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Research

Phytochemical Screening and Anti-Parkinsonian Activity of Hydroalcoholic Extract of the *Ruellia Tuberosa* Leaves in Rats.

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Abstract

Aim: This study aimed to evaluate the antiparkinsonian activity of the hydroalcoholic extract of *Ruellia tuberosa* leaves in rats using suitable animal models.
Methods: The *Ruellia tuberosa* plant was collected from the market and authenticated. The leaves were dried, ground, and extracted with 90% v/v ethanol and distilled water. The phytochemical analysis was conducted using various tests. Catalepsy was induced in rats using Rotenone and HART was administered orally to the treatment group. The behavioral changes were assessed using the hole board test for catalepsy, motor coordination test by rotarod, and locomotor activity by actophotometer.
Results: The hydroalcoholic extract of *Ruellia tuberosa* (HART) showed antiparkinsonian activity in rats. The catalepsy induced by Rotenone was reduced in the treatment group, as observed in the hole board test and motor coordination test. The locomotor activity was also improved in the treatment group.
Conclusion: The findings of this study suggest that the hydroalcoholic extract of *Ruellia tuberosa* leaves possesses antiparkinsonian activity and could be a potential therapeutic agent for the treatment of Parkinson's disease. Further studies are needed to explore the mechanism of action and safety of this extract.

Keywords: *Ruellia tuberosa*, antiparkinsonian activity, Rotenone, HART

INTRODUCTION

Parkinson's disease (PD) is indeed the second most common neurodegenerative disorder, following Alzheimer's disease. It affects millions of people worldwide. PD is characterized by symptoms such as bradykinesia (slowness of movement), muscle stiffness, resting tremor, and postural instability. These symptoms arise due to the gradual loss of dopamine-producing neurons in the substantia nigra, a midbrain region involved in movement control.

The highly effective blood-brain barrier (BBB) poses challenges for drug delivery to the brain, but nanotherapeutics show promise in crossing the BBB for targeted treatment [1]

Ruellia tuberosa, also known as Minnie root**,** fever root**,** snapdragon root**,** and sheep potato, belongs to the family Acanthaceae. It is native to Central America but has been introduced into Indian gardens as an ornamental plant (2). *Ruellia tuberosa* possesses thick, fusiform tuberous roots. These roots serve as a survival strategy during dry seasons, allowing the plant to store nutrients and water for sustenance (3). This explosive behavior gave the plant local name in English 'Cracker plant', This diverse phytochemical profile, including alkaloids, proteins, amino acids, flavonoids, glycosides, and terpenoids, hinted at the plant's multifaceted therapeutic potential (4).

Rotenone is a common pesticide used in agriculture. It is a mitochondrial complex-I inhibitor that can cause damage to the dopaminergic neurons by ROS generation and altering ATP production [8]. Rotenone is a well-established compound that is known to induce PD in animal models. Several animal models such as Drosophila, rats and mice have been investigated for the rotenone-induced PD model (5). In this study, we have Phytochemical Screening and Anti-Parkinsonian activity of Hydroalcoholic Extract of the Leaves of *Ruellia Tuberosa* in Rats.

METHODS

Collection and Authentication of plant

The entire *Ruellia tuberosa* plant was collected from market and specimen was submitted to the Sri Vijay Vidyalaya College of Arts and Science, Dharmapuri, Tamilnadu, with the authentication certificate.

Drying and grinding

The leaves of *Ruellia tuberosa* were washed, air-dried for 2 days, and crushed to a coarse powder. The powder obtained was passed through sieve No. 40 and used for further studies. (6)

Extraction

Dried coarse powder of *Ruellia tuberosa* leaves was extracted with a mixture of 90% v/v ethanol (50%) and distilled water (50%) in a 250 ml Soxhlet at 60°C. The solvent obtained was evaporated to remove excess of the solvent, concentrated, and then used for the study. The yield was observed to be 18% w/w. (6)

Phytochemical analysis

Preliminary phytochemical investigations were conducted employing various phytochemical tests and the phytochemical constituents were detected as elaborated by Safitri A (2020). The hydroalcoholic extract of the leaves of HART was dissolved in distilled water (q.s.) and used for the presence of phytoconstituents such as alkaloids, flavonoids, glucosides, saponins, steroids, tannins, and phenolic compounds. (6)

Animal

For this investigation, 80 ± 100 g of male Sprague Dawley rats, seven weeks of age, were employed. The rats were kept in groups of six animals per cage in a temperature-controlled environment (21–22°C) with a reversible light–dark cycle (12 h/12 h) had normally fed and water *ad libitum.* (7)

Pharmacological screening

Invitro Neuroprotective Effect Determination by MTT Assay

SHSY-5Y (Human Neuroblastoma cells) cell line was purchased from NCCS Pune and was maintained in Dulbecco's modified eagles' media (HIMEDIA) from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's Modified Eagles medium (DMEM) Sigma Aldrich, USA). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells was evaluated by direct observation of cells by an Inverted phase contrast microscope and followed by the MTT assay method.

Motor coordination test (rotarod test)

A motor coordination test was conducted using a rotarod apparatus. The animals were placed on the moving rod before the treatment and the rat stayed on the rod without falling for 120 s was chosen for the study. The time at which animals take for falling from the rotating rod was noted before and after the treatment. The starting speed of rotarod was adjusted to 4 rpm with the acceleration rate to 20 rpm. The maximum speed was 40 rpm.

Test for locomotor activity (Actophotometer)

The locomotor activity was measured using actophotometer. It consists of a cage which has six lights and six photocells, which are placed in the outer periphery of the bottom in such a way that single rat block only one beam at

a time. Photocell is activated when the rays of light fall on photocells, the beam of light is interrupted as and when animal crosses the light beam, number of cut interruptions was recorded for 10 min.

Test for Hole Board

The hole board test involves placing an animal on an elevated hole board apparatus, positioned 25 cm above the base. This setup induces anxiety in the animal as it encounters a novel environment, leading to characteristic head-poking behavior. A decrease in anxiety is reflected by increased exploration of the holes, while heightened anxiety results in a lower number of head-poking instances. The hole board apparatus comprises a wooden board (40*40cm) elevated 25 cm above the ground, featuring 16 holes of approximately 3 cm in diameter arranged symmetrically in a diamond pattern. Animals are positioned at the corner of the apparatus and observed for the next 5 minutes to record the number of head-dipping behaviors. A head dip is counted when the animal introduces its head into any hole of the box up to the level of the ears. Thorough cleaning of the apparatus is conducted between each subject to maintain consistency and accuracy in the observations.

RESULTS

Preliminary phytochemical screening of hydro-alcoholic extract of *Ruellia tuberosa*

The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, phytosterols, and terpenoids in a hydro-alcoholic extract of leaves of ***Ruellia tuberosa*** and it is rich in secondary metabolites which may contribute to its multi-pharmacological effects.

Table 1: Preliminary phytochemical screening of hydro-alcoholic extract of *Ruellia tuberosa*

S.no	Phytochemical constituents	Results
1	Alkaloids	+
2	Protein and Amino acids	+
3	Steroids	-
4	Flavonoids	+
5	Glycosides	+
6	Terpenoids	+
7	Sterols	-
8	Tannins	+

In-vitro Neuroprotective Effect Determination by MTT Assay

Table 2: Invitro Neuroprotective Effect Determination by MTT Assay

Sample Concentration (µg/ml)	OD I	OD II	OD III	Average Absorbance @ 540nm	Percentage Viability
Control	0.5993	0.6084	0.6048	0.6042	100
Rotenone	0.2912	0.301	0.2947	0.2956	48.93
Sample: **HART**					
1.5	0.3231	0.3138	0.3147	0.3172	52.50
3.1	0.3465	0.3384	0.3349	0.3399	56.26
6.25	0.3645	0.3548	0.3674	0.3622	59.95
12.5	0.3847	0.3759	0.3786	0.3797	62.58
25	0.274	0.2858	0.2829	0.2809	46.49

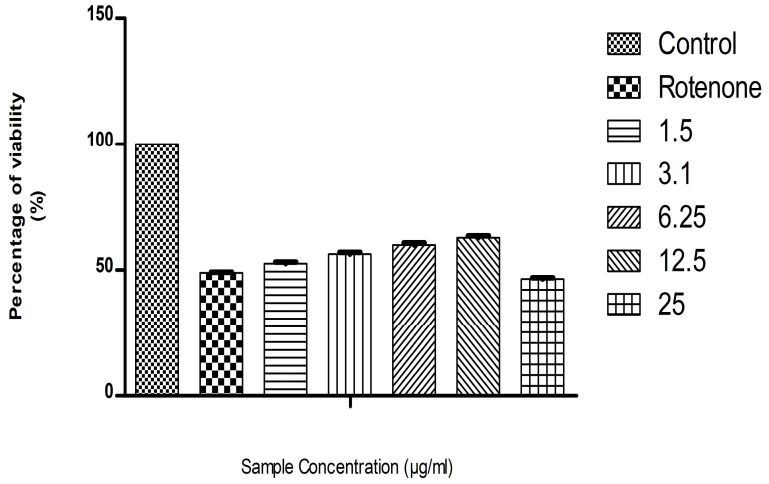

Fig 1: Invitro Neuroprotective Effect Determination by MTT Assay

Graphical representation depicting the neuroprotective effect of Sample 2 on SHSY5Y cell line by MTT assay. Along the Y axis Percentage viability, Along X axis varied concentration of Sample 2. All experiments were done in triplicates and results were represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. ***p < 0.001 compared to control groups, **p < 0.01 compared to control groups.

Screening of Anti-Parkinsonian Activity of Hart: [Rotenone Model]
Rotarod Test

Table 3: Effect of HART on Muscle Grip Strength

Groups	Time spent on Rotarod(sec)		
	Day 3	Day 5	Day 7
Vehicle Control	125±1.15	123±1.44	135±1
ROTENONE	65.667±1.20***	68±1.52***	46.333±9.98***
ROTENONE+ Levodopa	55±2.08***	88.333±1.20***	109.67±6.75***
ROTENONE+ HART (200mg/kg)	42.667±2.603***	66.333±0.88***	96±7.48***
ROTENONE+ HART (400mg/kg)	56±2.08***	71.333±6.634***	116.33±3.52***

Actophotometer Test

Table 4: Effect of Hart on Spontaneous Locomotor Activity

Groups	Locomotive score(sec)		
	Day 3	Day 5	Day 7
Vehicle control	395.33±4.46	416±2.08	392±1.15
ROTENONE	199.33±8.04***	171.33±1.85***	156.67±1.42***
ROTENONE+ Levodopa	175±1.16***	226.33±1.45***	255.33±2.42***
ROTENONE+ HART (200mg/kg)	169±1.73***	196±5.13***	221.33±2.90***
ROTENONE+HART (400mg/kg)	176.33±2.02***	219.33±2.17***	241.33±2.18***

Hole Board Test

Table 5: Effect of HART on alertness

Groups	No. of head dipping's		
	Day3	Day5	Day7
Vehicle control	36±3.06	35±4.52	28±2.42
ROTENONE	26.667±1.76**	26.333±2.95***	16±2.51***
ROTENONE+ Levodopa	29±1**	25±1.73**	23.333±0.66ns
ROTENONE+ HART (200mg/kg)	16.667±1.76***	13.667±0.43***	19.667±3.01***
ROTENONE+HART (400mg/kg)	18±0.08***	15.333±4.03***	26.333±0.31***

DISCUSSION

The hydro-alcoholic extract of *Ruellia tuberosa* leaves exhibited a diverse array of phytochemical constituents, indicating its potential as a rich source of secondary metabolites. Notably, the presence of alkaloids, proteins, amino acids, flavonoids, glycosides, and terpenoids underscores the complexity and bioactivity of the extract. This botanical composition suggests a promising foundation for multi-pharmacological effects. This preliminary phytochemical screening offers valuable insights into the chemical composition of *Ruellia tuberosa*, laying the groundwork for further exploration of its therapeutic potential. The combined presence of these phytoconstituents suggests that the hydro-alcoholic extract could potentially offer a spectrum of bioactivities. The comprehensive evaluation through various neurobehavioral tests provides compelling evidence of its potential neuroprotective effects in a rotenone-induced Parkinson's disease model. The MTT assay results highlight its ability to enhance cell viability, suggesting a promising capacity to counteract rotenone-induced cytotoxicity. The dose-dependent response further underscores the potential therapeutic efficacy of HART. In the Rota Rod test, the observed improvement in muscle grip strength with both Levodopa and HART, particularly at 400 mg/kg, indicates a protective effect against motor impairment induced by rotenone. This not only supports the potential neuroprotective role of HART but also positions it alongside Levodopa, a standard treatment for Parkinson's disease. The Actophotometer test, assessing spontaneous locomotor activity, reveals a significant reduction in locomotive scores induced by rotenone. The subsequent improvement in locomotor activity with HART, especially at higher doses, reinforces its neuroprotective potential. This aligns with the broader goal of preserving motor function in the face of neurodegenerative challenges. The Hole Board test, evaluating alertness through head dipping, highlights a decline in alertness in the rotenone group. The reversal of this trend with Levodopa and HART, particularly at 400 mg/kg, suggests an enhancement in alertness and further supports the neuroprotective effects of HART against Parkinson's disease-associated symptoms.[8-10]

SUMMARY AND CONCLUSION

In the pharmacological screening, the In-Vitro MTT assay showcased a dose-dependent increase in cell viability, suggesting the extract's neuroprotective capabilities against rotenone-induced cytotoxicity. Moving to In-Vivo studies, the Rota Rod test demonstrated a significant improvement in muscle grip strength, particularly at 400 mg/kg, signifying protection against rotenone-induced motor impairment. Enhanced locomotor activity, observed in the Actophotometer test, reinforced the neuroprotective potential of the plant extract. The Hole Board test further underlined its efficacy by showcasing an enhancement in alertness, particularly at 400 mg/kg. This study's significance lies in the promising neuroprotective effects exhibited by the plant extract, offering a potential avenue for the development of Parkinson's disease treatments.

In conclusion, this exploration of a plant-based approach to Parkinson's treatment not only provides valuable insights into the neuroprotective potential of the selected plant but also emphasizes the importance of investigating natural sources for novel therapeutic agents. Further molecular studies and clinical trials are warranted to delve deeper into the mechanisms and clinical applicability of this plant extract in Parkinson's disease management.

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Conflict of interest statement

We declare that we have no conflict of interest.

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