



Exploring the Neuroprotective Behaviour and Hepatoprotective Effects of Plumbagin in MPTP-Induced Parkinson's Disease

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, leading to motor symptoms like tremors and

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bradykinesia, as well as non-motor symptoms such as sleep disturbances and autonomic dysfunction. Recent research suggests a link between PD and liver dysfunction, including altered metabolism and elevated liver enzymes. Plumbagin, recognized for its antioxidant and neuroprotective properties, may offer therapeutic benefits. This study explores its effects on both motor and non-motor symptoms in MPTP-treated mice, as well as its impact on liver function through serum enzyme analysis. The aim is to assess the neuroprotective and hepatoprotective potential of plumbagin in countering MPTP-induced neurodegeneration and liver dysfunction.

Methodology: Thirty mice (22-30g, 8-12 weeks old) were randomly assigned to five groups of six each. The experiment spanned 28 days, with the mice housed under controlled conditions and provided standard feed and water. Behavioral tests, including the Marble Burying and Tail Suspension tests, were conducted to assess anxiety and depressive-like behavior. Serum biochemistry, including ALT, AST, and BUN, was measured using standard ERBA kits to evaluate liver function health.

Results: In the marble burying and tail suspension tests, MPTP-induced mice (DC group) exhibited significantly higher anxiety and depressive-like behaviors compared to the sham and plumbagin groups. Plumbagin treatment (PD) and levodopa (LS) significantly reduced these behavioral symptoms. Liver function markers (ALT, AST, ALP) and BUN levels were elevated in DC mice, indicating liver and kidney damage, while plumbagin treatment mitigated these effects, showing values comparable to the standard treatment (LS).

Conclusion: This study demonstrates that MPTP exposure affects both the central nervous system and liver function, leading to anxiety, depression, and liver damage, as evidenced by elevated liver enzymes. Plumbagin showed protective effects, reducing these behavioral and biochemical changes through its antioxidant properties, suggesting its potential as a therapeutic agent against MPTP-induced neurotoxicity and hepatotoxicity. These findings suggest that future research should investigate plumbagin's clinical potential for treating Parkinson's disease and liver dysfunction, offering promise for its use in developing neuroprotective therapies.

Keywords: Parkinson disease; plumbagin; liver; neuro toxicity.

1. INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder primarily characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), with its causes linked to a combination of genetic, environmental and aging factors [1]. Motor symptoms like bradykinesia, muscle rigidity, tremors and postural instability are key indicators of Parkinson's disease, but non-motor symptoms such as sleep disorders, dementia, sensory abnormalities, and autonomic dysfunction also play a significant role [2]. Experimental models for Parkinson's disease largely fall into two categories: neurotoxin-induced and genetic. The neurotoxin MPTP, a commonly used agent in Parkinson's disease animal models, replicates many aspects of the disease by causing selective dopaminergic neuronal damage [3]. Plumbagin, a naphthoquinone found in *Plumbago zeylanica*, has long been used in traditional medicine for its antioxidant, anti-inflammatory, and neuroprotective properties [4]. Recent studies suggest a potential connection between CNS disorders like PD and liver dysfunction, with Parkinson's disease patients showing altered

liver metabolism, mitochondrial dysfunction and elevated AST/ALT levels [5]. Given these findings, this study aims to explore the effects of plumbagin on motor and non-motor symptoms in MPTP-treated mice, as well as its impact on liver function, as indicated by key serum enzyme levels.

2. MATERIALS AND METHODS

2.1 Animals

A total of 30 mice, each weighing between 22-30 g and aged 8-12 weeks, were obtained from Jeeva Science, Hyderabad. They were housed in polypropylene cages under a 12-hour light/dark cycle in the Department of Veterinary Pharmacology and Toxicology, CVSc, Rajendranagar, Hyderabad., maintaining a temperature of 22–24°C. Before the experiment, the mice were allowed to acclimatize for one week. Throughout the study, they were provided with commercial standard pellet feed and ad libitum reverse osmosis water. The mice were evenly divided into 5 groups of 6, with the experiment lasting 28 days, and each group receiving different treatments as outlined in the study plan.

2.2 Experimental Design

Table 1. List of treatments used for the study

Group	Treatments
1	Sham control (SC) - Normal saline for 28 days.
2	Disease control (DC) - induction with MPTP@ 25 mg/kg BW, IP route, on the last 4 consecutive days of the experiment.
3	Plumbagin <i>per se</i> (PS): 10 mg/kg BW, IP dose for 28 days.
4	Plumbagin dose: Pretreated with Plumbagin @ 10mg/kg BW, IP route for 28 days + MPTP@ 25 mg/kg BW, IP route, on last 4 consecutive days of experiment.
5	Levodopa Standard: Pretreated with Levodopa @20 mg/kg BW, IP route for 28 days + MPTP@ 25 mg/kg BW, IP route, on last 4 consecutive days of the experiment.

2.3 Neurobehavioral Analysis

Marble Burying Test: The mice were housed in test cages containing 5 cm deep wood chip bedding. Prior to testing, 20 glass marbles (15 mm in diameter) were evenly arranged in a 4 × 5 pattern within the cage. After 30 minutes, the number of marbles buried up to two-thirds of their height was recorded [6].

Tail Suspension Test: The mouse's tail was secured approximately 1 cm from its end using a clamp, suspending it upside down about 15 cm above the ground. Initially, the mouse attempted to escape the uncomfortable position, but after a period of activity, it displayed intermittent immobility, indicating a state of despair. The immobility time was recorded during the final 4 minutes of a 6-minute test [6].

2.4 Serum Biochemistry

Alanine Transaminase (ALT) (ERBA SGPT KIT)

Procedure: ALT was estimated by IFCC (International Federation of Clinical Chemistry & Laboratory Medicine) method using a diagnostic kit manufactured by Erba diagnostics. The 1000µl reagent was taken in a clean cuvette (rinsed with distilled water) and 100 µl of serum sample was added and mixed well and reading was taken immediately at 37°C and wavelength at 340nm. Then the optical density values were noted down and calculated.

Table 2. Serum Biochemistry assay

Pipette	Volume
Working solution	1000 µl
Test sample	100 µl

Mixed well and aspirated. After the initial delay of 60 seconds, recorded the absorbance of the test at an interval of 30 seconds for the next 90 seconds at 340 nm.

Calculation:

The activity of ALT in IU/l = Δ Abs /min x 1749
Units of activity: IU/L

Aspartate Transaminase (AST): (ERBA SGOT KIT)

Procedure: Using a diagnostic kit made by Erba Diagnostics, AST was calculated using the IFCC (International Federation of Clinical Chemistry & Laboratory Medicine) method. The reagent of working solution (R1) 160 µl was taken in a cuvette and 40 µl of working solution (R2) added later. serum sample 10 µl was added and mixed well and reading was taken immediately at 37°C and wavelength at 340nm. Then the optical density values were noted down and calculated.

Table 3. Aspartate Transaminase assay

Pipette	Volume
Working solution (R1)	160 µl
Working solution (R2)	40 µl
Test sample	10 µl

Mixed well and aspirated. After the initial delay of 60 seconds, recorded the absorbance of the test at an interval of 30 seconds for the next 90 seconds at 340 nm.

Calculation:

The activity of AST in IU/L = Δ Abs /min x 3339

Units of activity: IU/L

Alkaline Phosphatase (ALP): (ERBA SGOT KIT)

Procedure: ALT was measured using a diagnostic kit from Erba Diagnostics, following the IFCC method. In a 96 well plate, 30 µl of working solution (R1) was mixed with 30 µl of working solution (R2), followed by 10 µl of serum sample. The mixture was read immediately at 37°C with a wavelength of 340 nm, and optical density values were recorded for calculation.

Table 4. Alkaline Phosphatase assay

Pipette	Volume
Working solution (R1)	30 µl
Working solution (R2)	30 µl
Test sample	10 µl

Mixed well and aspirated. After the initial delay of 60 seconds, recorded the absorbance of the test at an interval of 30 seconds for the next 90 seconds at 340 nm.

Calculation:

The activity of ALP in IU/L = Δ Abs /min x 3249

Units of activity: IU/L

Blood Urea Nitrogen (BUN): (ERBA UREA (BUN) KIT)

Procedure:

Table 5. Blood Urea Nitrogen assay

	Reagent blank	Standard	Sample
Working reagent	1.000 ml	1.000 ml	1.000 ml
Sample	-	-	0.010 ml
Standard	-	0.010 ml	-
Distilled water	0.010 ml	-	-

Mixed well, measured the initial absorbance after 30 sec (A1), and reread it after 1 min (A2).

$$\Delta A_{sam} = (A2-A1)/min$$

Calculation: The final values were calculated from the standard curve using Microsoft Excel.

2.5 Statistics

The experimental results were expressed as the mean ± standard error (SE) values. Statistical

analysis was carried out utilizing Graph Pad Prism Software version 5.0. This encompassed a one-way analysis of variance, followed by Tukey's multiple comparison test. The significance of the observations was established at a significance level of $p < 0.05$.

3. RESULTS

In the marble burying test, MPTP-induced DC group (14.04±0.98) tended to leave a significantly ($P < 0.01$) higher number of unburied glass marbles compared to those left by sham group (SC) (7.75±0.14) and plumbagin perse (PS) (6.64±0.33) mice. However, treatment group plumbagin dose (PD) (10.45±0.16), and levodopa standard (LS) (8.12±0.24) mice exhibited significantly ($P < 0.05$, $P < 0.01$) a lower number of unburied marbles compared to that of group DC mice. Furthermore, no significant difference was noticed among groups PD and LS, as well as between groups SC and PS. The results are presented in Fig. 1.

MPTP-induced DC mice (176.2±3.01) presented significantly ($P < 0.001$) increased immobility time spent compared to sham group (131.1±1.84) and perse (137.4±2.4). However, plumbagin treatment group (155.3±2.3) and standard group (146.8±1.2) exhibited significantly ($P < 0.05$, $P < 0.01$, respectively) lowered immobility time when compare to DC mice. There was no significant difference in immobility time levels between PD group and LS group. (Fig. 1).

The ALT activity in DC (46.22±2.1) was elevated, albeit significantly different from SC (17.80±2.1). Conversely, treatment group PD (35.68±1.4) exhibited less significant variance compared to DC, and values were similar to standard treatment LS (35.77±2.2). Meanwhile, the value in PS (16.90±1.2) was similar to that of SC. (Fig. 2).

The AST activity in DC (73.58±3.0) was higher than in SC (27.50±2.8), but the difference was highly significant. Conversely, treatment PD group (47.88±3.7) exhibited significant difference compared to DC, and similar values were noticed in LS (41.14±2.6). The AST value in PS (26.36±3.5) was comparable to SC (Fig. 2).

The ALP in group 2 (61.35±3.3) was higher significant difference compared to group 1 (34.03±2.4). On the other hand, treatment group 4 (46.20±2.6) showed no considerable difference compared to group 2. The value in group 4 (0.87±0.20) was comparable to group 5 (43.80±3.8) (Fig.2).

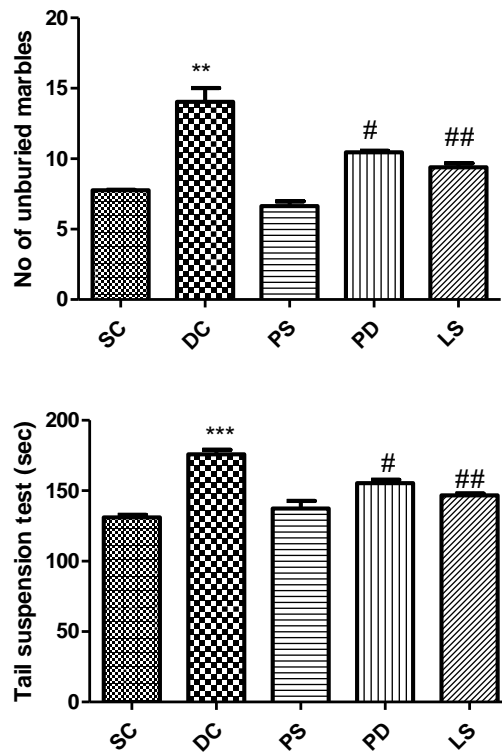


Fig. 1. Graphical representation of Marble test and Tail suspension test

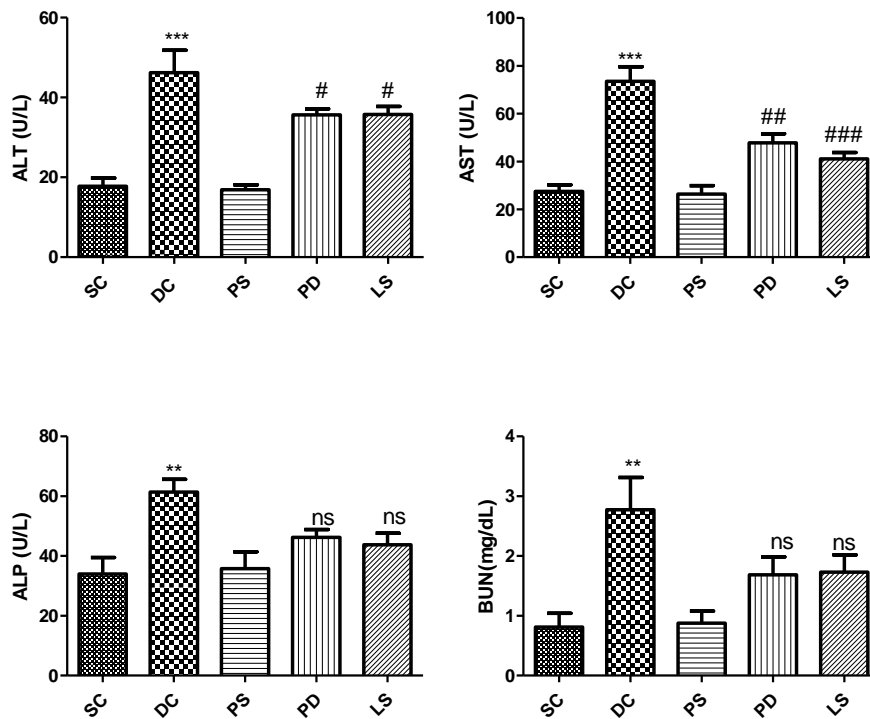


Fig. 2. Graphical representation of ALT, AST, ALP and BUN

The concentration of BUN in groups 1 to 5 were 0.81 ± 0.2 , 2.7 ± 0.5 , 0.87 ± 0.2 , 1.68 ± 0.3 and 1.73 ± 0.28 , respectively. Group 2 displayed marginally higher values than group 1, although the difference was statistically significant. Similarly, treatment groups 4 exhibited no significant difference compared to group 2. The value for group 3 was comparable to that of group 1 (Fig. 2).

4. DISCUSSION

Neuro Behavioural: Behavioural tests in this study were performed to evaluate the effects of MPTP on the central nervous system (CNS) and its role in psychological and cognitive symptoms, such as anxiety, depression, and cognitive deficits [7]. Anxiety-like behavior was measured using the marble burying test, while the tail suspension test revealed that DC mice exhibited significantly longer periods of immobility, suggestive of depressive-like behavior. In the marble burying test, DC mice left a notably higher number of unburied marbles compared to the sham group, indicating anxiety-related responses likely due to the effects of MPTP on the substantia nigra. Recent research suggests that MPTP triggers the release of inflammatory mediators, which can cross the blood-brain barrier, causing systemic inflammation and contributing to immune-mediated neuronal dysfunction. Activated immune cells, impaired endothelial cells in the BBB, along with microglia and astrocytes, are linked to depressive symptoms in Parkinson's disease [8]. Moreover, CNS immune activation can influence the serotonin-kynurenine pathway, which is significant in depression by increasing the expression of Indoleamine 2,3-dioxygenase (IDO) under the influence of pro-inflammatory cytokines. This leads to reduced serotonin levels, a key factor in depressive behavior and a primary target for antidepressant treatments [9]. Impaired adult neurogenesis and increased neuronal degeneration were also noted. Hyperactive microglia may cause abnormal synaptic degradation in the hippocampus, diminishing neuronal excitability and synaptic plasticity [10]. These observations align with those reported by Datta et al. (2020) [5]

Hepatotoxicity: Increasing clinical evidence highlights the connection between the central nervous system (CNS) and liver metabolism, particularly in Parkinson's disease patients [11]. MPTP can affect the liver through two mechanisms—first, by crossing the blood-brain

barrier (BBB) and directly inhibiting complex I of liver cells [12], and second, through disruptions in the brain's dopaminergic system [13]. In this study, liver function impairment was evident, as shown by elevated serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) following the initial administration of MPTP, with further deterioration after repeated doses. Under normal conditions, AST and ALT levels in the bloodstream are low, and their increase indicates liver damage, making them reliable markers for assessing liver function [14].

Alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are enzymes found in hepatocytes and serve as sensitive indicators of liver function and hepatotoxicity. These enzymes are generally localized in the cytoplasm, and their presence in the bloodstream typically suggests liver damage [15]. In this study, significant elevations in ALT, AST, ALP, and blood urea nitrogen (BUN) levels were observed in the DC group, likely due to increased bacterial translocation and altered protein metabolism, leading to hepato-biliary damage and compromised liver function. This damage triggers the release of pro-inflammatory cytokines and results in cellular injury, further disrupting hepatocyte integrity, as evidenced by the high enzyme levels. These findings align with earlier studies Shojaie et al. 2020 [16].

Conversely, plumbagin treatment significantly reduced the elevated liver enzymes in a dose-dependent manner in the PD and LS groups, showing substantial improvement when compared to the DC group. However, no significant difference was found between the PD and LS groups. This effect is likely due to plumbagin's antioxidant properties, which protect liver cells from damage caused by toxic substances by neutralizing free radicals and reducing oxidative stress. This protection results in decreased liver cell injury, thereby lowering levels of ALT, AST, ALP, and BUN, indicating the hepatoprotective effects of plumbagin [17].

5. CONCLUSION

In summary, this study highlights the dual impact of MPTP on both the central nervous system and liver function. MPTP exposure led to significant behavioural changes, including anxiety-like and depressive behaviours, which are likely a result of neuroinflammation, immune activation. These

findings emphasize the wider implications of MPTP on cognitive and psychological health. Simultaneously, MPTP caused liver dysfunction, as indicated by elevated levels of liver enzymes (ALT, AST, ALP), signalling liver damage. This damage likely stems from inflammation and disrupted liver metabolism. Notably, the administration of plumbagin showed a protective effect, reducing these enzyme levels through its antioxidant properties and thereby mitigating liver injury. This suggests that plumbagin has potential as a therapeutic agent for addressing both the neurotoxic and hepatotoxic effects of MPTP. These insights offer valuable perspectives for further research into the interconnected impacts of neurotoxins on multiple organ systems.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

The experimental protocol was approved by the Institutional Animal Ethics Committee (Approval No. 33/26/C.V.Sc., Hyd. IAEC).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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