

## IMPACT OF HYDROALCOHOLIC CONCENTRATE OF *HEMIDESMUS INDICUS* AGAINST 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE PROMPTED PARKINSONISM IN MICE

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### ABSTRACT

**Objective:** The objective of this study was to evaluate the protective effect of *Hemidesmus indicus* against 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mice model.

**Methods:** A total of 18 male Swiss Albino mice were divided into three groups (n=6). Hydroalcoholic concentrate of *H. indicus* (HAHI) at 200 and 400 mg/kg dosages, 30 min before the MPTP (20 mg/kg, intraperitoneal organization) treatment, was regulated for 7 days, and behavioral assessment was made by rotarod test, grip strength test, locomotor activity, and catatonia behavioral study. Appraisal of cell reinforcement catalysts, such as thiobarbituric acid reactive substance (TBARS), superoxide dismutase, catalepsy, glutathione (GSH) peroxidase, and GSH, were also assessed to screen the neurotoxicity incited by MPTP.

**Results:** In the present investigation, the neuroprotective impacts of the hydroalcoholic concentrate of *H. indicus* were assessed, Which is known for its monoamine oxidase activity, stimulant, anti-convulsant activity and a section of it is used as nerve tonic Frozen at 8 degree C. The cell viability analysis and IC50 determination in SH-SY5Y neuroblastoma cell lines were performed by MTT assay, and neuronal red uptake assay and HAHI were found to have a significant IC50 value in SH-SY5Y cells.

**Conclusion:** The outcomes demonstrated that HAHI essentially enhanced the behavioral studies, striatal neurotransmitter content, and antioxidant status in a dose-dependent manner lessened TBARS level.

**Keywords:** Parkinson's Disease, *Hemidesmus indicus*, 1-methyl-4-phenyl-1,2,3,6-Tetrahydropyridine, *Substantia nigra*.

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### INTRODUCTION

Parkinson's disease (PD) is a dynamic neurodegenerative disorder with a loss of dopamine carrying neurons in the *Substantia nigra* and is commanded by four cardinal signs: Tremors, bradykinesia, inflexibility, and postural disturbances. Other neurobehavioral irregularities incorporate personality changes (indifference, the absence of certainty, frightfulness, and tension), dementia, depression, restlessness, sexual variation, absence of facial expression, disabled gulping, diminished arm swinging, and other mechanized developments [1]. It has been demonstrated that, due to oxidative stress, neuronal death in PD happens due to free radical damage[2,41]. In general, a combination of treatment of manufactured medications is more viable in treating PD. In any case, the delayed utilization of this medication prompts antagonistic complexities such as unusual developments, conduct impacts, change in engine execution, mind flights, hypotension, and perplexity [3]. Henceforth, nowadays, homegrown treatment has picked up a considerable measure of significance in PD which limits the symptoms and gives wanted outcomes.

Plants assume an imperative job in the presentation of new helpful specialists and have got much consideration as wellsprings of naturally dynamic substances. Various plants mentioned in Ayurveda medicine are utilized for the treatment of nervous disorders like Alzheimer's disease, meningitis and Huntington's disease.

*Hemidesmus indicus* (anantmoool) is belonging to the Apocynaceae family regularly found in India, especially among various territories of West Bengal. It is a slim, laticiferous, semi-erect imperiled bush, particularly known for its medicinal properties [4]. The plant *H. indicus* was accounted to have some essential pharmacological

activities such as anticancerous [5], antidiarrheal [6], antivenom [7], hepatoprotective [8], nootropic action [9], wound healing, and anti-inflammatory activities [10].

### METHODS

#### Extraction procedure

The entire plant of *H. indicus* was obtained from the coastal areas of Ongole, Andhra Pradesh. Plant validation (Voucher example number-PARC/2011/789) was done by Prof. Jayaraman. P, Ph.D., Plant Anatomy Research Center, Tambaram, Chennai - 45. The gathered roots were cleaned, air dried at room temperature, and ground into a fine powder with a blender and kept in a profound cooler until utilization. The powder was separated with ethanol utilizing Soxhlet extractor and was concentrated by rotary evaporator at 40°C and kept in a cool and dry place.

#### In vitro cell culture

The SH-SY5Y neuroblastoma cells (NCCS, Pune, India) were cultured in DMEM:HAM medium with 100 UI/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum at 37°C in 5% CO<sub>2</sub>. Cells were seeded in 10×10<sup>3</sup> cells/well density in 96-well plates for 48 h.

#### MTT assay

SH-SY5Y cells were initially treated with the extract of various concentrations (0.05–5 µg/ml) and incubated for 24 h with 5% CO<sub>2</sub>, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT dye) was added. The purple-colored formazan crystals formed after 3 h of incubation were treated with 100 µl of dimethyl sulfoxide. The absorbance was measured at 570 nm Table 1 by a microplate reader, and the results were expressed as the percentage of viable cells [22].

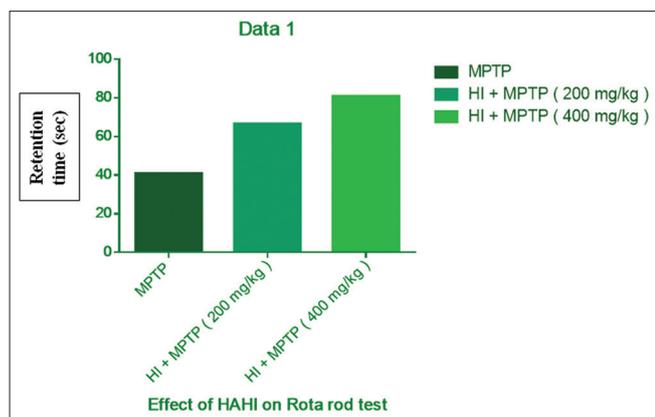


Fig. 1: Effect of hydroalcoholic extract of *Hemidesmus indicus* on rotarod test

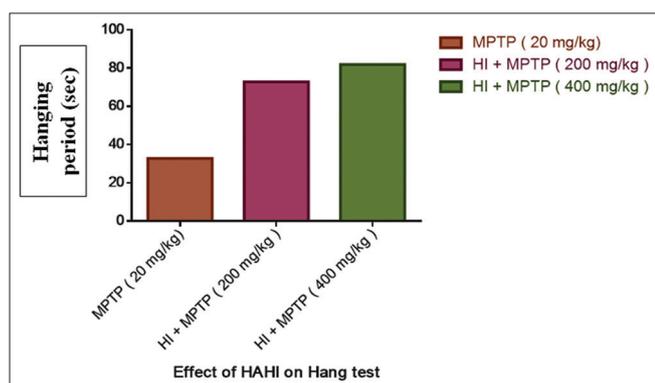


Fig. 2: Effect of hydroalcoholic extract of *Hemidesmus indicus* on Hang test

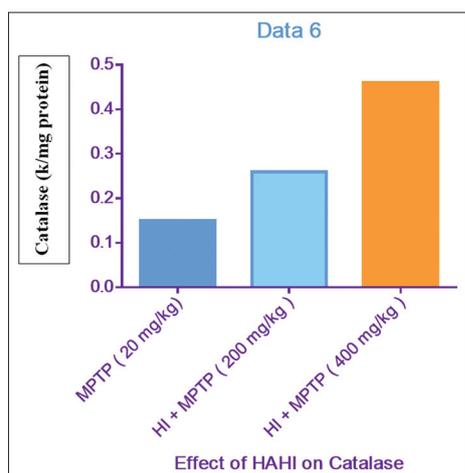


Fig. 3: Effect of the hydroalcoholic extract of *Hemidesmus indicus* on catalase

**Neutral red uptake assay**

This assay is based on the evaluation of uptake of the neutral red dye by the lysosomes of the viable cells [23]. SH-SY5Y cells treated with various concentrations of extract were incubated with 150 µl of neutral red dye in serum-free medium for 3 h at 37°C and washed with phosphate-buffered saline, and EtOH/AcOH/H<sub>2</sub>O (50%/1%/49%) was added with shaking for 60 min. Absorbance was recorded at 540–630 nm Table 2, and the cell viability was expressed as a percentage.

SH-SY5Y neuroblastoma cells are an important tool which has been widely used as a neurodegenerative disease model. These cells

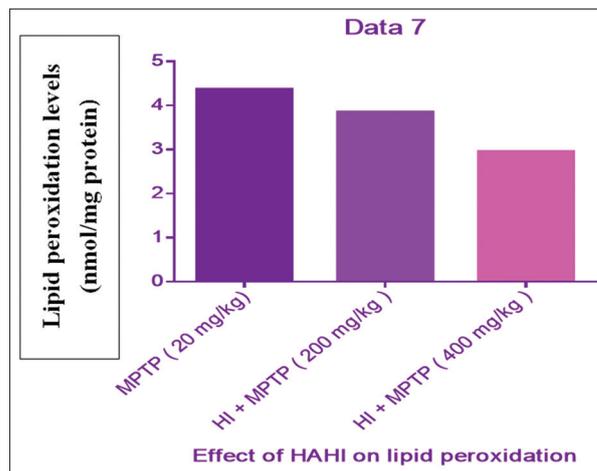


Fig. 4: Effect of the hydroalcoholic extract of *Hemidesmus indicus* on lipid peroxidation

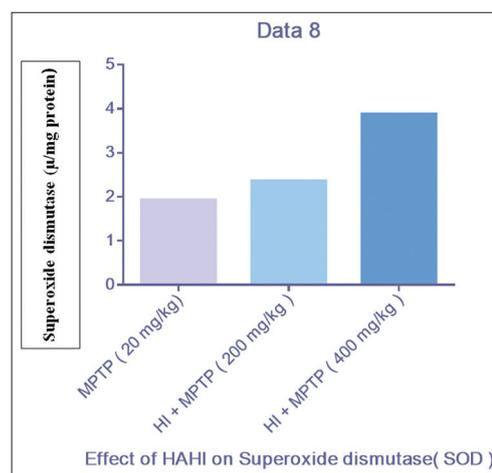


Fig. 5: Effect of the hydroalcoholic extract of *Hemidesmus indicus* on superoxide dismutase

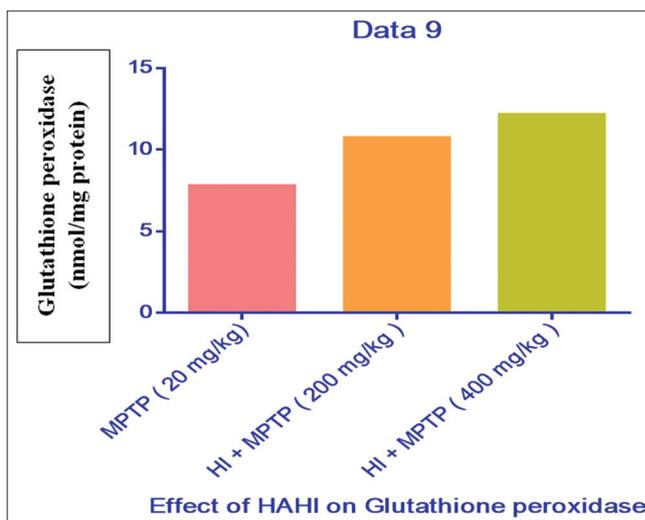


Fig. 6: Effect of the hydroalcoholic extract of *Hemidesmus indicus* on glutathione peroxidase

have the capability to differentiate into neuron-like cells which are morphologically and biochemically similar to neurons [31,32]. For the present study, the SH-SY5Y cells were treated with concentrations of charantin ranging from 0.05 mg/mL to 5 mg/mL which showed

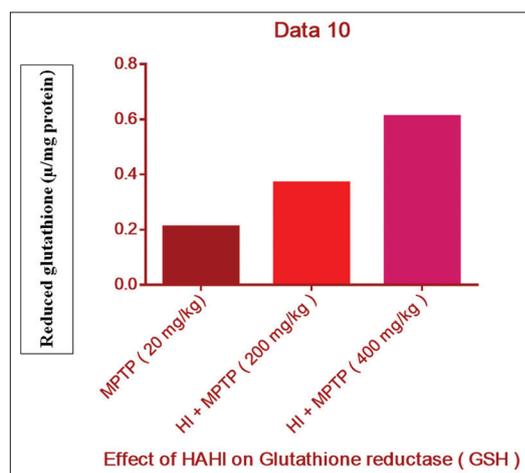


Fig. 7: Effect of the hydroalcoholic extract of *Hemidesmus indicus* on glutathione reductase

Table 1: MTT assay

Method	Sample	Concentration µg/ml	Cell viability %
Cell viability %	Extract	0	100
		0.05	92.5
		0.1	84.3
		0.5	78.56
		1	71.44
		5	28.12

Table 2: Neutral red uptake assay

Method	Sample	Concentration µg/ml	Cell viability %
Cell viability %	Extract	0	100
		0.05	93.15
		0.1	83.28
		0.5	76.49
		1	71.65
		5	22.64

none of the toxic effects. It may cause some toxic effects only at higher doses.

## BEHAVIORAL ASSESSMENTS

### Animals and experimental design

18 male Swiss Albino mice of 25 – 35 g were procured from the Kings Institute Chennai and acclimatized for 7 days. The animals will be fed with commercially available food and maintained under standard condition of temperature (25 C ± 5 C), with a relative humidity ( 55 ± 10% ) and 12/12hr light /dark cycle. The experimental protocols were approved by the Institutional Animal Ethical Committee of CPCSEA ( Committee for the Purpose of control and Supervision of Experiments of Animals). The approval number is (IAEC/130/2010).

The animals were divided into three groups (n=6): Group I - 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg/day, i.p, 1 day), Group II - MPTP + hydroalcoholic root separate (20 mg/kg/day, i.p, 1 day + 200 mg/kg/day, p.o, 6 days), and Group III - MPTP + hydroalcoholic root removal (20 mg/kg/day, i.p, 1 day + 400 mg/kg/day, p.o., 6 days).

Parkinsonism was induced by administering four infusions of MPTP (20 mg/kg; i.p) at 2-h interim. The medication treatment was given on beginning days, 30 min preceding the principal infusion of MPTP, and once day for an additional 6 days of investigation period. After the 7 days of treatment, all the three groups underwent motor integration tests. Cervical dislocation was performed and the striatal tests were

isolated by detaching the cerebrum, homogenized in super cold phosphate buffer saline, and utilized for biochemical appraisals [12].

### Rotarod test

An automated rotarod (Inco) was utilized to measure the motor coordination on the 7<sup>th</sup> day. The animals were kept on the rotating bar at a speed of 10 rpm at 5 min interim and experienced 10 trails before the beginning of the trial. The control mouse stayed on the bar for 180 s Fig.1. In a similar way, the time of the tumble from the pole was noted with a cutoff time of 3 min [13,14,42].

### Grip strength

Male mice with a normal weight of 18–30 g were utilized in this investigation. The animals were placed on a metallic wire suspended 30 cm over the floor, on which they quickly get to handle on with the forepaws and afterward were discharged to hang. The animals which climbed the wire in 5 s were chosen for the trial and were tested each 15 min Fig 2. The animals unfit to contact the danger with their rear appendages within 5 s or tumble off from the metallic wire were considered as disabled [15].

### Spontaneous locomotor activity

The animals were individually placed in the enclosure of the actophotometer, moved and interfered with a light emission falling on the photograph cell, and a count was recorded as the “basal action score.” After 30 min and 60 min of the oral administration of the vehicle or standard or concentrate, each mouse was retested for an action of about 10 min and the distinction in the basal action was recorded. At last, the rate diminished in the locomotor movement was computed [16-18].

### Catalepsy test

The animals were kept on a flat surface with both hind limbs being placed on a square wooden block (3 cm tallness), and latency to move was computed in seconds Table 3. Haloperidol was utilized to initiate the catatonia impact, and the stages were learned at 30, 60, 90, and 120 min after administration of the concentrate. In stage I, the animal stays stationary, and just a slight push causes brief developments (score - 0). In stage II, even a drive never again causes developments in the animal (score - 0.5). In stage III, forelimbs of the animals were placed on a square 3 cm high, and still, it did not make any developments (score - 1). In stage IV, one of its forelimbs is placed on a square of 9 cm high and the other forelimb is permitted to hang free and the animal keeps up a settled position (score - 2) [19-21].

### Beam walking test

At first, the mice were made to walk on a beam 80 cm long, 3 cm wide, raised 30 cm with metal backings to an objective box. After 30 min of standard and test medications, the mouse was placed toward one side of the beam and let to walk to the objective box. If the mice fall off, they were once again placed on it for a time period of 60 s. As a proportion of motor coordination shortage, the number of foot slips Table 4 of one or both hind limbs was taken into account [24-26].

### Evaluation of dopamine, 3,1-dihydroxy phenylacetic acid (DOPAC), and homovanillic acid (HVA) levels

The dissected brain samples were weighed, frozen at 8°C till the assay and homogenized in 1ml ice-cooled 0.1 mmol/L Perchloric acid solution containing 0.2 µg/ml L-isoproterenol hydrogen and 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA). Tissue homogenates were centrifuged at 15,000×g at 40°C for a ½ h, and the supernatant was filtered and stored at –8°C until assay. HPLC with an electrochemical detector and 25 cm × 0.5 cm I.D column was used in the assessment of dopamine, DOPAC, and HVA levels Table 5. The sample peak received is compared with the standard peak and expressed in microgram per gram of tissue weight [27,28].

### Estimation of lipid peroxidation

To 1 ml of tissue homogenate, 30% trichloroacetic acid (TCA) and 1 ml of 0.8% thiobarbituric acid reagent were added and centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was examined at 535 nm at room temperature against the blank [29].

Table 3: Effect of hydroalcoholic extract of *Hemidesmus indicus* on catatonia behavioral study

S.No	Group	Dose (/kg)	Cataleptic scores at different time points			
			30	60	90	120
1	MPTP	20 mg/kg	10.37±1.12	20.98±1.72	31.09±2.72	44.20±3.72
2	HI+MPTP	200 mg/kg	7.092±0.81	9.58±0.06	10.46±0.04	8.03±0.03
3	HI+MPTP	400 mg/kg	11.59±0.72	13.06±0.03	12.60±5.79	11.82±4.12

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Table 4: Effect of hydroalcoholic extract of *Hemidesmus indicus* on beam walking assay

S.No	Groups (n=6)	Dose (/mg)	Meantime to complete the task (min)	Mean number of foot slips
1	MPTP	20 mg/kg	22.73±3.30	4.04±0.72
2	HI+MPTP	200 mg/kg	17.96±0.93	2.82±0.36
3	HI+MPTP	400 mg/kg	10.58±2.18	1.63±0.04

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Table 5: Effect of hydroalcoholic extract of *Hemidesmus indicus* on dopamine, DOPAC, and HVA levels

S.No	Group (n=6)	Dose	Dopamine	DOPAC	HVA (mg/g of brain tissue)
1	MPTP	20 mg/kg	3.68±0.01	1.26±0.15	0.55±0.01
2	HI+MPTP	200 mg/kg	4.73±0.14	1.66±0.04	0.75±0.01
3	HI+MPTP	400 mg/kg	8.88±0.18	1.94±0.01	0.95±0.05

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, HVA: Homovanillic acid, DOPAC: Dipalmitoylphosphatidylcholine

The content material of thiobarbituric acid reactive materials (TBARS), expressed as "n" formed per milligram of protein within the tissue, was calculated Fig 4.

#### Assay of superoxide dismutase (SOD)

SOD was assessed using the inhibition of the formation of nicotinamide adenine dinucleotide (NADH)-phenazine methosulfate nitroblue tetrazolium formazan. NADH after the 90 s of incubation and the reaction becomes terminated by the addition of glacial acetic acid. The coloration formed at the end of the reaction was extracted into the butanol layer and measured at 520 nm Fig 5 [30].

#### Assay of catalase

The tissue is homogenated in isotonic buffer (pH - 7.4) and centrifuged at 1000 rpm for 10 min. 20 µl of 100-fold diluted tissue supernatant brought to 980 µl of the assay mixture; the assay mixture consists of 900 µl of 10 mmol/L of H<sub>2</sub>O<sub>2</sub>, 50 µl of Tris HCl buffer (pH - 8), and 30 µl of water. The degree of decomposition of H<sub>2</sub>O<sub>2</sub> becomes monitored spectrophotometrically at 240 nm Fig 3 [29,30].

#### Assay of glutathione (GSH) peroxidase (GPx)

About 0.1 ml of the diluted tissue was incubated at 37°C with response combination mixture consisting of 0.2 ml of each EDTA, sodium azide, and H<sub>2</sub>O<sub>2</sub>. 0.5 ml of TCA was added to this aggregate to stop the response and then centrifuged at 2000 rpm. 4 ml of disodium hydrogen phosphate and 0.5 ml dithiobis (2-nitrobenzoic acid) (DTNB) have been added to 0.5 ml of supernatant, and the color formation changed was recorded at 420 nm in a spectrophotometer Fig 6 [31-33,37].

Activity was expressed as µ moles of GSH oxidized/min/mg protein.

#### Assay of reduced GSH

To 2 ml of the tissue homogenate, add KCl, 4 ml of cold distilled water, and 1ml of 50 % TCA. The contents were centrifuged at 3000 rpm for 15 min, and from this, 2 ml of the supernatant was taken and 4 ml of 0.4 M Tris buffer (pH 8.9) and 0.1 ml of 0.01 M DTNB were added; the absorbance was read at 412 nm in opposition to the blank reagent. For blank readings, 2 ml of distilled water was used.

Total GSH change was calculated using the following formula:

$$Co = (A^*D)/E$$

Where A is an absorbance at 412 nm Fig 7, D is dilution component, and E is the molar extinction coefficient (C = 13000M<sup>-1</sup>CM<sup>-1</sup>); Co is the attention of GSH [34-38].

#### Statistical analysis

The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnett's test. p<0.05 was considered as statistically significant.

## RESULTS

### DISCUSSION

In recent years, there are more investigations on MPTP-induced neurotoxicity which was reported that it might be a poison which might lead to Parkinsonism. Przedborski *et al.* suggested that MPP<sup>+</sup>, a lively metabolite of MPTP, gets gathered in the *S. nigra* pars compacta neurons, inhibits ATP manufacturing, and stimulates superoxide radical formation [40]. These radicals react with nitrogen to supply peroxy nitrile which damages proteins like tyrosine hydroxylase through nitration which consequently inhibits dopamine manufacturing.

SH-SY5Y neuroblastoma cells are an important tool which has been widely used as a neurodegenerative disease model. These cells have the capability to differentiate into neuron-like cells which are morphologically and biochemically similar to neurons [31,32]. For the present study, the SH-SY5Y cells treated with concentrations of hydroalcoholic concentrate of *H. indicus* (HAHI) ranging from 0.05 mg/mL to 5 mg/mL showed none of the toxic effects. It may cause some toxic effects only at higher dose ranges.

MPTP administered mice subjected to the rotarod test revealed a substantial lack of neuromuscular coordination and the terrible performance in the hang test. The narrow beam maze used to check the balance and running overall performance turned into considerably altered through MPTP treatment. In the present study, a significant reduction of striatal lipid peroxidation with extended antioxidant fame becomes determined in MPTP-induced animals. MPTP also caused the catatonic reaction. *H. indicus* extract (HAHI) prevented motor impairment in a dose-dependent way and also reduced the latency strength of catatonic behavior.

The animals which were administered 400 mg/kg of HAHI had a huge development than those which received lower doses. Maximum cataleptic scores were observed after 60 and 90 min of administration. The scoring was drastically reduced after 60 min with the test drug HAHI at the doses examined (200 mg/kg and 400 mg/kg).

Under normal physiological conditions, free radicals produced through metabolism can be inactivated by free radical scavenging system. In PD, the environment within the SN is conducive to the formation of cytotoxic free radicals. These free radicals react instantaneously with membrane lipids and cause lipid peroxidation and cell death, which indicates that it is due to the excessive oxidative state in SN. Increased oxidative strain results in over intake of SOD and Gpx.

The decreased stage of reduced GSH in MPTP-treated experimental animals indicated that there was an extended generation of free radicals and the decreased GSH depleted during the process of oxidative strain. A large reduction of striatal dopamine turned into at least in part averted when mice have been dealt with HAHI at each dose and the beneficial impact as the maximum for 400 mg/kg dose.

It has been mentioned that monoamine oxidase (MAO) inhibition became obvious with *H. indicus* extract due to the presence of coumarin liquids of Asclepiadaceae family [42]. Possibly due to the inhibition of MAO, it may be said that the dopamine degree enhancing is assured by way of this plant extract. In the present study, HAHI notably reduced the MPTP prompted lipid peroxidation (TBARS) in a dose-dependent way.

MPTP decreased SOD, catalase, and brain GSH ranges in dose-dependent way than the ordinary range. These stages were significantly covered on HAHI treatment. In end, it can be proved that HAHI can be effectively employed inside the treatment of PD due to its neuroprotective and antioxidant property.

## CONCLUSION

*H. indicus* is a historically used medicinal plant with anti-inflammatory activity, antiviral, antibacteriostatic, anticancer, and antispasmodic properties, and it is far used as a nerve tonic to enhance reminiscence. The above study has been explored to take a look at its anti-Parkinsonism interest which became proved to a degree. The initial phytochemical screening of the hydroalcoholic extract of *H. indicus* showed the presence of coumarin liquid, hemidesmin-1, and hemidesmin-2. In the behavioral study, the MPTP-induced mice confirmed a development in motor coordination and the muscular strength which dealt with the plant extract of *H. indicus*. The dopamine, DOPAC, and HVA levels and the antioxidant property have been observed using the *H. indicus* remedy. Hence, it can be stated that the anti-Parkinsonism activity of *H. indicus* might be due to the enhancement of dopaminergic neurons and also may be due to the MAO inhibiting interest of coumarin present inside the plant, which further desires a detailed study.

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## AUTHORS CONTRIBUTION

- Dr. V. Chitra - Principle investigator.
- Evelyn Sharon. S - Performed the animal studies.
- Manasa. K - Performed cell culture studies.
- Shatabdi Choudhury - Performed the statistical studies.

## CONFLICTS OF INTEREST

The authors certify that they have no conflicts of interest.

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