance, reducing the limb dragging time to 21.29 seconds. After treatment with standard L-Dopa, limb dragging moderately decreases to 23.16 seconds. LEV treatments (low and high doses with MPTP) display intermediate improvements (26.42 and 24.29 seconds, respectively). Statistical significance confirms the protective effects of these treatments on motor function.

S. No.	Groups	Average time of limbs dragging (sec)	p-value
1.	Control (Vehicle)	20.51±6.003	0.0025*
2.	Control MPTP (30 mg/kg, i.p)	37.85±4.292	
3.	LEV (54 mg/kg)	21.29±4.021	0.001*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	23.16±5.465	0.0115*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	26.42±5.72	0.0223*
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	24.29±4.713	0.0075*

[Table/Fig-8]: Effect of Levetiracetam (LEV) on balance beam walk test (limb dragging) in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

Superoxide Dismutase (SOD) Activity: This [Table/Fig-9] illustrates the effect of treatments on SOD activity, measured in U/mg, which is a marker of antioxidant defence. The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). The control group (vehicle) shows an SOD activity of 0.4153 U/mg. MPTP significantly reduces SOD levels to 0.239 U/mg, indicating oxidative stress. LEV at a high dose without MPTP slightly improves SOD (0.3608

S. No.	Groups	SOD (U/mg)	p-value
1.	Control (Vehicle)	0.415±0.07393	0.067*
2.	Control MPTP (30 mg/kg, i.p)	0.239±0.03195	
3.	LEV (54 mg/kg)	0.360±0.04152	0.0442*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	0.378±0.1038	0.0435*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	0.280±0.03474	0.8593
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	0.373±0.06277	0.0222*

[Table/Fig-9]: Effect of Levetiracetam (LEV) on SOD in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

U/mg). L-Dopa with MPTP and LEV treatments (low and high doses combined with MPTP) restore SOD activity (0.378, 0.2807, and 0.3732 U/mg, respectively). The p-values indicate statistically significant differences, suggesting the potential of these treatments to reduce oxidative damage caused by MPTP.

Glutathione Peroxidase (GPX) Activity: The [Table/Fig-10] shows the effects of various treatments on GPX activity (mcg/mg/min) in a controlled experiment. The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). The Control (Vehicle) group has the highest GPX activity at 3.634. The control MPTP (30 mg/kg) group shows significantly reduced GPX activity at 2.625. The LEV - High Dose, L-Dopa + MPTP, LEV - Low Dose + MPTP, and LEV - High Dose + MPTP groups display varying levels of GPX activity, all higher than the MPTP group alone but lower than the Vehicle control, with statistical significance noted by p-values. LEV and L-Dopa treatments partially mitigate MPTP-induced reductions in GPX activity.

S. No.	Groups	GPX (mcg/mg/min)	p-value
1.	Control (Vehicle)	3.634±0.1805	0.0043*
2.	Control MPTP (30 mg/kg, i.p)	2.625±0.2152	
3.	LEV (54 mg/kg)	3.517±0.2214	0.004*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	3.565±0.2656	0.0082*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	3.363±0.3822	0.0152*
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	3.436±0.3979	0.0096*

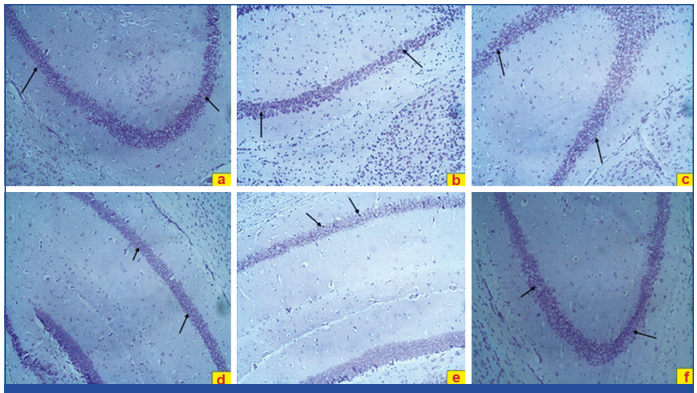
[Table/Fig-10]: Effect of Levetiracetam (LEV) on GPX in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

The [Table/Fig-11] depicts the effects of various treatments on mean nitrite levels (mM/mg). The p-value was determined using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Specific intergroup comparisons were performed between group 2 (G2) and (G1, G3, G4, G5, G6). The control (vehicle) group had the lowest nitrite level at 1.767 mM/mg. The control MPTP (30 mg/kg) group shows a significant increase in nitrite levels at 2.997 mM/mg. The LEV - High Dose, L-Dopa + MPTP, LEV - Low Dose + MPTP, and LEV - High Dose + MPTP groups show decreases in nitrite levels. Each treatment group's results indicate statistical significance, suggesting that LEV and L-Dopa treatments can mitigate the MPTP-induced increase in nitrite levels.

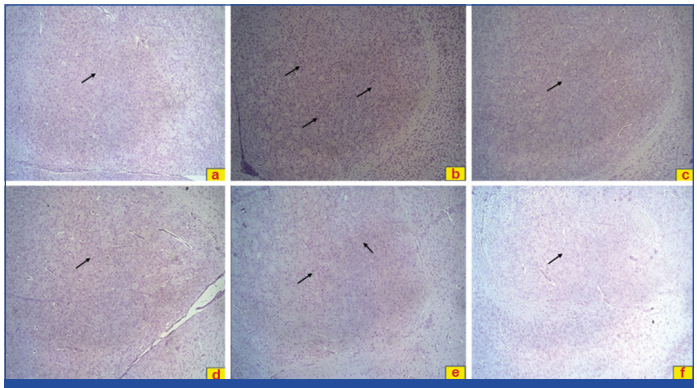
S. No.	Groups	Nitrite (mM/mg)	p-value
1.	Control (Vehicle)	1.767±0.1079	0.0003*
2.	Control MPTP (30 mg/kg, i.p)	2.997±0.631	
3.	LEV (54 mg/kg)	1.628±0.1026	<0.0001*
4.	L-Dopa (20 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	1.997±0.2914	0.0028*
5.	LEV (27 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	2.11±0.1873	0.0017*
6.	LEV (54 mg/kg, i.p) + MPTP (30 mg/kg, i.p)	2.041±0.1658	0.0011*

[Table/Fig-11]: Effect of Levetiracetam (LEV) on Nitrite in MPTP-induced PD C57BL/6J mice.
•p-value was observed between group 2 (G2) and (G1, G3, G4, G5, G6)

The histological findings from the hippocampus and striatum regions of the brain for all groups are depicted in [Table/Fig-12a-f] and [Table/Fig-13a-f]. In the hippocampus, Group A (Control - Vehicle) showed normal histological architecture with densely packed and well-defined neuronal cells, indicating healthy brain tissue. In contrast, Group B (Control MPTP - 30 mg/kg, i.p.) revealed significant neuronal degeneration and disrupted cell arrangement, consistent with MPTP-induced neurotoxicity that mimics PD-like pathology. Group C (LEV 54 mg/kg) displayed a structure similar to the control, suggesting LEV's potential neuroprotective effect.



[Table/Fig-12]: Histological analysis of C57BL/6J mice brain exposed to different doses of Levetiracetam (LEV): a) Normal control; Arrow denotes normal histological appearance of neuronal cell in Hippocampus region of brain; b) MPTP treated; Arrow denotes neuronal cell degeneration in hippocampus region of brain; c) LEV treated 54 mg/kg; Arrow indicates mild neuronal cell degeneration in hippocampus region of brain; d) L-Dopa + MPTP; Arrow indicates normal histological appearance of neuronal cell in Hippocampus region of brain; e) LEV (27 mg/kg, i.p) + MPTP; Arrow indicates mild neuronal cells degeneration in hippocampus region of brain; f) LEV (54 mg/kg, i.p) + MPTP: Arrow indicates normal histological appearance of neuronal cell in Hippocampus region of brain. (Haematoxylin and Eosin stain with magnification 10X).



[Table/Fig-13]: Histological analysis of C57BL/6J mice brain exposed to different doses of Levetiracetam (LEV): a) Control (Vehicle): Normal treated; Arrow denotes normal histological appearance of neuronal cell in Striatums region of brain; b) MPTP treated; Arrow denotes moderate densely stained neuronal cell in Striatum region of brain; c) LEV (54 mg/kg); Arrow indicates normal histological appearance of neuronal cell in Striatum region of brain; d) L-Dopa (20 mg/kg, i.p) + MPTP; Arrow indicates normal histological appearance of neuronal cell in Striatum region of brain; e) LEV (27 mg/kg, i.p) + MPTP : Arrow indicates mild densely stained neuronal cell in Striatum region of brain; f) LEV (54 mg/kg, i.p) + MPTP: Arrow indicates normal histological appearance of neuronal cell in Striatum region of brain. (Haematoxylin and Eosin stain with magnification 10X).

Likewise, Group D (L-Dopa 20 mg/kg + MPTP) maintained near-normal histological features, indicating that L-Dopa can mitigate MPTP-induced damage. Group E (LEV 27 mg/kg + MPTP) shows mild degeneration, implying partial neuroprotection at a lower LEV dose, whereas Group F (LEV 54 mg/kg + MPTP) closely resembled the control structure, reinforcing the protective effect of a higher LEV dose. Similarly, in the striatum, Group G (Control) displayed normal histological features with evenly distributed and preserved neuronal cells. Group H (MPTP 30 mg/kg) revealed a moderate increase in densely stained neurons, indicative of neurodegeneration and dopaminergic neuronal loss. Group I (LEV 54 mg/kg) again showed normal histology, confirming its non-toxic nature. Groups-J (L-Dopa + MPTP) and Group L (LEV 54 mg/kg + MPTP) both exhibited near-normal striatal architecture, further supporting their neuroprotective roles. However, Group K (LEV 27 mg/kg + MPTP) presented mild dense staining, reflecting only partial protection at a lower dose. Collectively, these results underscore the dose-dependent neuroprotective effects of LEV in both the hippocampus and striatum, supporting its potential therapeutic application in mitigating MPTP-induced neurodegeneration.

DISCUSSION

In a study using a PD model, LEV was administered at low (27 mg/kg) and high (54 mg/kg) doses and compared with the standard treatment,

L-Dopa (30 mg/kg). The high dose of LEV showed superior efficacy over the low dose in behavioural assessments and demonstrated comparable effectiveness to L-Dopa in improving motor and neurological function. Neurohistopathological analysis further revealed that the low dose of LEV resulted in only mild recovery of damaged neurons in the substantia nigra and striatum, whereas the high dose achieved moderate neuronal recovery, comparable to L-Dopa protective or restorative effects on these critical brain regions involved in motor control. These findings suggest that LEV's neuroprotective potential is dose-dependent and may hold therapeutic promise similar to that of L-Dopa for neurodegenerative conditions.

In the present study, the OFT demonstrated that LEV treatment enhanced the exploratory behaviour of mice, as indicated by an increased number of square crossings. In the rota-rod test, mice treated with LEV exhibited improved motor coordination, as they remained on the rod for a longer duration compared to the MPTP control group. Similarly, in the beam walk test, LEV-treated mice took significantly less time to cross the 50 cm beam, further supporting its positive effect on motor coordination. These behavioural assessments clearly highlight the potential of LEV in alleviating PD symptoms and improving motor functions.

In addition to the behavioural studies, biochemical assays were conducted to measure antioxidant enzyme levels, specifically SOD and GPX, along with the oxidative stress marker nitrite. The results showed a significant increase in SOD and GPX levels, while nitrite levels decreased, indicating a significant reduction in oxidative stress. These biochemical findings also suggest neuronal recovery following MPTP-induced damage. Collectively, both the behavioural and biochemical data demonstrate that LEV exhibits neuroprotective effects in the MPTP-induced Parkinson's model.

In a study by Kadoguchi N et al., the antidepressant mirtazapine was investigated for its antiparkinsonian effects in a mouse model [20]. The findings revealed that mirtazapine, at higher doses, significantly increased the latency to fall in the rota-rod test and reduced beam-walking time in the beam walk test when compared to the control group. These results suggest that mirtazapine may have therapeutic potential for alleviating Parkinson's symptoms, which aligns with the outcomes of the current study. Similarly, Rai SN et al., explored the antiparkinsonian potential of ursolic acid, reporting substantial improvements in motor coordination [21]. This was evidenced by enhanced walking performance and increased rota-rod activity in an MPTP-induced Parkinson's model. Ursolic acid also shortened the time required to traverse a narrow beam in the beam walk test compared to the MPTP control group.

Additionally, Khatri DK and Juvekar AR evaluated curcumin for its effects against Parkinson's disease [22]. Their study showed that curcumin enhanced locomotor activity and stabilised mood, as indicated by increased rearing behaviour. Mice treated with curcumin stayed on the rota-rod significantly longer than the rotenone-induced Parkinson's group. Moreover, curcumin improved antioxidant defences by boosting SOD and glutathione levels, while reducing oxidative stress, as indicated by lower lipid peroxidation levels. The improvement in motor function observed in their study aligns with findings that LEV can significantly improve motor impairments caused by MPTP.

Nagarajan S et al., examined how ferulic acid pretreatment alleviates motor impairments and histopathological changes induced by MPTP in C57BL/6 mice [23]. Their histopathological findings indicated that MPTP caused neuron loss in the substantia nigra, attributed to inflammation and apoptosis. Following treatment with ferulic acid at doses of 20, 40, and 80 mg/kg, there was a reduction in microglial cells and an increase in intact neuronal cells. Similarly, this study also demonstrates dose-dependent neuroprotective effects of LEV in both the hippocampus and striatum, supporting its potential therapeutic application in mitigating MPTP-induced neurodegeneration.

LEV, primarily used as an anticonvulsant for epilepsy, has shown significant promise in neuroprotective applications. Its ability to mitigate oxidative stress and inflammation makes it a potential therapeutic option for various neurological disorders. Oxidative stress, a major factor in diseases like epilepsy, Alzheimer's disease, and stroke, results from an imbalance between free radicals and the body's antioxidant defences [24,25]. LEV helps reduce this stress by lowering ROS levels and enhancing the activity of antioxidant enzymes. Additionally, its anti-inflammatory properties help limit the release of pro-inflammatory cytokines, further protecting neurons [26].

Studies in animal models have shown that LEV reduces neuronal loss and improves outcomes in brain injury. Its ability to improve cognitive function by normalising abnormal neuronal activity, especially in Alzheimer's disease [26,27], underscores its broader neuroprotective potential. While previous studies have provided limited preclinical data on the neuroprotective effects of LEV in PD models, there remains a significant gap in understanding its full potential as an antiparkinsonian agent. The current study addresses this gap by offering comprehensive insights into the neuroprotective effects of LEV in a PD mice model, thereby expanding the knowledge base in this area.

Limitation(s)

One limitation of this study is the reliance on animal models, which may not fully replicate the complexities of PD in humans. Additionally, the study exclusively examined the neuroprotective effects of LEV without investigating possible synergies with other therapeutic agents. Future research should aim to conduct clinical trials to verify the efficacy of LEV in human subjects. Investigating the long-term effects and optimal dosing regimens of LEV, as well as its potential interactions with existing Parkinson's treatments, would provide a more comprehensive understanding of its therapeutic potential. Exploring the molecular pathways underlying its neuroprotective effects could also yield insights into new therapeutic targets for PD.

CONCLUSION(S)

In conclusion, LEV demonstrates significant antiparkinsonian effects by enhancing motor coordination and improving behaviour in a mouse model of PD. The biochemical analyses further support its neuroprotective potential, showing that LEV effectively reduces oxidative stress while elevating antioxidant levels. These findings imply that LEV not only alleviates PD-related motor symptoms but also preserves dopaminergic neuronal function due to its antioxidative effects. Future studies into LEV's long-term effects and mechanisms of action are necessary in order to potentially apply the drug in clinical settings for neurodegenerative illnesses.

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