RESEARCH PAPER

Combined Neuroprotective Effects of *Nardostachys jatamansi* and *Tinospora cordifolia* in a Chronic Rotenone-Induced Rat Model of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by selective degeneration of dopaminergic neurons in the substantia nigra pars compacta, resulting in dopamine depletion and characteristic motor deficits. Oxidative stress, mitochondrial dysfunction, and neuroinflammation are key interrelated pathogenic mechanisms. The present study investigated the neuroprotective efficacy of standardized hydroalcoholic extracts of *Nardostachys jatamansi* (NJ) and *Tinospora cordifolia* (TC), administered individually and in combination, against chronic rotenone-induced Parkinsonism in Wistar rats. Rotenone (2 mg/kg, s.c.) was administered for 35 days. Treatment groups received NJ (400 mg/kg), TC (400 mg/kg), or their combination (200 mg/kg each) orally. Behavioral performance, oxidative stress markers, neurochemical parameters, pro-inflammatory cytokines, histopathology, and tyrosine hydroxylase (TH) immunohistochemistry were evaluated. Rotenone produced marked motor impairment, depletion of antioxidant defenses, elevation of lipid peroxidation and nitrite, reduction in dopamine and its metabolites, increased TNF-α, IL-1β and IL-6, neuronal architectural damage, and significant loss of TH-positive neurons. Both individual extracts attenuated these alterations; however, the combination treatment produced superior numerical improvement across the majority of endpoints and approached the efficacy of the standard drug (levodopa + carbidopa). These findings indicate that the NJ–TC combination exerts multi-target neuroprotection and warrants further investigation as a potential therapeutic strategy for Parkinsonian neurodegeneration.

Keywords: Parkinson's disease, rotenone, *Nardostachys jatamansi*, *Tinospora cordifolia*, oxidative stress, neuroinflammation, dopamine, neuroprotection.

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1. Introduction

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease and is clinically defined by bradykinesia, resting tremor, rigidity, and postural instability [1,2]. The pathological hallmark is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, accompanied by the accumulation of α-synuclein-containing Lewy bodies [1,3]. Multiple interconnected mechanisms, including mitochondrial complex-I dysfunction, excessive generation of reactive oxygen and nitrogen species, impaired antioxidant defenses, neuroinflammation, and defective autophagy, contribute to dopaminergic neuronal vulnerability [3,4].

Rotenone, a naturally occurring pesticide and classical inhibitor of mitochondrial complex I, has been widely used to model PD in rodents. Chronic systemic administration of rotenone reproduces several key features of the human disease, including nigrostriatal dopaminergic degeneration, oxidative stress, neuroinflammation, α-synuclein aggregation, and motor deficits [5–7]. Consequently, the rotenone model is relevant for evaluating candidate neuroprotective agents.

Medicinal plants have attracted increasing attention as sources of multi-target neuroprotective compounds. *Nardostachys jatamansi* (Valerianaceae), commonly known as Jatamansi, has been investigated for its potential to improve motor performance, restore antioxidant enzyme activities, reduce lipid peroxidation, and protect dopaminergic neurons in experimental models of PD. Its proposed mechanisms include modulation of the Nrf2 pathway and inhibition of NLRP3 inflammasome signaling [8,9]. *Tinospora cordifolia* (Menispermaceae), commonly known as Guduchi or Giloy, possesses antioxidant, anti-inflammatory, and immunomodulatory properties. Previous investigations have explored its effects on neuroinflammation, pro-inflammatory cytokines, tyrosine hydroxylase expression, oxidative stress, and mitochondrial dysfunction in experimental Parkinsonian models [10,11].

Although both plants have been investigated individually for their potential neuroprotective effects, their combined administration requires systematic evaluation in a chronic rotenone model. Therefore, the present study was designed to compare the effects of NJ and TC individually and in combination on behavioral, biochemical, neurochemical, inflammatory, histopathological,

and immunohistochemical parameters in rotenone-induced Parkinsonism in rats.

2. Materials and Methods

2.1 Plant Extracts

Standardized hydroalcoholic extracts of *Nardostachys jatamansi* (rhizome) and *Tinospora cordifolia* (stem) were purchased from a commercial supplier of herbal extracts. The extracts were obtained with supplier-provided authentication and standardization information concerning their major phytoconstituents. The purchased extracts were stored in airtight containers at 4°C until use and suspended in 0.5% carboxymethyl cellulose (CMC) for oral administration.

2.2 Chemicals and Reagents

Rotenone, levodopa, carbidopa, and analytical-grade chemicals required for biochemical assays were procured from Sigma-Aldrich (St. Louis, MO, USA) or equivalent suppliers, as applicable. ELISA kits for TNF-α, IL-1β, and IL-6 were obtained from commercial sources. Primary antibodies against tyrosine hydroxylase and other reagents required for immunohistochemistry were purchased from established vendors.

2.3 Experimental Animals

Adult male Wistar rats weighing 180–200 g were housed under standard laboratory conditions, with a temperature of 22 ± 2°C, relative humidity of 55 ± 5%, and a 12-hour light/dark cycle. Animals were provided with standard pellet diet and water *ad libitum*. They were acclimatized for one week before the commencement of the experiment. All experimental procedures were required to comply with the approval of the Institutional Animal Ethics Committee (IAEC) and the applicable guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4 Experimental Design and Treatment Schedule

Animals were randomly divided into six groups, with six animals in each group.

Group I (Normal Control): Animals received vehicle (0.5% CMC, p.o.) and sunflower oil (s.c.).

Group II (Rotenone Control): Animals received rotenone at a dose of 2 mg/kg, subcutaneously, dissolved in sunflower oil, once daily for 35 days.

Group III (Standard Drug): Animals received rotenone along with levodopa (100 mg/kg, p.o.) and carbidopa (10 mg/kg, p.o.).

Group IV (*Nardostachys jatamansi*): Animals received rotenone along with NJ extract at a dose of 400 mg/kg, orally.

Group V (*Tinospora cordifolia*): Animals received rotenone along with TC extract at a dose of 400 mg/kg, orally.

Group VI (Combination Treatment): Animals received rotenone along with NJ extract (200 mg/kg, p.o.) and TC extract (200 mg/kg, p.o.).

Rotenone was administered subcutaneously once daily for 35 consecutive days. Plant extracts and the standard drug were administered orally one hour before rotenone administration throughout the experimental period. The final protocol should specify whether the standard drug was administered daily and confirm that the treatment schedule, vehicle administration, and timing were consistently followed.

2.5 Behavioral Assessments

Behavioral assessments were conducted at the end of the 35-day treatment period in a quiet room during the light phase of the light/dark cycle.

Rotarod Test: Motor coordination and balance were assessed using an accelerating rotarod apparatus. Animals were trained for three consecutive days before the final assessment. The latency to fall from the rotating rod was recorded in seconds [12].

Pole Test: Bradykinesia and motor initiation were evaluated by placing each animal head-up on top of a vertical pole and recording the time required to descend to the base [13].

Open-Field Test: Locomotor activity and exploratory behavior were assessed in a square open-field arena over a period of five minutes. The number of squares crossed and the frequency of rearing were recorded [14].

Catalepsy Bar Test: Catalepsy was assessed by placing the animal's forepaws on a horizontal bar positioned 9 cm above the surface and recording the latency until both paws were removed [15].

Grip Strength Test: Neuromuscular strength was measured using a digital grip strength meter, and the peak force generated was recorded in grams [16].

2.6 Biochemical Estimations

At the end of the experimental period, animals were sacrificed under deep anesthesia in accordance with the approved animal protocol. Brains were rapidly dissected on ice, and the relevant midbrain and striatal regions were homogenized in ice-cold phosphate buffer (pH 7.4) for biochemical analysis.

Superoxide Dismutase (SOD): SOD activity was determined using a spectrophotometric method based on the inhibition of nitroblue tetrazolium reduction [17].

Catalase: Catalase activity was estimated spectrophotometrically by monitoring the decomposition of hydrogen peroxide at 240 nm [18].

Reduced Glutathione (GSH): Reduced glutathione levels were estimated using 5,5′-dithiobis-(2-nitrobenzoic acid), commonly known as Ellman's reagent [19].

Malondialdehyde (MDA): Lipid peroxidation was assessed using the thiobarbituric acid reactive substances (TBARS) assay [20].

Nitrite: Nitrite content was determined using the Griess reagent as an indirect indicator of nitric oxide production [21].

Protein concentrations in tissue homogenates were determined using the Bradford method. Assay

conditions, sample preparation, calibration procedures, and units of expression should be specified in accordance with the methods actually performed.

2.7 Neurochemical Analysis

The concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in striatal tissue were quantified using high-performance liquid chromatography (HPLC) with electrochemical detection, following an established analytical protocol [22]. Results were expressed as ng/mg tissue.

2.8 Estimation of Pro-inflammatory Cytokines

The concentrations of TNF-α, IL-1β, and IL-6 in brain homogenates were determined using commercially available sandwich ELISA kits according to the manufacturers' instructions [10]. Sample preparation, standard curves, assay sensitivity, and the units used for reporting cytokine concentrations should be documented. Cytokine concentrations should be expressed according to the assay output and the normalization procedure actually used.

2.9 Histopathological Examination

Brain tissues were fixed in 10% neutral buffered formalin, dehydrated through graded alcohol concentrations, embedded in paraffin, and sectioned at a thickness of 5 µm. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope to assess neuronal morphology, cellular integrity, and tissue architecture [23]. Histopathological observations should be documented using representative photomicrographs and a predefined assessment procedure.

2.10 Tyrosine Hydroxylase Immunohistochemistry

Paraffin-embedded brain sections were subjected to immunohistochemical staining for tyrosine hydroxylase using a specific primary antibody, followed by a suitable secondary detection system and diaminobenzidine (DAB) visualization. TH-positive neurons in the substantia nigra were assessed under a light microscope [24]. The antibody details, antigen retrieval procedure, dilution, incubation conditions, negative controls, and method of neuronal counting should be reported. Cell counts should be obtained using a consistent and predefined sampling procedure.

2.11 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM), with six animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple-comparison test to compare each treatment group with the rotenone control group. A *p*-value of less than 0.05 was considered statistically significant. The statistical method should be selected according to the experimental design and the assumptions of the data. For repeated measurements, such as body weight recorded across multiple time points, an appropriate repeated-measures or mixed-effects analysis may be more suitable than one-way ANOVA. Statistical significance should only be reported when confirmed by analysis of the underlying individual-animal data.

3. Results

3.1 Effect on Body Weight

Chronic administration of rotenone was associated with a progressive reduction in body weight throughout the 35-day experimental period. On day 0, mean body weights were comparable across all groups, ranging from approximately 180 to 182 g. By day 35, the rotenone control group exhibited a marked decline in body weight to 141.33 ± 0.80 g, whereas the normal control group showed an increase to 191.16 ± 1.55 g. The standard-drug group recorded a final body weight of 200.16 ± 0.79 g. Both individual plant extracts attenuated the observed body-weight reduction. The final body weights in the NJ and TC groups were 191.83 ± 0.47 g and 195.66 ± 0.66 g, respectively. The combination-treated group recorded a final body weight of 198.30 ± 0.66 g, numerically approaching the value observed in the standard-drug group (Table 1).

Table 1. Effect of different treatments on body weight (g)

Group	Treatment	Day 0	Day 35
I	Normal Control	180.66 ± 0.49	191.16 ± 1.55
II	Rotenone Control	181.17 ± 0.79	141.33 ± 0.80
III	Standard Drug	180.83 ± 0.60	200.16 ± 0.79
IV	*N. jatamansi*	181.33 ± 0.88	191.83 ± 0.47
V	*T. cordifolia*	181.83 ± 0.60	195.66 ± 0.66
VI	NJ + TC	181.16 ± 0.79	198.30 ± 0.66

Values are expressed as mean ± SEM (n = 6).

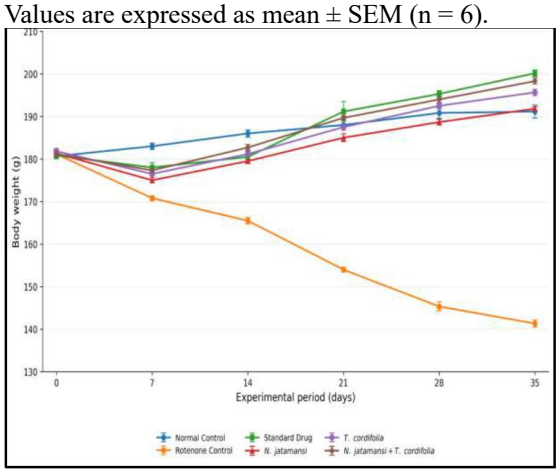


Figure 1. Effect on body weight

3.2 Behavioral Assessments

Rotarod Performance: Rotenone administration reduced the rotarod latency from 280.10 ± 3.27 s in the normal control group to 41.83 ± 3.17 s in the rotenone control group. Standard-drug treatment increased the latency to 236.50 ± 6.44 s. Treatment with NJ and TC increased the values to 175.30 ± 3.56 s and 201.80 ± 6.06 s, respectively. The combination-treated group recorded a latency of 221.50 ± 7.31 s, representing the highest numerical value among the plant-treated groups (Table 2).

Pole Test: The time required to descend the pole increased from 4.25 ± 0.50 s in normal controls to 20.78 ± 1.47 s in rotenone-treated animals. Standard-drug treatment reduced the descent time to 7.73 ± 0.30 s. NJ and TC treatments reduced the descent times to 11.13 ± 0.52 s and 10.15 ± 0.34 s, respectively. The combination group recorded a descent time of 8.41 ± 0.37 s (Table 2).

Open-Field Test: Locomotor activity, expressed as the number of squares crossed, decreased from 95.50 ± 4.16 in the normal control group to 27.33 ± 3.89 in the rotenone control group. Standard-drug treatment increased the number of squares crossed to 67.83 ± 3.56. NJ and TC treatments produced values of 52.66 ± 4.26 and 60.16 ± 3.55, respectively, while the combination group recorded 63.66 ± 3.93. Rearing frequency followed a similar pattern, with the combination treatment producing the highest numerical recovery among the plant-treated groups (Table 2).

Table 2. Effect of treatments on motor coordination and spontaneous activity

Parameter	Normal Control	Rotenone Control	Standard Drug	*N. jatamansi*	*T. cordifolia*	NJ + TC
Rotarod latency (s)	280.10 ± 3.27	41.83 ± 3.17	236.50 ± 6.44	175.30 ± 3.56	201.80 ± 6.06	221.50 ± 7.31
Pole test (s)	4.25 ± 0.50	20.78 ± 1.47	7.73 ± 0.30	11.13 ± 0.52	10.15 ± 0.34	8.41 ± 0.37
Squares crossed	95.50 ± 4.16	27.33 ± 3.89	67.83 ± 3.56	52.66 ± 4.26	60.16 ± 3.55	63.66 ± 3.93
Rearing frequency	22.00 ± 0.93	4.83 ± 0.79	17.66 ± 0.84	8.00 ± 0.57	12.00 ± 0.57	15.66 ± 0.98

Values are expressed as mean ± SEM (n = 6).

Catalepsy and Grip Strength: Catalepsy duration increased from 2.50 ± 0.34 s in the normal control group to 96.00 ± 5.02 s in the rotenone control group. Standard-drug treatment reduced the duration to 28.66 ± 2.81 s. NJ and TC treatments reduced catalepsy duration to 56.83 ± 3.01 s and 48.00 ± 3.39 s, respectively. The combination group recorded the lowest catalepsy duration among the plant-treated groups, at 37.33 ± 0.88 s. Grip strength decreased to 250.80 ± 15.66 g in the rotenone group, compared with 625.10 ± 18.49 g in normal controls. The combination treatment produced a grip strength of 534.60 ± 7.99 g, the highest value among the plant-treated groups (Table 3).

Table 3. Effect of treatments on catalepsy and grip strength

Parameter	Normal Control	Rotenone Control	Standard Drug	*N. jatamansi*	*T. cordifolia*	NJ + TC
Catalepsy time (s)	2.50 ± 0.34	96.00 ± 5.02	28.66 ± 2.81	56.83 ± 3.01	48.00 ± 3.39	37.33 ± 0.88
Grip strength (g)	625.10 ± 18.49	250.80 ± 15.66	547.60 ± 7.79	464.10 ± 16.05	517.00 ± 10.25	534.60 ± 7.99

Values are expressed as mean ± SEM (n = 6).

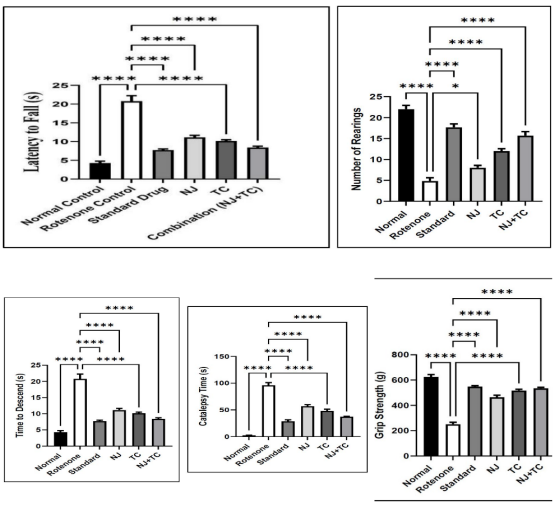


Figure 2. Effect on behavioral parameters

3.3 Oxidative and Nitrosative Stress Parameters

Rotenone administration was associated with substantial changes in oxidative and nitrosative stress markers. SOD activity decreased from 15.20 ± 0.41 to 5.46 ± 0.36 U/mg protein, catalase activity decreased from 59.70 ± 1.76 to 22.16 ± 2.06 U/mg protein, and GSH levels decreased from 8.30 ± 0.24 to 2.96 ± 0.20 µmol/mg protein. Conversely, MDA increased from 1.83 ± 0.21 to 7.20 ± 0.21 nmol/mg protein, while nitrite increased from 1.60 ± 0.13 to 6.28 ± 0.21 µmol/mg protein. The standard-drug

group exhibited improved antioxidant marker values and lower MDA and nitrite levels than the rotenone control group. Among the plant-treated groups, the combination treatment recorded the highest SOD activity (12.20 ± 0.27 U/mg protein) and GSH level (6.24 ± 0.24 µmol/mg protein), together with lower MDA (3.49 ± 0.11 nmol/mg protein) and nitrite (2.63 ± 0.16 µmol/mg protein) values (Table 4).

Table 4. Effect of treatments on oxidative and nitrosative stress markers

Parameter	Normal Control	Rotenone Control	Standard Drug	*N. jatamansi*	*T. cordifolia*	NJ + TC
SOD (U/mg protein)	15.20 ± 0.41	5.46 ± 0.36	12.88 ± 0.37	9.83 ± 0.52	10.63 ± 0.54	12.20 ± 0.27
Catalase (U/mg protein)	59.70 ± 1.76	22.16 ± 2.06	49.11 ± 1.83	42.31 ± 2.80	44.56 ± 2.01	47.40 ± 2.40
GSH (µmol/mg protein)	8.30 ± 0.24	2.96 ± 0.20	6.54 ± 0.27	5.47 ± 0.22	5.79 ± 0.18	6.24 ± 0.24
MDA (nmol/mg protein)	1.83 ± 0.21	7.20 ± 0.21	3.16 ± 0.11	4.32 ± 0.13	3.79 ± 0.15	3.49 ± 0.11
Nitrite (µmol/mg protein)	1.60 ± 0.13	6.28 ± 0.21	2.51 ± 0.15	4.27 ± 0.13	4.03 ± 0.09	2.63 ± 0.16

Values are expressed as mean ± SEM (n = 6).

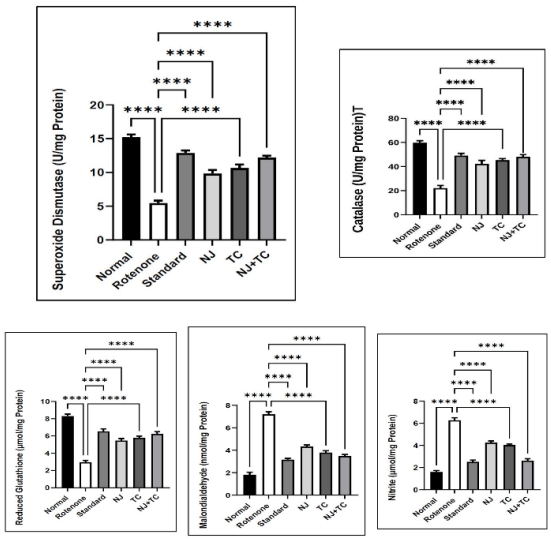


Figure 3. Effect on oxidative and nitrosative stress markers

3.4 Neurochemical Parameters

Rotenone administration was associated with a substantial reduction in striatal dopamine, DOPAC, and HVA levels. Dopamine decreased from 12.40 ± 0.30 to 3.41 ± 0.26 ng/mg tissue, DOPAC decreased from 2.62 ± 0.16 to 0.70 ± 0.03 ng/mg tissue, and HVA decreased from 1.78 ± 0.06 to 0.40 ± 0.04 ng/mg tissue. The combination-treated group exhibited dopamine, DOPAC, and HVA levels of 9.40 ± 0.27, 2.15 ± 0.08, and 1.33 ± 0.04 ng/mg tissue, respectively. These values were numerically higher than those observed with either individual plant extract and approached the values observed in the standard-drug group (Table 5).

Table 5. Effect of treatments on dopamine and its metabolites (ng/mg tissue)

Parameter	Normal Control	Rotenone Control	Standard Drug	*N. jatamansi*	*T. cordifolia*	NJ + TC
Dopamine	12.40 ± 0.30	3.41 ± 0.26	9.78 ± 0.26	7.15 ± 0.25	8.35 ± 0.23	9.40 ± 0.27
DOPAC	2.62 ± 0.16	0.70 ± 0.03	2.26 ± 0.11	1.59 ± 0.07	1.73 ± 0.05	2.15 ± 0.08
HVA	1.78 ± 0.06	0.40 ± 0.04	1.41 ± 0.03	0.99 ± 0.03	1.24 ± 0.04	1.33 ± 0.

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Values are expressed as mean ± SEM (n = 6).

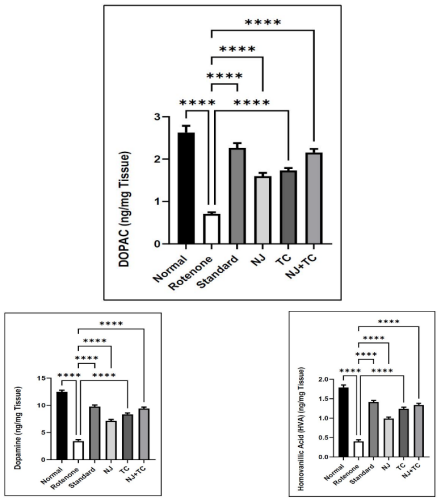


Figure 4. Effect on dopamine and its metabolites

3.5 Neuroinflammatory Markers

Rotenone administration was associated with increased concentrations of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. TNF-α increased to 91.76 ± 1.44 pg/mg protein, IL-1β to 75.15 ± 2.20 pg/mg protein, and IL-6 to 70.88 ± 1.19 pg/mg protein in the rotenone control group. The combination treatment recorded TNF-α, IL-1β, and IL-6 values of 45.58 ± 2.11, 33.01 ± 1.10, and 27.88 ± 0.75 pg/mg protein, respectively. These values were numerically lower than those observed in either individual plant-treatment group, indicating a more pronounced reduction in the measured inflammatory markers (Table 6).

Table 6. Effect of treatments on pro-inflammatory cytokines (pg/mg protein)

Parameter	Normal Control	Rotenone Control	Standard Drug	*N. jatamansi*	*T. cordifolia*	NJ + TC
TNF-α	23.48 ± 0.96	91.76 ± 1.44	41.51 ± 1.78	60.45 ± 1.81	51.31 ± 2.15	45.58 ± 2.11
IL-1β	18.05 ± 0.38	75.15 ± 2.20	29.21 ± 1.40	46.50 ± 1.47	37.86 ± 0.44	33.01 ± 1.10
IL-6	16.13 ± 0.42	70.88 ± 1.19	25.85 ± 0.91	40.81 ± 1.18	33.81 ± 0.94	27.88 ±

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Values are expressed as mean ± SEM (n = 6).

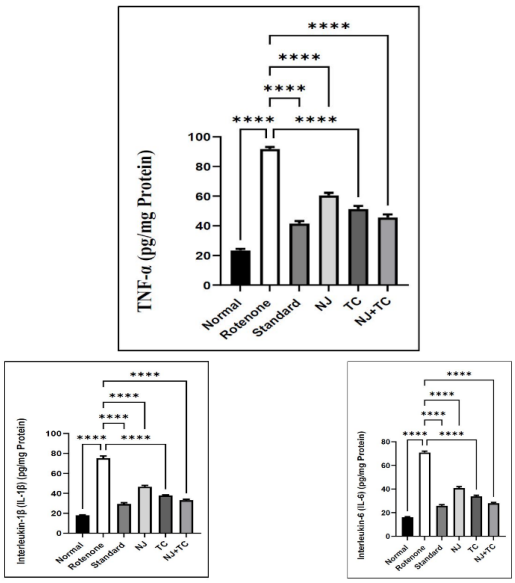


Figure 5. Effect on pro-inflammatory cytokines

3.6 Histopathology and Tyrosine Hydroxylase Immunohistochemistry

H&E staining showed comparatively well-preserved neuronal architecture in the normal control group. In contrast, the rotenone control group exhibited neuronal degeneration and disruption of tissue architecture. The standard-drug group showed comparatively better preservation, while the individual plant-treated groups exhibited varying degrees of morphological preservation. The combination-treated group demonstrated comparatively better preservation of neuronal morphology than either monotherapy. The number of TH-positive neurons decreased from 122.10 ± 2.05 in the normal control group to 41.50 ± 1.33 in the rotenone control group. Treatment with the standard drug increased the count to 109.30 ± 2.91. NJ and TC treatments produced counts of 77.16 ± 2.30 and 88.16 ± 2.40, respectively. The combination group recorded 88.66 ± 2.89 TH-positive neurons, a value numerically higher than that observed with NJ or TC individually (Table 7).

Table 7. Effect of treatments on TH-positive neuron count

Group	Treatment	TH-positive neurons
I	Normal Control	122.10 ± 2.05
II	Rotenone Control	41.50 ± 1.33
III	Standard Drug	109.30 ± 2.91
IV	*N. jatamansi*	77.16 ± 2.30
V	*T. cordifolia*	88.16 ± 2.40
VI	NJ + TC	88.66 ± 2.89

Values are expressed as mean ± SEM (n = 6).

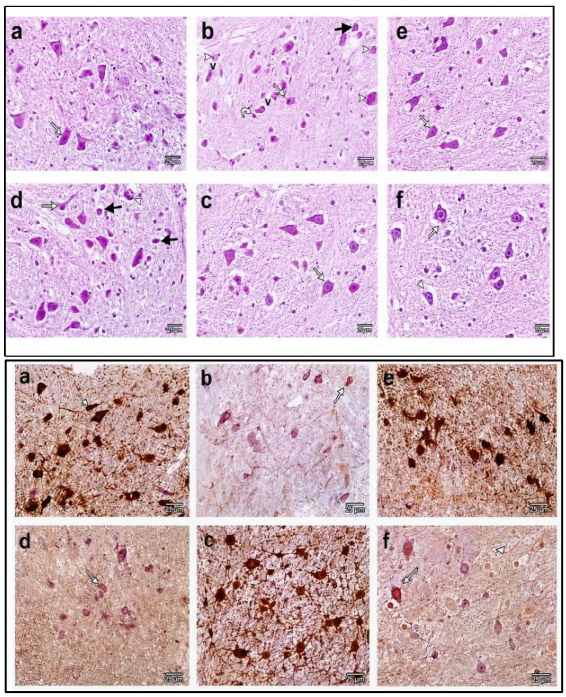


Figure 6. Histopathology and TH immunohistochemistry

4. Discussion

The present study evaluated the effects of *Nardostachys jatamansi* and *Tinospora cordifolia*, administered individually and in combination, in a chronic rotenone-induced rat model of Parkinson's disease. The observed changes in motor behavior, oxidative stress markers, neurochemical parameters, inflammatory cytokines, histopathology, and TH-positive neuronal counts were consistent with the expected features of rotenone-induced neurotoxicity. Rotenone inhibits mitochondrial complex I and can promote oxidative stress, inflammatory responses, and nigrostriatal dopaminergic injury [5–7].

Both NJ and TC treatments were associated with improvements in several measured endpoints compared with the rotenone control group. The observed effects of NJ are consistent with investigations into its antioxidant and neuroprotective potential, including possible modulation of Nrf2-associated antioxidant responses and NLRP3 inflammasome signaling [8,9]. Similarly, the changes observed following TC administration are consistent with investigations of its antioxidant and anti-inflammatory properties in experimental models of neurodegeneration [10,11]. However, the specific molecular pathways responsible for the observed effects were not directly established by the present set of measurements.

The combination of NJ and TC produced greater numerical improvements than either monotherapy across several behavioral, oxidative stress, neurochemical, and inflammatory endpoints. This pattern may reflect complementary biological activities. NJ may contribute to antioxidant defense, whereas TC may contribute to the modulation of inflammatory responses. Nevertheless, these mechanisms should be regarded as potential explanations rather than confirmed findings unless supported by direct pathway measurements. The improvement in dopamine, DOPAC, and HVA levels was accompanied by better motor performance in the combination-treated group. These findings suggest an association between the preservation of dopaminergic neurochemistry and improved behavioral outcomes. The observed increase in TH-positive neuron counts also supports the possibility of protection against dopaminergic neuronal injury. However, definitive claims of neuronal preservation require appropriate anatomical sampling, validated counting procedures, and consistent immunohistochemical assessment.

Although the combination treatment showed favorable numerical results, these data alone do not establish pharmacological synergy. Demonstrating synergy would require a suitable experimental design and formal statistical interaction analysis. Furthermore, the proposed mechanisms should be verified through additional molecular studies. The findings therefore provide a preliminary rationale for further investigation of the combined use of NJ and TC in experimental Parkinsonism.

5. Conclusion

Chronic rotenone administration was associated with motor impairment, altered oxidative and nitrosative stress markers, reduced striatal dopamine and its metabolites, elevated pro-inflammatory cytokines, neuronal architectural damage, and reduced TH-positive neuron counts. Treatment with *Nardostachys jatamansi* and *Tinospora cordifolia*, individually and in combination, was associated with improvements in several of these endpoints. The combination treatment generally produced more favorable numerical outcomes than either plant extract administered individually.

These findings support further investigation of the combined neuroprotective potential of NJ and TC in experimental models of Parkinson's disease. Future studies should include confirmatory statistical analysis, standardized extract characterization, dose-response evaluation, direct investigation of proposed molecular pathways, and formal assessment of combination effects before conclusions regarding synergy or translational therapeutic potential can be established.

References

1. Bloem, B. R., Okun, M. S., & Klein, C. (2021). Parkinson's disease. *The lancet*, *397*(10291), 2284-2303.
2. Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., ... & Lang, A. E. (2017). Parkinson disease. *Nature reviews Disease primers*, *3*(1), 17013.
3. Dauer, W., & Przedborski, S. (2003). Parkinson's disease: mechanisms and models. *Neuron*, *39*(6), 889-909.

4. Greenamyre, J. T., & Hastings, T. G. (2004). Parkinson's--divergent causes, convergent mechanisms. *Science*, *304*(5674), 1120-1122.
5. Cannon, J. R., Tapias, V., Na, H. M., Honick, A. S., Drolet, R. E., & Greenamyre, J. T. (2009). A highly reproducible rotenone model of Parkinson's disease. *Neurobiology of disease*, *34*(2), 279-290.
6. Sherer, T. B., Kim, J. H., Betarbet, R., & Greenamyre, J. T. (2003). Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and α-synuclein aggregation. *Experimental neurology*, *179*(1), 9-16.
7. Johnson, M. E., & Bobrovskaya, L. (2015). An update on the rotenone models of Parkinson's disease: their ability to reproduce the features of clinical disease and model gene–environment interactions. *Neurotoxicology*, *46*, 101-116
8. Li, J., et al. (2023). *Nardostachys jatamansi* and levodopa combination alleviates Parkinson's disease symptoms in rats through activation of Nrf2 and inhibition of NLRP3 signaling pathways. *Pharmaceutical Biology, 61*(1), 1134–1146.
9. Bian, L., et al. (2021). Nardosinone improves motor and cognitive symptoms in experimental Parkinsonism by regulating dopamine D2 receptor. *Frontiers in Pharmacology, 12*, 718944.
10. Birla, H., et al. (2019). *Tinospora cordifolia* suppresses neuroinflammation in Parkinsonian mouse model. *NeuroMolecular Medicine, 21*(1), 42–53.
11. Singh, S., et al. (2023). Neuroprotective effects of *Tinospora cordifolia* via reducing oxidative stress and mitochondrial dysfunction against rotenone-induced PD mice. *ACS Chemical Neuroscience, 14*(17), 3077–3087.
12. Jones, B. J., & Roberts, D. J. (1968). The quantitative measurement of motor incoordination in naive mice using an accelerating rotarod. *Journal of Pharmacy and Pharmacology, 20*(4), 302–304.
13. Ogawa, N., Hirose, Y., Ohara, S., et al. (1985). A simple quantitative bradykinesia test in MPTP-treated mice. *Research Communications in Chemical Pathology and Pharmacology, 50*(3), 435–441.
14. Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psychological Bulletin, 83*(3), 482–504.
15. Sanberg, P. R., Bunsey, M. D., Giordano, M., & Norman, A. B. (1988). The catalepsy test: Its ups and downs. *Behavioral Neuroscience, 102*(5), 748–759.
16. Meyer, O. A., Tilson, H. A., Byrd, W. C., & Riley, M. T. (1979). A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. *Neurobehavioral Toxicology, 1*(3), 233–236.
17. Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry & Biophysics, 21*(2), 130–132.
18. Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology, 105*, 121–126.
19. Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics, 82*(1), 70–77.
20. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*(2), 351–358.
21. Green, L. C., Wagner, D. A., Glogowski, J., et al. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Analytical Biochemistry, 126*(1), 131–138.
22. Magnani, C., et al. (2000). HPLC determination of catecholamines in biological samples. *Journal of Chromatography B, 745*, 123–130.
23. Bancroft, J. D., & Gamble, M. (Eds.). (2008). *Theory and practice of histological techniques*. Elsevier health sciences.
24. Düzel, E., Bunzeck, N., Guitart-Masip, M., Wittmann, B., Schott, B. H., & Tobler, P. N. (2009). Functional imaging of the human dopaminergic midbrain. *Trends in neurosciences*, *32*(6), 321-328.