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EXPLORING THE ANTI PARKINSONIAN POTENTIAL OF SOLANUM INCANUM: AN IN-VIVO APPROACH

Srinivasan Raghu[*1], Palani Hemavathy[2]

[1*]Associate professor, Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Vinayak Mission, Research Foundation (DU)Salem, Tamilnadu, India, E-mail: sragusrinivasan@gmail.com, Phone no: 9443713961
[2]Department of Pharmacology, Vinayaka Mission's College of Pharmacy

ABSTRACT

Objective: To evaluate the neuroprotective and antiparkinsonian potential of ethanolic extract of Solanum incanum (EESI) against rotenone-induced Parkinson's disease models, assessing its effects on cell viability, oxidative stress, antioxidant defenses, α-synuclein aggregation, motor behavior, and nigrostriatal neuronal integrity.

Methodology: In vitro studies: Human neuroblastoma SH-SY5Y cells were treated with rotenone to model dopaminergic neurotoxicity. Cells were pretreated or co-treated with EESI and assessed for cell viability, markers of oxidative stress, and intracellular glutathione levels.

In vivo studies: Wistar rats received rotenone to induce parkinsonian pathology. Animals were administered EESI at 50 and 100 mg/kg. Behavioral assessments for motor coordination were conducted. Brain tissues were analyzed for antioxidant enzyme activities, lipid peroxidation, α-synuclein expression, and histopathological evaluation of nigrostriatal dopaminergic neurons.

Results: In vitro: EESI significantly increased viability of rotenone-exposed SH-SY5Y cells and reduced oxidative stress parameters while restoring glutathione levels.

In vivo: EESI-treated rats showed improved motor coordination compared with rotenone-only controls. EESI enhanced endogenous antioxidant defenses, decreased lipid peroxidation, lowered α-synuclein expression, and preserved nigrostriatal dopaminergic neurons on histopathology. Effects were dose dependent, with greater protection at 100 mg/kg.

Conclusion: Ethanolic extract of Solanum incanum exhibited neuroprotective and antiparkinsonian effects in rotenone-induced in vitro and in vivo models, likely via antioxidant, anti-aggregation, and neuroprotective mechanisms, supporting its potential as an adjunct therapy for Parkinson's disease.

KEYWORDS: Solanum incanum, Parkinson's disease, rotenone, neuroprotection

1. INTRODUCTION

The CNS also progressively deteriorates with age, making neurons sensitive to mitochondrial and environmental toxins like rotenone and paraquat, which are implicated in neurodegeneration [1–3]. Mitochondrial dysfunction is characteristic of many neurodegenerative diseases, including PD, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis [4].

PD is a progressive disease of dopaminergic neuron degeneration in the substantia nigra pars compacta and dopamine depletion in the striatum, associated with motor impairment and Lewy body formation [5–7]. Experimental models of PD induced by neurotoxins like rotenone, MPTP, and 6-hydroxydopamine (6-OHDA) mimic PD pathology, with rotenone being an effective mitochondrial complex I inhibitor that produces progressive dopaminergic degeneration and Parkinsonism-like signs [8–10]

Pharmacologic treatments currently available such as levodopa, dopamine agonists, MAO-B blockers, COMT inhibitors, anticholinergics, and amantadine—alleviate symptoms but are hampered by long-term side effects and failure to arrest disease progression [11–13]. These limitations have led to interest in plant medicines with antioxidant, neuroprotective, and anti-inflammatory effects. Plant members of the Solanaceae family, i.e., Atropa belladonna, Withania somnifera, and Hyoscyamus niger, have proved to have anticholinergic and neuroprotective effects in Parkinsonism [14–16].

Solanum incanum L., a medicinal plant traditionally used, possesses solasodine, solanine, flavonoids, and phenolic compounds with antioxidant, anti-inflammatory, and neuroprotective activities [17–19]. These phytoconstituents indicate its possible antiparkinsonian activity. Hence, the current study proposes to investigate the antiparkinsonism activity of Solanum incanum.

METHODOLOGY

Plant Collection and Extraction

Leaves of Solanum incanum were harvested from Palayamkottai, Tirunelveli, during November and identified (Reg. No. XCH-40670, St. Xavier's College). Air-dried leaves were powdered (500 g) and extracted with 95% ethanol by Soxhlet extraction for 6–8 h until completion. The extract was concentrated under reduced pressure, giving 14 g semisolid ethanolic extract (EESI), kept at 4 °C. Ethanol has been used extensively for the extraction of phytochemicals because it is effective for the recovery of alkaloids, flavonoids, and phenolics with neuroprotection [20,21].

Phytochemical Screening
EESI underwent phytochemical screening. Alkaloids, flavonoids, glycosides, terpenoids, phenols, and steroids were present, whereas tannins and saponins were not present. Research emphasizes flavonoids and alkaloids of Solanum species as potential antioxidant and anti-inflammatory agents related to neurodegenerative disorders [22,23].

In vitro Neuroprotective Assay
SH-SY5Y neuroblastoma cells were grown in DMEM and 10% FBS. Cells were incubated with rotenone (10 µM) and co-administered with EESI (5–120 µg/mL). Cell viability was measured after 24 h using MTT assay, and ROS content using DCFH-DA probe. SH-SY5Y cells are popular in PD research owing to their dopaminergic characteristics [24,25].

Pharmacological Studies:
The study was approved by the Vinayaga Mission Institutional Animal Ethics Committee (CPCSEA Reg. No. 6684/PO/Re/S/02/CCSEA) and carried out under approval P.COL/83/2025/IAEC/VMCP.

Acute Toxicity
Female Swiss albino mice were treated with EESI (300 and 2000 mg/kg, p.o.) and were observed for 14 days. The experiment was conducted according to OECD guideline 423 [26,27].

In vivo Anti-Parkinson's Activity
Male Wistar rats were grouped into five sets (n=6): control, rotenone control, rotenone + levodopa, rotenone + EESI (50 mg/kg), and rotenone + EESI (100 mg/kg). Rotenone (2 mg/kg, s.c.) was given for 30 days. Behavioural assessment comprised rotarod, pole test, and elevated plus maze. Rotenone is a proven PD inducer that replicates mitochondrial impairment and α-synuclein pathology [28,29].

Biochemical and Molecular Analysis
Post-treatment, brain homogenates were analyzed for lipid peroxidation (MDA), glutathione (GSH), and superoxide dismutase (SOD). α-Synuclein was determined by ELISA. Oxidative imbalance and α-synuclein aggregation are characteristics of PD progression [30,31].

Histopathology
Brains were fixed, sectioned, and stained with H&E to analyze neuronal morphology in substantia nigra and striatum. Neurodegeneration in rotenone models is well established [32].

Statistics
Values were presented as mean ± SD. One-way ANOVA with Tukey's post hoc test was used, and $p<0.05$ was taken to be significant [33].

RESULTS AND DISCUSSION

Phytochemical Profile
Phytochemical screening established alkaloids, flavonoids, glycosides, phenols, terpenoids, and steroids (Table 1). These results concur with reports that Solanum phytoconstituents such as solasodine and flavonoids have antioxidant and neuroprotective effects [22]

Table 1: Secondary metabolite and their presence

S.No	Secondary metabolites	Presence
	Alkaloids	+++
	Flavonoids	+++
	Tannins	-
	Saponins	-
	Glycosides	+
	Terpenoids	+
	Phenols	++
	Steroids	+

+ indicates present, - indicate present absence

In vitro Neuroprotective Effect
EESI substantially recovered cell viability (82.4 ± 3.1% vs. 41.5 ± 2.8% in rotenone, $p<0.01$) and decreased ROS at 100 µg/mL Figure 1and Table 2. Comparable results were encountered with flavonoid-enriched extracts shielding SH-SY5Y cells from rotenone [24,25].

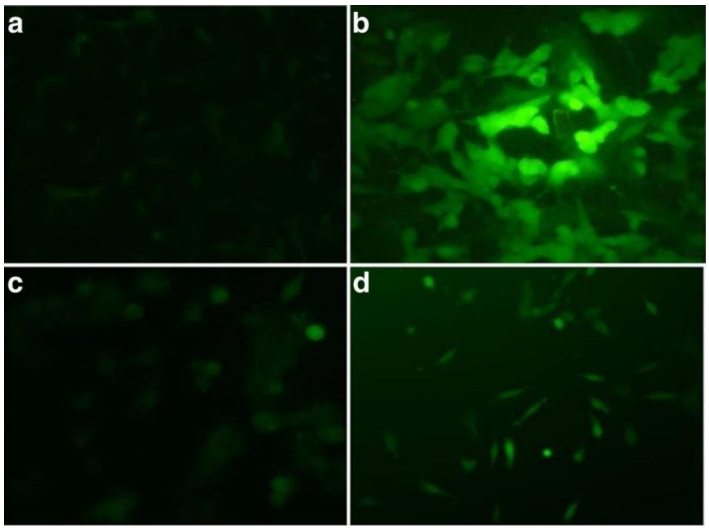


Figure 1. Effect of EESI on SH-SY5Y viability and ROS

Table 2: Invitro neuro effective determination by MTT

Sample concentration (µg/ ml)	Absorbance values(Mean) N=3	Percentage viability
Control	0.3190	100
Rotenone	0.1583	49.62
5	0.1731	54.26
10	0.1994	62.50
20	0.2450	76.80
40	0.2106	66.00
80	0.1935	60.65
120	0.1842	57.74

Acute Toxicity

EESI up to 2000 mg/kg was devoid of mortality and behavioral toxicity, ensuring safety for in vivo application [26].

Behavioral Assessments in Rats

Rotenone caused impairment of motor and cognitive performance. EESI significantly enhanced rotarod, pole test, and elevated plus maze performance comparable to levodopa Table 3,4 and 5. This is in agreement with plant-derived neuroprotectants ameliorating dopaminergic function [30,31].

Rotenone-treated rats exhibited a remarkable reduction in open-arm time and entries, reflecting enhanced anxiety-like behavior and cognitive impairments. Levodopa and ethanolic extract of solanum incanum extract treatment (particularly higher doses) significantly restored these parameters, reflecting possible anxiolytic and cognitive improvements in Parkinsonism. The extract-treated groups promoted open-arm exploration, reflecting alleviation of neurotoxicity-evoked anxiety and cognitive impairment.

Rotenone treatment significantly increases T_turn and T_total, indicating bradykinesia and motor impairment. L-Dopa (Control drug) facilitates motor function. Extract at 50mg/kg and 100 mg/kg illustrates dose-dependent facilitation of motor function.

Table 3. Behavioral effects of EESI in the rota rod test.

Groups	Rotarod Test (Sec) (Mean ± SD)		
	Day 1	Day 4th	Day 7th
Control	70.667 ± 3.613	71.500 ± 2.377	70.000 ± 2.520
Rotenone (Negative Control)	25.833± 2.623*	24.833 ± 2.432*	21.833 ± 2.276*
Levodopa (30 mg/kg)	51.000±3.011***	53.667±2.826***	55.833±1.880***
EESI (50 mg/kg)	27.000 ± 3.120**	31.500 ± 2.430**	33.167 ± 1.732**
EESI (100 mg/kg)	41.167± 1.286**	43.500 ± 1.896**	45.333 ± 2.311**

Values are expressed as Mean ± SD, (*P<0.05 vs control group, **P<0.01 vs rotenone treated group, ***P<0.001 vs rotenone treated group)

Table 4: Behavioral effects of EESI in Elevated plus maze method.

Groups	Time Spent in Open Arms (s) (Mean ± SD)	Time Spent in Closed Arms (s) (Mean ± SD)	Entries into Open Arms (Mean ± SD)
Control	30.5 ± 2.1	110.5 ± 5.2	8.2 ± 1.3
Rotenone (2 mg/kg, i.p.)	12.8 ± 1.5*	150.3 ± 7.1*	3.5 ± 0.7*
Standard (Levodopa 10 mg/kg)	28.2 ± 2.4**	115.8 ± 4.9**	7.5 ± 1.1**
EESI (50 mg/kg)	22.4 ± 2.2**	125.6 ± 5.8**	6.1 ± 1.0**
EESI (100 mg/kg)	26.3 ± 2.0**	118.2 ± 5.2**	7.1 ± 1.2**

Values are expressed as Mean ± SD (*P < 0.05 vs. Control; ***P < 0.01 vs. Rotenone-treated group)*

Table 5: Behavioral effects of EESI in pole test

Groups	T_turn (sec)	T_total (sec)
Control	2.5 ± 0.3	5.2 ± 0.5
Rotenone (2 mg/kg, i.p.)	6.8 ± 0.6*	12.5 ± 0.8*
Standard (Levodopa 10 mg/kg)	3.2 ± 0.4***	6.0 ± 0.5***
EESI (50 mg/kg)	5.4 ± 0.5**	10.2 ± 0.7**
EESI (100 mg/kg)	4.0 ± 0.4**	8.3 ± 0.6**

Values are expressed as Mean ± SD (*P < 0.05 vs control, **P < 0.01 vs rotenone treated group, ***P < 0.001 vs rotenone treated group)

Biochemical Parameters

Rotenone increased MDA and decreased GSH and SOD. EESI dose-dependently restored antioxidant defenses (Table 6). This concurs with findings that polyphenolic extracts reduce oxidative stress in PD models [28,29].

Lipid Peroxidation (MDA): Raised substantially in the Rotenone group (p < 0.001 vs Control), reflecting oxidative stress. EESI (100 mg/kg) and Levodopa lowered MDA levels significantly (p < 0.001 vs Rotenone).

SOD Activity: Reduced substantially in the Rotenone group (p < 0.001 vs Control), reflecting decreased antioxidant defense. EESI (100 mg/kg) improved SOD levels significantly (p < 0.001 vs Rotenone).

GSH Levels: Reduced in Rotenone-treated rats (p < 0.001 vs Control), whereas EESI (50 mg/kg & 100 mg/kg) markedly reversed the levels of GSH (p < 0.001 vs Rotenone).

Marked increase (p < 0.001), Slight increase (p < 0.01), Marked decrease (p < 0.001), Slight decrease (p < 0.01).

Table 6: Biochemical analysis of anti parkinsonism animal Model

Groups	Lipid Peroxidation (MDA) (nmol MDA/mg protein)	SOD (U/mg protein)	GSH (µmol/mg protein)
Control	1.85 ± 0.12	8.65 ± 0.24	7.80 ± 0.30
Rotenone (Negative Control)	4.92 ± 0.30**	3.25 ± 0.18**	3.10 ± 0.22**
Levodopa (30 mg/kg)	2.60 ± 0.15**	7.10 ± 0.20***	6.80 ± 0.25***
EESI (50 mg/kg)	3.40 ± 0.22**	5.80 ± 0.19**	5.20 ± 0.28**
EESI (100 mg/kg)	2.90 ± 0.18**	6.70 ± 0.21***	6.40 ± 0.27***

Values are expressed as Mean ± SD (*P < 0.05, **P < 0.01, ***P < 0.001)

α-Synuclein and Histopathology

Rotenone increased α-synuclein expression and neuronal loss, while EESI reduced α-synuclein and preserved neuronal integrity (Figures 2 & 3). These findings corroborate evidence of phytochemicals reducing α-synuclein aggregation [32]. Photomicrographs show H&E-stained sections of the substantia nigra (left) and striatum (right) across treatment groups. Control: Normal neurons with intact morphology (black arrows). Rotenone: Neuronal shrinkage and degeneration (arrow).Solanum incanum 50 mg/kg: Moderate protection; some neurons remain degenerated. Solanum incanum 100 mg/kg: Marked improvement in neuronal structure, with nearly restored cytoarchitecture.

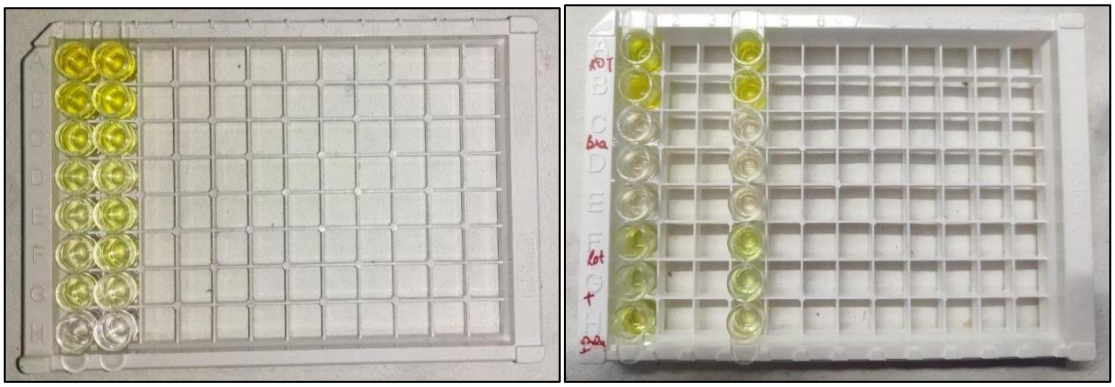
Photomicrographs show hematoxylin and eosin (H&E)-stained coronal brain sections at the level of the substantia nigra (left column) and striatum (right column) across various treatment groups.

Table 7: Alpha-synuclein levels on various treated groups

Treatment Groups	Alpha-Synuclein Levels (ng/mg protein) ± SD
Control	1.25 ± 0.12
Rotenone (Negative Control)	4.85 ± 0.28##
Levodopa (30 mg/kg)	2.10 ± 0.18**
EESI (50 mg/kg)	3.40 ± 0.22*

EESI (100 mg/kg)	2.60 ± 0.20**

Values are expressed as Mean ± SD (##P < 0.01 vs control, **P < 0.01 vs rotenone treated group, *P < 0.05 vs rotenone treated group)



(A) Standard **(B) Groups at 450 nm**

Fig 2: Alpha Synuclein level estimation using sandwich ELISA method

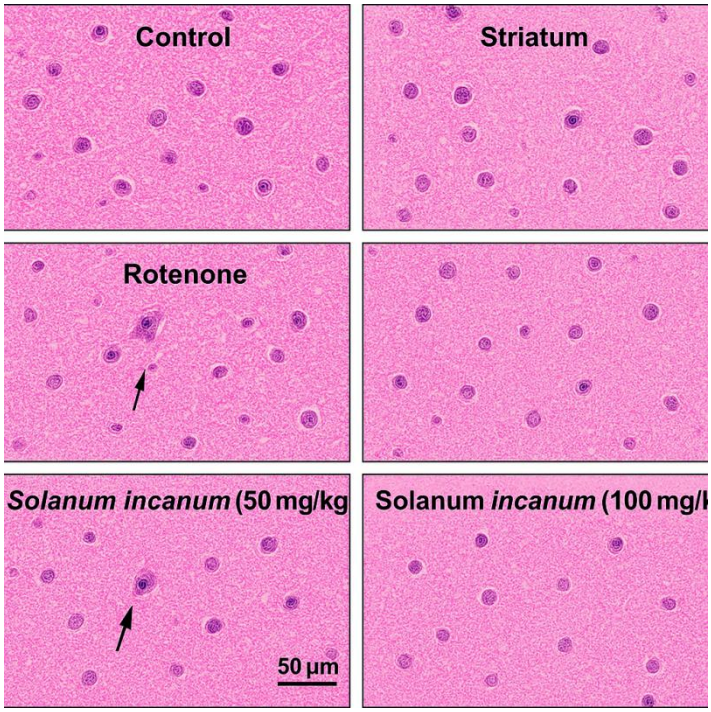


Figure 3 : Histopathological Analysis of the Substantia Nigra and Striatum in Rotenone-Induced Parkinsonism and Solanum incanum-Treated Rats

CONCLUSION

The extract replenished intracellular glutathione levels and showed potent anti-Parkinson's activity in vitro with the SH-SY5Y cell line model. Increased levels of α-synuclein lead to cellular impairment and death of dopaminergic neurons. Rotenone-intoxicated mice in this experiment had significantly elevated levels of α-synuclein, showing parkinsonism disease-like pathology. Ethanolic extract of Solanum incanum markedly decreased these levels, proving its neuroprotective action. Histopathology studies strengthen the claimed activity further. Interpretation of results obtained from behavioural assessment, in vitro and invivo studies confirms the antiparkinsonism activity of solanum incanum plant extract.

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