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

CHITRA V, MANASA K*, EVELYN SHARON S*, SHATABDI CHOUDHURY

Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology (SRM IST), Kanchipuram, Tamil Nadu, India. Email: k.manasa1@gmail.com

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 this barbituric acid reactive substance (TBARS), superoxide dismutase, catalase, 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 was made by rotarod test, grip strength test, locomotor activity, and catatonia behavioral study. Appraisal of cell reinforcement catalysts, such as this barbituric acid reactive substance (TBARS), superoxide dismutase, catalase, glutathione (GSH) peroxidase, and GSH, were also assessed to screen the neurotoxicity incited by MPTP.

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.
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/H2O (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 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...
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 12/12 hr 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 on 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 7th day. The animals were kept on the rotating bar at a speed of 10 rpm at 5 min interim and experienced 10 trials 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 cause 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 x 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].
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 H2O2, 50 µl of Tris HCl buffer (pH - 8), and 30 µl of water. The degree of decomposition of H2O2 becomes monitored at 1000 rpm for 10 min. 20 µl of 100-fold diluted tissue supernatant became determined in MPTP-induced animals. MPTP also caused reduction of striatal lipid peroxidation with extended antioxidant fame the balance and running overall performance turned into considerably improved in the hang test. The narrow beam maze used to check substantial lack of neuromuscular coordination and the terrible motoric dysfunction. MPTP administered mice subjected to the rotarod test revealed a motoric improvement.

**Statistical analysis**

The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnety’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+ 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 peroxynitrite which damages proteins like tyrosine hydroxylase through nitration which consequently inhibits dopamine manufacturing.

SH-SYSY 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 example, these cells have the capability to differentiate into neuron-like cells which are morphologically and biochemically similar to neurons [31,32]. For this study, the SH-SYSY 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. 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 improved 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.

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

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Dose (/kg)</th>
<th>Cataleptic scores at different time points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>MPTP</td>
<td>20 mg/kg</td>
<td>10.37±1.12</td>
</tr>
<tr>
<td>2</td>
<td>HI+MPTP</td>
<td>200 mg/kg</td>
<td>7.09±0.81</td>
</tr>
<tr>
<td>3</td>
<td>HI+MPTP</td>
<td>400 mg/kg</td>
<td>11.59±0.72</td>
</tr>
</tbody>
</table>

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

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

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

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

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

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

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

### 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+, 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 peroxynitrite which damages proteins like tyrosine hydroxylase through nitration which consequently inhibits dopamine manufacturing.

SH-SYSY 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 this study, the SH-SYSY 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. 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 improved 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-4 hydroxylated metabolites 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.

ACKNOWLEDGMENT

The authors are grateful to the Dean of SRM College of Pharmacy, SRM Institute of Science and Technology, for her continued support. They also thank the Vice Principal and Head of Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology.

AUTHORS CONTRIBUTION

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

CONFLICTS OF INTEREST

The authors certify that they have no conflicts of interest.

REFERENCES


34. Spitz DR, Oberley LW. Measurement of mnSOD and cuZnSOD activity in mammalian tissue homogenates. Curr Protoc Toxicol 2001;Chapter 7:Unit7.5.


