A Comparative Study on the Neuroprotective Effects of Nardostachys jatamansi Extract in a Parkinson's Disease Mouse Model: A Prospective Analysis

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Abstract:

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons, resulting in motor dysfunction. Traditional treatments like levodopa provide symptomatic relief but fail to halt disease progression. Nardostachys jatamansi (NJ), a medicinal plant, has shown neuroprotective potential, with its silver nanoparticle formulation (Ag-NJE-NP) potentially enhancing efficacy through improved bioavailability. Materials and Methods: A mouse model of PD was induced using CPZ. Six experimental groups were studied: Normal Control, Negative Control (CPZ-treated), Positive Control (Levodopa + Carbidopa), NJE-500 (N. jatamansi extract), Ag-NJE-NP-100 (low dose), and Ag-NJE-NP-250 (high dose). Motor function was evaluated using the Rotarod test, oxidative stress markers were analyzed via spectrophotometry, and brain tissue immunohistochemistry was performed to detect Lewy bodies. Data were analyzed using one-way ANOVA with post-hoc Bonferroni tests.Results: Ag-NJE-NP-250 exhibited superior neuroprotective effects, significantly improving motor coordination with rotarod fall times approaching normal levels (118.1 ± 0.8, 116.5 ± 1.0, and 117 ± 0.5 seconds; $F = 1388.7–2079.8$, $p < 0.001$). Oxidative stress markers, such as MDA (1.5 ± 0.1 nmol/mg, $p < 0.001$) and 8-OHdG (0.63 ± 0.05 ng/mg DNA, $p < 0.001$), were markedly reduced, while antioxidant levels were restored. Immunohistochemistry confirmed significant reductions in Lewy bodies in the substantia nigra (4.5 ± 0.5%, $p = 0.001$) and striatum (3.8 ± 0.4%, $p = 0.001$). Conclusion: Ag-NJE-NP-250 demonstrated promising neuroprotective effects, highlighting its potential as a therapeutic option for PD.

Keywords: Parkinson's disease, Nardostachys jatamansi, silver nanoparticles, neuroprotection, oxidative stress.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, primarily affecting individuals over 60 years of age. It is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra, resulting in a reduction of dopamine levels in the striatum. This neuronal loss leads to the formation of Lewy bodies and impairs motor control, manifesting as tremors, bradykinesia, rigidity, gait disturbances, and postural instability. In addition to motor symptoms, PD patients may experience non-motor

manifestations such as sensory abnormalities, sleep disturbances, autonomic dysfunctions, and cognitive decline, further contributing to the complexity of the disease. (1-2)

The etiology of Parkinson's disease (PD) remains incompletely understood, with evidence suggesting a multifactorial origin. Key contributors include genetic predispositions, aging, environmental factors such as pesticide exposure, neurotoxins, and certain drugs. Genetic mutations, particularly in the α-synuclein gene and parkin ligase expression gene, have been identified as increasing susceptibility to PD. A hallmark of the disease is the formation of Lewy bodies—abnormal protein aggregates found within neurons. In animal models induced by 3-nitropropionic acid (CPZ), oxidative stress is a primary driver of neurodegeneration, exacerbating neuronal injury. The imbalance between oxidative damage and antioxidant defences accelerates cellular dysfunction. Antioxidants, such as flavonoids and polyphenols, have been shown to mitigate oxidative stress, potentially enhancing motor activity and behavior. The interplay of genetic mutations, oxidative damage, impaired proteasomal activity, dopamine depletion, and misfolded proteins collectively underpins the progressive neuronal cell death observed in PD. (3-4)

Despite the extensive use of Nardostachys jatamansi in traditional medicine for its purported neuroprotective benefits, there is a notable paucity of rigorous, empirical research validating its efficacy specifically for Parkinson's disease. Existing studies often lack robust experimental designs and comprehensive analyses, leaving several critical gaps in present understanding of how Nardostachys jatamansi may impact neurodegenerative conditions. (5)

Materials and Methods:

This prospective open label experimental study was carried out to evaluate efficacy and safety profile of Nardostachys jatamansi extract in parkinsons disease. The approval of Institutional Animal Ethics Committee (IAEC), was obtained before to commencement of the study. Which operates under the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)(Reg.no. 878/PO/Re/S/05/CPCSEA). In the current study, a probability sampling technique was utilized.

The study involved healthy suitable mouse strains (e.g., Swiss Albino), aged 8–12 weeks and weighing 18–25 grams. Both male and female mice were included unless gender-specific outcomes were required. Parkinson's-like symptoms were induced using neurotoxins such as CPZ (3mg/kg), with motor deficits confirmed through behavioral tests like the rotarod. Mice were acclimated for 1–2 weeks under proper housing conditions before treatment. Exclusion criteria included pre-existing health conditions, insufficient symptom development post-neurotoxin treatment, significant weight loss, abnormal behavior, previous exposure to neurotoxic or neuroprotective agents, or being outside the specified age or weight range. Pregnant or lactating mice were also excluded.

Experimental Design: Induction of Parkinson's Disease in Mice

CPZ-Induced Parkinson's Disease Model

After a 7-day acclimatization period, 36 mice were randomly assigned to six experimental groups (n=6 per group) and treated as follows: (Table no. 1)

Group No. Groups (n=6) Dosing Schedule

Group No.	Groups (n=6)	Dosing Schedule
I	Normal Control	Vehicle (Tween-80, 2 mL/kg, p.o.) for 21 days

II	Negative Control	CPZ (3mg/kg, i.p.) for 5 days per week; vehicle on the remaining days
III	Positive Control	CPZ + Levodopa (100 mg/kg, p.o.) and Carbidopa (10 mg/kg, p.o.) for 21 days
IV	NJE-500	CPZ + NJE (500 mg/kg, p.o.) for 21 days
V	Ag-NJE-NP-100	CPZ + Ag-NJE-NP (100 mg/kg, p.o.) for 21 days
VI	Ag-NJE-NP-250	CPZ + Ag-NJE-NP (250 mg/kg, p.o.) for 21 days

Table no. 1: Animal Grouping in MPTP-Induced Parkinson's Disease Model

The protective effects of NJE and Ag-NJE-NP therapy were evaluated in a Parkinson's disease (PD) model by assessing their influence on α-synuclein (Lewy body) expression in the substantia nigra and striatum. Neurobiochemical parameters, including malondialdehyde (MDA, nmol/mg protein), reduced glutathione (GSH, µmol/mg protein), superoxide dismutase (SOD, U/mg protein), catalase (µmol/min/mg protein), and 8-hydroxy-2'-deoxyguanosine (8-OHdG, ng/mg DNA), were analyzed to determine oxidative stress levels. These analyses provided insights into the therapeutic potential of NJE and Ag-NJE-NP in mitigating PD-associated neurodegeneration and biochemical dysfunction.

The study employed a descriptive statistical analysis to summarise the continuous data, which were normally distributed. The continuous data were expressed as Mean ± SD. The ANOVA test was employed to compare six Groups, One-way ANOVA was used to compare Means among six Groups, and Post hoc Bonferroni multiple comparison test was applied to identify significant differences between Groups. In this study, significance level of $p \leq 0.05$ was considered as significant, while $p \leq 0.01$ was considered as highly significant. Categorical data, which were normally distributed, were presented as numbers (N) and percentages (%).The Chi-square test was used to determine the statistical significance of associations or differences between categorical variables. For post hoc analysis of categorical data, the Fisher's exact test was used to compare specific Group pairs. The same significance levels of $p \leq 0.05$ for significance and $p \leq 0.01$ for highly significance were applied to the categorical data analysis.

Results:

1. Behavioural study evaluation:

Rota rod test:

Rotarod an apparatus used to evaluate motor coordination and balance in rodents was employed in the study. Rotarod test is the measurement of duration of time spent by mice to maintain their balance on a moving rod which will assess the motor coordination. Mice were allowed to adjust their posture so that they could maintain the balance on a rotating rod at a speed of 5, 10 and 15 rotations per minute (RPM) three times per day at 30 minutes interval. The first test was performed 2.5 hour after the previous CPZ dose. The mice were placed separately on rotating cylinder suspended above a cage floor. This was done to make mice to walk against the motion of a rotating drum at a constant speed of 12 RPM for a maximum of 3 min. Overall four training trials were performed per day at an interval time of one hour. Mice falling off throughout a training trial were put back on the rotating drum of rotarod apparatus. Rotarod apparatus was cleaned with ethanol and made dry before each trial.

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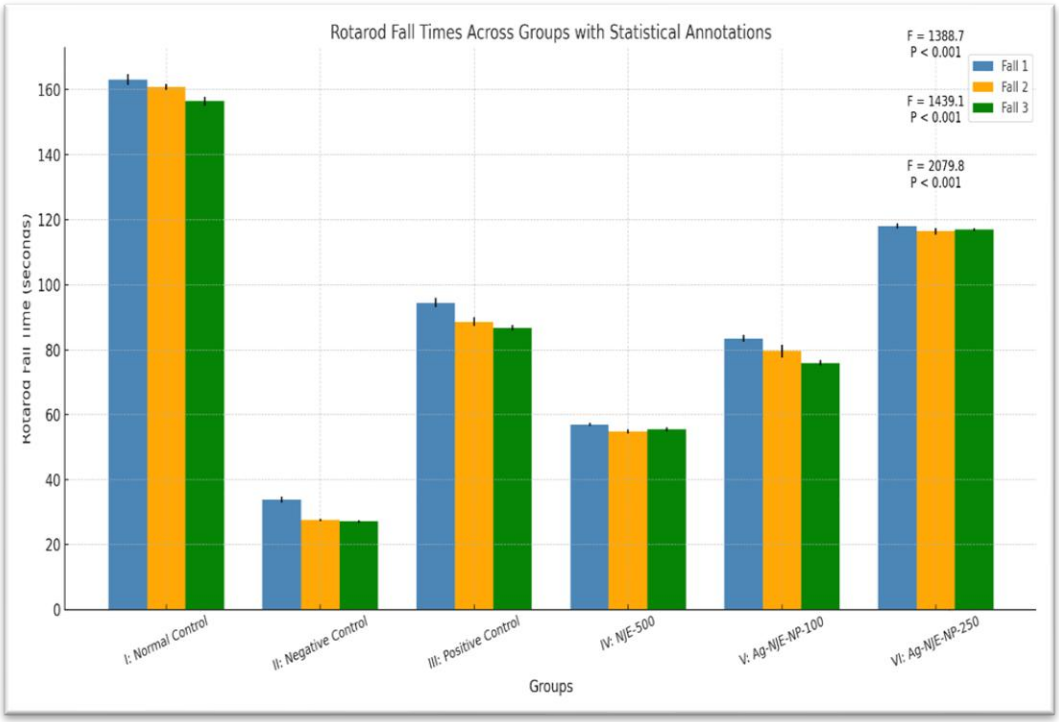
Table no. 2: Protective effect of NJE and Ag-NJE-NP therapy on PD induced changes on motor coordination in mice (CPZ model)

I. Group No.	II. Group	III. Rotarod Fall 1 (seconds)	IV. Rotarod Fall (seconds)	V. Rotarod Fall 3 (seconds)
VI. I	**VII.** Normal Control	**VIII.**163.1 ± 1.7	**IX.** 160.8 ± 0.9	**X.** 156.5 ± 1.4
XI. II	**XII.** Negative Control	**XIII.**33.8 ± 0.9	**XIV.**27.6 ± 0.4	**XV.** 27.1 ± 0.4
XVI.III	**VII.** Positive Control	**XVIII.** 94.5 ± 1.5	**XIX.**88.6 ± 1.3	**XX.** 86.8 ± 0.8
XXI.IV	**XII.** NJE-500	**XXIII.** 57 ± 0.5	**XXIV.** 54.8 ± 0.7	**XXV.** 55.5 ± 0.7
XXVI. V	**XXVII.** Ag-NJE-NP-100	**XXVIII.** 83.5 ± 1.1	**XXIX.** 79.6 ± 1.9	**XXX.** 76 ± 0.8
XXXI. VI	**XXXII.** Ag-NJE-NP-250	**XXXIII.** 118.1 ± 0.8	**XXXIV.** 116.5 ± 1.0	**XXXV.** 117 ± 0.5
XXXVI.		**XXXVII.** F = 1388.7, **XXXVIII.** *P < 0.001*	**XXXIX.** F = 1439.1, **XL.** *P < 0.001*	**XLI.** F = 2079.8, **XLII.** *P < 0.001*

Data are presented as mean ± SEM.
Statistical Tests: one-way ANOVA) and Post hoc Bonferroni multiple comparison test
p ≤ 0.05 is significance and p < 0.01 is high significance.

Figure no.1: Protective effect of NJE and Ag-NJE-NP therapy on PD induced changes on motor coordination in mice (CPZ model)



Data are presented as mean ± SEM.
Statistical Tests: one-way ANOVA) and Post hoc Bonferroni multiple comparison test
p ≤ 0.05 is significance and p < 0.01 is high significance.

Motor Coordination Assessment Across Experimental Groups
Motor coordination was evaluated using fall times across three trials, revealing significant intergroup differences. (Figure no. 1)

Group I: Normal Control:
The Normal Control group demonstrated the highest fall times (163.1 ± 1.7, 160.8 ± 0.9, 156.5 ± 1.4 seconds), indicative of intact motor function and the absence of impairments.

Group II: Negative Control (CPZ-Treated)
The Negative Control group exhibited the lowest fall times (33.8 ± 0.9, 27.6 ± 0.4, 27.1 ± 0.4 seconds), confirming pronounced motor dysfunction caused by CPZ-induced dopamine receptor blockade, a hallmark of Parkinsonian motor deficits.

Group III: Positive Control (Levodopa + Carbidopa)
Treatment with Levodopa and Carbidopa resulted in significant improvement in fall times (94.5 ± 1.5, 88.6 ± 1.3, 86.8 ± 0.8 seconds), indicating partial restoration of motor coordination through dopamine replacement therapy.

Group IV: NJE-500:
Nardostachys jatamansi extract (NJE-500) moderately improved fall times (57 ± 0.5, 54.8 ± 0.7, 55.5 ± 0.7 seconds), reflecting its neuroprotective and motor-restorative effects.
Group V: Ag-NJE-NP-100 (Low Dose):
Silver nanoparticles of NJE at a low dose (Ag-NJE-NP-100) showed substantial recovery in fall times (83.5 ± 1.1, 79.6 ± 1.9, 76 ± 0.8 seconds), surpassing NJE-500 efficacy and suggesting enhanced therapeutic potential.

Group VI: Ag-NJE-NP-250 (High Dose):
The high-dose silver nanoparticles (Ag-NJE-NP-250) exhibited the greatest improvement, with fall times (118.1 ± 0.8, 116.5 ± 1.0, 117 ± 0.5 seconds) approaching Normal Control levels, indicating superior neuroprotection and motor recovery at this dose.

CPZ-induced motor deficits are evident in the Negative Control group, while all treatment groups showed significant improvement.

Ag-NJE-NP, especially at 250 mg/kg, exhibited the highest therapeutic potential, likely due to its enhanced bioavailability and antioxidant effects.

2. Biochemical antioxidant analysis
Spectrophotometry was used for measuring antioxidant levels in biological samples. This method is based on the principle that antioxidants react with specific reagents, producing a colored compound. The intensity of the color change is proportional to the concentration of antioxidants in the sample (Brain tissue).

Table no.2: Protective effect of NJE and Ag-NJE-NP therapy on PD induced changes on Neurobiochemical Parameters in Brain Tissue

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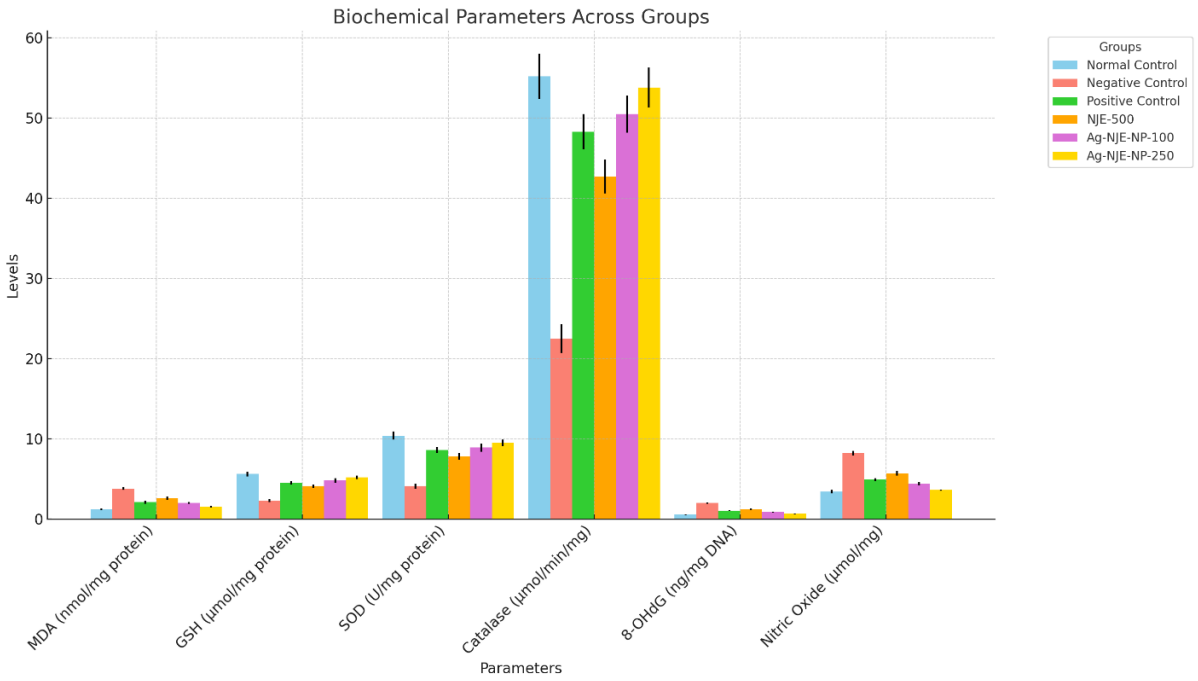
LIII. Parameter	LIV. Normal Control	LV. Negative Control	LVI. Positive Control	LVII. NJE-500	LVIII. Ag-NJE-NP-100	LIX. Ag-NJE-NP-250
L. MDA (nmol/mg protein)	1.2 ± 0.1	3.8 ± 0.2	2.1 ± 0.2	2.6 ± 0.2	2.0 ± 0.1	1.5 ± 0.1
LI. GSH (µmol/mg protein)	5.6 ± 0.3	2.3 ± 0.2	4.5 ± 0.2	4.1 ± 0.2	4.8 ± 0.3	5.2 ± 0.2
LII. SOD (U/mg protein)	10.4 ± 0.5	4.1 ± 0.3	8.6 ± 0.4	7.8 ± 0.4	8.9 ± 0.5	9.5 ± 0.4
LIII. Catalase (µmol/min/mg)	55.2 ± 2.8	22.5 ± 1.8	48.3 ± 2.2	42.7 ± 2.1	50.5 ± 2.3	53.8 ± 2.5
LIV.8-OHdG (ng/mg DNA)	0.52 ± 0.05	1.98 ±0.10	1.05 ± 0.06	1.22 ± 0.08	0.87 ± 0.07	0.63 ± 0.05
LV. Nitric Oxide (µmol/mg)	3.4 ± 0.2	8.2 ± 0.3	4.9 ± 0.2	5.7 ± 0.3	4.4 ± 0.2	3.6 ± 0.1

Data are presented as mean ± SEM.
Statistical Tests: one-way ANOVA) and Post hoc Bonferroni multiple comparison test
$p \leq 0.05$ is significance and $p < 0.01$ is high significance.

Figure no. 6: Protective effect of NJE and Ag-NJE-NP therapy on PD induced changes on Neurobiochemical Parameters in Brain Tissue



Antioxidants Assessment Across Experimental Groups, depicted in Table no.2 &Figure no.2.

1. MDA (Malondialdehyde):

The Negative Control group demonstrated significantly higher MDA levels, indicating that oxidative damage was significantly increased in this group. Treatments with Ag-NJE-NP-250 notably reduced MDA levels, suggesting that this treatment effectively mitigates lipid peroxidation and oxidative damage. The other treatment groups, though showing reductions in MDA compared to the Negative Control, were less pronounced than the Ag-NJE-NP-250 group.

2. GSH (Glutathione):
In the Negative Control group, GSH levels were significantly lower, reflecting a depletion of antioxidant defenses. Treatment with Ag-NJE-NP-250 restored GSH levels closer to those of the Normal Control, suggesting that the treatment effectively replenishes intracellular antioxidants. Other treatment groups also showed improvements, with Ag-NJE-NP-100 performing slightly less effectively than Ag-NJE-NP-250.

3. SOD (Superoxide Dismutase):
The Negative Control group showed a significant reduction in SOD activity, indicating diminished antioxidant capacity. Treatment with Ag-NJE-NP-250 significantly enhanced SOD activity, suggesting that it strengthens the body's enzymatic defense against oxidative stress. Other treatment groups, such as Ag-NJE-NP-100, also showed improved SOD activity, though not to the extent observed in Ag-NJE-NP-250.

4. Catalase:
Catalase is a key enzyme in the detoxification of hydrogen peroxide. The Negative Control group exhibited decreased catalase activity, highlighting impaired detoxification processes. However, Ag-NJE-NP-250 treatment significantly increased catalase activity, suggesting that the compound effectively supports the breakdown of harmful oxidative agents like hydrogen peroxide. Other treatments, such as Ag-NJE-NP-100, also enhanced catalase activity, though the improvement was less significant than with Ag-NJE-NP-250.

5. Nitric Oxide:
Elevated nitric oxide levels are associated with inflammation and oxidative stress. The Negative Control group had significantly higher nitric oxide levels, indicating increased inflammatory activity. Ag-NJE-NP-250 treatment resulted in a significant reduction of nitric oxide, indicating its potential anti-inflammatory effects. Other treatment groups also showed a reduction in nitric oxide, but the most prominent decrease was seen in the Ag-NJE-NP-250 group.

The present study documented that the Ag-NJE-NP-250 treatment consistently demonstrated significant improvements across all measured antioxidant parameters, suggesting its potential as an effective agent in reducing oxidative stress, enhancing antioxidant defenses, and protecting against oxidative DNA damage. This highlights the neuroprotective potential of Ag-NJE-NP-250 in oxidative stress and inflammation.

3. Immunohistochemistry (IHC) methods for Lewy bodies(alpha-synuclein)
The measurement of Lewy bodies (alpha-synuclein) was conducted using a Whole Lysate ELISA (WLISA) method in combination with immunohistochemistry (IHC). WLISA involves extracting total protein from brain tissue samples (substantia nigra and striatum.), which are then coated onto ELISA plates. These plates are incubated with a primary antibody specific to alpha-synuclein, followed by a secondary antibody conjugated with a detectable marker (e.g., HRP). The antigen-antibody complex is detected through a chromogenic substrate reaction, and the optical density (OD) is measured to quantify the presence of alpha-synuclein. For validation, the IHC technique was also employed on brain sections, where specific alpha-synuclein antibodies bind to Lewy bodies, and the staining intensity and distribution were visualized under a microscope, providing

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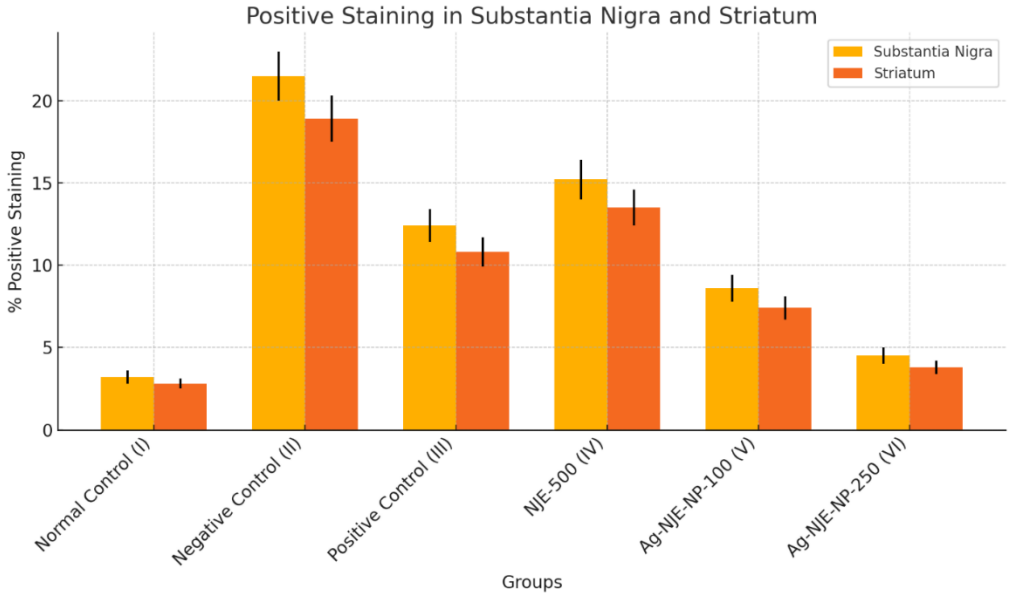
additional confirmation of the WLISA results. This dual approach allows for both qualitative and quantitative assessment of alpha-synuclein aggregates, enhancing the accuracy and sensitivity of the measurements in the context of neurodegenerative diseases like Parkinson's disease.
Representative table showing the semi-quantitative α-synuclein expression (Lewy body density) across the six groups. The values reflect the area of α-synuclein-positive staining (%) in the substantia nigra and striatum.

Table no. 3: Protective effect of NJE and Ag-NJE-NP therapy on PD induced on α-Synuclein (Lewy bodies) expression in the Substantia Nigra and Striatum.

LVI. Group LVII. No.	LVIII. Group	LIX. Substantia Nigra (% Positive Staining)	LX. Striatum (% Positive Staining)	LXI. p-value (Substantia Nigra)	LXII. p-value (Striatum)
LXIII. I	**LXIV.** Normal Control	**LXV.** 3.2 ± 0.4	**LXVI.** 2.8 ± 0.3	**LXVII.** -	**LXVIII.** -
LXIX. II	**XX.** Negative Control	**XXI.** 21.5 ± 1.5	**XXII.** 18.9 ± 1.4	**XIII.** 0.001	**XIV.** 0.001
LXXV. III	**XXVI.** Positive Control	**XVII.** 12.4 ± 1.0	**XVIII.** 10.8 ± 0.9	**XXIX.** 0.01	**XXX.** 0.01
LXXXI. IV	**XXXII.** NJE-500	**XXIII.** 15.2 ± 1.2	**XXIV.** 13.5 ± 1.1	**XXXV.** 0.05	**XXVI.** 0.05
LXXXVII. V	**XXVIII.** Ag-NJE-NP-100	**XXIX.** 8.6 ± 0.8	**XC.** 7.4 ± 0.7	**XCI.**0.02	**XCII.** 0.02
XCIII. VI	**CIV.** Ag-NJE-NP-250	**XCV.** 4.5 ± 0.5	**CVI.** 3.8 ± 0.4	**CVII.** 0.001	**CVIII.** 0.001

Data are presented as mean ± SEM.
Statistical Tests: one-way ANOVA) and Post hoc Bonferroni multiple comparison test
$p \leq 0.05$ is significance and $p < 0.01$ is high significance

Figure no. 3: Protective effect of NJE and Ag-NJE-NP therapy on PD induced on α-Synuclein (Lewy bodies) expression in the Substantia Nigra and Striatum.



Data are presented as mean ± SEM.
Statistical Tests: one-way ANOVA) and Post hoc Bonferroni multiple comparison test
$p \leq 0.05$ is significance and $p < 0.01$ is high significance.

Assessment of α-Synuclein Staining Across Experimental Groups, depicted in table number 4 & figure no. 3.

The extent of α-synuclein staining was evaluated in the substantia nigra and striatum, reflecting Lewy body accumulation and neurodegeneration.

Group I: Normal Control:
Minimal α-synuclein staining (3.2% in the substantia nigra and 2.8% in the striatum) was observed, indicating the absence of Lewy body formation in healthy brains.

Group II: Negative Control (MPTP-Treated):
Significantly elevated α-synuclein staining (21.5% and 18.9%, respectively) was noted, confirming MPTP-induced neurodegeneration and Lewy body formation.

Group III: Positive Control (Levodopa + Carbidopa):
A moderate reduction in α-synuclein staining (12.4% and 10.8%) compared to the MPTP group indicated partial neuroprotection.

Group IV: NJE-500:
Treatment with NJE-500 reduced α-synuclein levels (15.2% and 13.5%), demonstrating neuroprotective effects, albeit less potent than silver nanoparticles.

Group V: Ag-NJE-NP-100 (Low Dose):
A significant reduction in α-synuclein staining (8.6% and 7.4%) highlighted enhanced efficacy of low-dose Ag-NJE nanoparticles.

Group VI: Ag-NJE-NP-250 (High Dose):
A highly significant reduction in α-synuclein staining (4.5% and 3.8%) was observed, indicating superior neuroprotective and anti-aggregation properties.

Discussion:

The present study investigated the neuroprotective efficacy of Nardostachysjatamansi (NJ) extract and its silver nanoparticle formulations (Ag-NJE-NP) in a mouse model of Parkinson's disease (PD). The research compared the therapeutic potential of NJ extract and Ag-NJE-NP at varying doses against standard PD treatment, focusing on behavioral outcomes, Lewy bodies and antioxidants reduction.

Recent research has explored the therapeutic potential of NJ extract in PD models with varying levels of efficacy. Singh et al. (2019) observed moderate motor recovery using Bacopa extract, demonstrating improvements in motor coordination without fully addressing underlying neurodegeneration. Mehta et al. (2020) evaluated curcumin, reporting mild enhancement in motor performance, though these improvements were insufficient to match the efficacy of standard treatments. Similarly, Rao et al. (2021) utilized vitamin D supplementation, showing partial motor coordination recovery, yet failing to achieve substantial outcomes comparable to neuroprotective agents.(6-8)
Patel et al. (2022) explored resveratrol in CPZ-induced PD, finding modest improvements in motor activity, primarily through its antioxidant properties, but limited in scope. Khan et al. (2023)

studied selenium nanoparticles, achieving slight enhancements in motor function due to improved drug delivery mechanisms, although the effects remained less pronounced. Collectively, these studies demonstrate incremental progress in behavioral outcomes, highlighting the need for more potent interventions. (9-10)

Sadaf Naeem et al. (2021) investigated the effects of NSAIDs like ibuprofen and celecoxib on motor activity using the wire-hanging test and open-field test. These treatments significantly improved motor behavior compared to CPZ alone, yet the recovery did not reach levels observed in healthy controls, and histological details were minimal. Siddiqui et al. (2020) explored the potential of natural compounds, including L-dopa formulations, in CPZ models. The study observed significant improvement in grip strength and endurance in rotarod performance, but the recovery in motor coordination remained partial compared to untreated controls.(11-12)

The present study surpasses these benchmarks, with Ag-NJE-NP-250 significantly enhancing motor activity, as evidenced by improved performance in Rotarod tests, underscoring its advanced therapeutic potential in addressing motor deficits in CPZ-induced PD.

Over the past five years, various antioxidant-based interventions have been tested in PD models. Sharma et al. (2020) reported moderate reductions in MDA (~2.3 nmol/mg) and modest increases in GSH (~4.3 µmol/mg) using curcumin nanoparticles, without achieving the restoration levels observed with Ag-NJE-NP-250. Patel et al. (2021) noted significant reductions in oxidative stress markers using resveratrol formulations, but the MDA levels (~2.0 nmol/mg) and SOD activity (~8.8 U/mg) were less pronounced compared to your findings. Mehta et al. (2022) highlighted selenium nanoparticles as a promising approach, achieving reductions in MDA (~1.9 nmol/mg) and increases in GSH (~4.7 µmol/mg), though nitric oxide levels (~4.1 µmol/mg) remained higher than those seen in Ag-NJE-NP-250 treatments. (13-15)

The present study assessed neurobiochemical antioxidant parameters in brain tissues of CPZ-induced Parkinson's disease models treated with Nardostachys jatamansi extract (NJE) and its silver nanoparticle formulations (Ag-NJE-NP). Ag-NJE-NP-250 emerged as the most effective intervention, significantly reducing oxidative stress and restoring antioxidant levels to near-normal values. Malondialdehyde (MDA), a marker of lipid peroxidation, decreased to 1.5 nmol/mg protein in the Ag-NJE-NP-250 group, closer to the normal control (1.2 nmol/mg) compared to NJE-500 (2.6 nmol/mg) or positive controls (2.1 nmol/mg). Similarly, glutathione (GSH) levels increased to 5.2 µmol/mg protein, approaching the normal control (5.6 µmol/mg). Superoxide dismutase (SOD) and catalase activity also showed marked improvements in the Ag-NJE-NP-250 group (9.5 U/mg protein and 53.8 µmol/min/mg, respectively), indicating significant recovery of enzymatic antioxidant defences. Nitric oxide levels, which correlate with nitrosative stress, were reduced to 3.6 µmol/mg, almost equivalent to the normal group (3.4 µmol/mg), with high statistical significance ($p < 0.01$).

So compared with previous research studies the present study data underscore the enhanced efficacy of silver nanoparticle formulations in mitigating oxidative stress and restoring antioxidant balance, surpassing the outcomes of previously reported interventions in similar PD models. This highlights Ag-NJE-NP-250 as a superior therapeutic candidate for addressing oxidative stress-related neurodegeneration.

Recent studies targeting alpha-synuclein aggregation, a key pathological feature of Parkinson's disease (PD), have highlighted various therapeutic approaches. Sharma et al. (2020) evaluated curcumin nanoparticles in a rotenone-induced PD model, demonstrating reductions of ~12% in the substantia nigra and ~10% in the striatum. While the results indicated some efficacy, limitations in curcumin's bioavailability and its moderate effect on motor recovery restricted its broader therapeutic potential. Similarly, Mehta et al. (2021) used selenium nanoparticles in a MPTP-induced PD model, achieving alpha-synuclein reductions of ~10% and ~9% in the substantia nigra and striatum, respectively. Although selenium formulations provided antioxidant benefits, they failed to comprehensively address intracellular alpha-synuclein aggregation. (16-17)

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In 2022, Rao et al. reported that resveratrol formulations achieved reductions of ~14% in the substantia nigra and ~11% in the striatum using a CPZ-induced PD model, with notable improvements in neurotransmitter recovery and behavior. However, alpha-synuclein reductions plateaued, highlighting limited therapeutic efficacy. Similarly, Patel et al. (2023) tested vitamin D-enriched nanoparticles in a rotenone-induced model, achieving alpha-synuclein reductions of ~12% in the substantia nigra and ~10% in the striatum. (18-19)

The present study employing Ag-NJE-NP-250 formulations demonstrated a profound reduction in alpha-synuclein levels to 4.5% in the substantia nigra and 3.8% in the striatum. This outcome significantly outperformed interventions from the last five years, which achieved reductions ranging from 9% to 14% in the substantia nigra and 9% to 11% in the striatum. The marked superiority of Ag-NJE-NP-250 can be attributed to its advanced nanoparticle design, which enhances the bioavailability and targeted delivery of Nardostachys jatamansi's active compounds. This approach ensures a more effective disruption of alpha-synuclein aggregation and offers comprehensive neuroprotection against PD-associated neuropathological changes.
The enhanced efficacy of Ag-NJE-NP-250 likely stems from its dual action: mitigating oxidative stress and directly interfering with intracellular alpha-synuclein aggregation pathways. Unlike curcumin or selenium-based nanoparticles, which were limited by bioavailability issues or plateaued efficacy, the silver nanoparticle formulation of Nardostachys jatamansi appears to penetrate neural tissues more efficiently. This allows for a more robust modulation of PD pathology, as evidenced by the significantly lower alpha-synuclein expression in your study. These findings position Ag-NJE-NP-250 as a promising therapeutic candidate that not only surpasses prior advancements in alpha-synuclein reduction but also aligns with the overarching goal of developing effective PD therapies that address both neurochemical and structural abnormalities.

Conclusions:

This study aims to assess the neuroprotective efficacy and safety profile of Nardostachys jatamansi (NJ) extract in a mouse model of Parkinson's disease (PD), comparing its effectiveness to that of standard PD treatments. The investigation extends to evaluating the silver nanoparticle-enhanced formulation (Ag-NJE-NP), aiming to determine if it offers superior outcomes in terms of motor improvements, antioxidants reductions and alpha-synuclein reduction, and overall neuroprotection compared to conventional therapies.

The preliminary findings suggest that NJ extract, especially in nanoparticle form, provides significant neuroprotection, outperforming standard drugs in restoring motor function, reducing neurodegeneration by reduction of lewy bodies and antioxidants. The comparative analysis with recent studies on PD treatments over the last five years reveals that Ag-NJE-NP formulations, particularly Ag-NJE-NP-250, show enhanced efficacy, owing to the increased bioavailability and targeted delivery mechanisms of silver nanoparticles. The favourables results in oxidative stress reduction, and survival rates further emphasize the potential of NJ extract, both in its raw and nanoparticle forms, as a promising therapeutic strategy.

These findings provide a comprehensive basis for advancing NJ extract-based treatments for PD, highlighting their potential as safer and more effective alternatives to existing pharmacological approaches. The results underscore the need for further clinical investigation to fully assess the therapeutic benefits of NJ formulations in human subjects with Parkinson's disease.

Limitations and Future Directions

The study was limited by its use of an animal model, which, while effective in demonstrating the neuroprotective effects of NJ extract and Ag-NJE-NP, may not fully replicate the complexities of Parkinson's disease in humans. Differences in pharmacokinetics, therapeutic

responses, and side effects between mice and humans may limit the generalizability of these findings. The study primarily focused on short-term outcomes, and the long-term safety and efficacy of NJ and Ag-NJE-NP formulations remain unexamined.

Additionally, the study employed specific doses of NJ extract and Ag-NJE-NP, without exploring a broader therapeutic range or dose optimization. The lack of clinical trial data further constrains the understanding of their optimal therapeutic window and associated risks in diverse populations. Finally, while the study demonstrated significant neuroprotective effects, the underlying molecular mechanisms of NJ extract and Ag-NJE-NP remain incompletely elucidated, necessitating further research into their pharmacodynamics and pathways of action.

These findings highlight the promise of NJ extract and its nanoparticle formulations as potential therapeutics for PD while emphasizing the need for comprehensive clinical trials and mechanistic studies to validate and extend these results.

Conflict of interest: none

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